COLLEGE OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY

NAVSARI AGRICULTURAL UNIVERSITY, NAVSARI

Introduction

The College of Veterinary Science and Animal Husbandry was established on 1st July, 2008 with the funding from the Chief Minister's Ten Point Programme (Vanbandhu Kalyan Yojana) and Government of Gujarat under the flagship of Navsari Agricultural University by the Late Vice Chancellor - Dr. R.P.S. Ahlawat.

The college building was inaugurated on January 1st, 2012 by 'Padma Vibhushan' awardee, Hon'ble M.P. (RajyaSabha) and 'Father of Green Revolution' Dr. M.S. Swaminathan, in the solemn presence of Hon'ble Minister Shri Dileep Sanghani (Cabinet Minister of Agriculture and Co-operation, Gujarat state) and Hon'ble Minister Shri Mangubhai Patel (Minister of Tribal Development and Forest and Environment, Gujarat state). The college building spans in the area of 12,500 m^2 built at the cost of Rs 14.5 crores. The building is divided in to five blocks, mainly 'A block' which is administrative building, B, C and D blocks, which has 13 departments and E block, which has four class rooms, two examination halls, a library room, an exhibition hall etc. All the departments and class rooms are having permanently fixed LCD projectors with *wi-fi* internet facility. All the departments and administrative blocks are also well equipped with computer and internet facilities, CCTV, *Shalihotra* Conference Hall, RO water cooler, computer room for students etc. All the departments of the college are having most of the infrastructural facilities, equipments as well as laboratories facilities as per VCI requirements. Along with that there are several state of art instruments in various departments of the college.

Strategic South Gujarat location of this veterinary college caters the necessity of livestock farmers / owners and pet owners especially of tribal belt of this region.

Objectives of the institute

- As a principal mandate to impart education at undergraduate, postgraduate and doctorate levels in the field of Veterinary and Animal Husbandry.
- To carry out region specific need based research in Veterinary and Animal Sciences and subsequently devise specific package of practices
- To effectively collaborate in the technology transfer applicable to animal health / production practices and recommend suitably to the tribal belt of South Gujarat.
- To educate students especially of tribal area in the field of Veterinary Science so as to improve their family livelihood.

Goals of the College:

- To impart best knowledge to the students
- To carry out applied as well as basic research

- To transfer the technology / latest research recommendations with respect to veterinary and animal husbandry practices at the grass root level.
- To be one of the best veterinary college in India
- To adapt Vansada Taluka of Navsari District and double its income in a span of 5 years

Teaching

The college imparts education at Bachelor's level, Master's level and Doctorate level. BVSc. & AH education is carried out as per the VCI norms of 2008 and MVSc and PhD education follows ICAR 2010 rules. The college has got all the 17 departments and has adopted the course curriculum as recommended by the Veterinary Council of India (VCI) for the veterinary colleges of India. Moreover, all the facilities including HRD for the college are being developed in conformity with the norms of VCI. At present, totally there are 334 students are getting their education at various level. The first undergraduate batch of the college passed out in year 2013. Till date, total 121 students have earned their Bachelor's degree, while 73 and 17 students have completed their Master's and PhD degree, respectively from the college. The departments are well equipped with laboratory facilities and other specialized equipments. Laboratory facilities/teaching aids are continuously being improved to provide latest technology. This is the first college of India to get VCI recognition after enactment of VCI 2008. Currently, the college has total 59 teaching faculties including 8 Professors, 6 Asso. Prof and 45 Asst. Professors in the various departments.

Clinical Services

Teaching Veterinary Clinical Complex was started on 1st Oct., 2009 by Hon'ble Shri. Dileep Sanghani, Minister for Agriculture Cooperation, Animal Husbandry, Fisheries and Cow breeding and Hon'ble Mangubhai Patel, Minister of Forest and Environment and Tribal Development Department of Government of Gujarat in presence of Member of Parliament Hon'ble Shri C. R. Patil for learning of the students as well as to cater the animal owners of surrounding area. A separate building was inaugurated on 53rd Gujarat Gaurav Din 1st May, 2013 as Clinical complex by the-then Hon'ble CM of the state Shri Narendra Modi. All the clinical departments are working in this building with state-of-art infrastructural facilities as a referral animal clinic. The building has been planned with the latest facilities for animal treatment.

Veterinary Clinical Research and Experiential Learning Complex (VCRELC) was instigated in the year of 2009 with the following objectives:

- To provide health coverage and disease diagnostic research in on-station and on-farm situations
- To conduct veterinary clinical camps to provide 'on the spot' treatment and diagnosis deemed necessary on the pattern of Cooperative dairies
- To provide experiential training to veterinary students viz. interns & postgraduate scholars,
- To create epizootiological research database for brucellosis, leptospirosis, helminthiasis and other economically important infectious diseases and
- To educate the farmers on disease prophylactic and containment measures.

Within a very short span since the inception of this project, more than 15000 animal owners have been benefited by this unit. Moreover the number of sick animals reporting daily to the OPD, has been increasing drastically owing to successful treatment carried out after appropriate disease diagnosis using the imaging techniques and laboratory facilities. The refresher courses for vets and para-vets viz. ASCAD trainings in clinical subjects are organized on a regular basis. The ambulatory services are also rendered to the dairy farmers in the vicinity of Navsari Agricultural University since January, 2011.

Research and Extension

All the staff members are actively involved in the area specific need based research. Till date, various departments of the college have completed more than 80 Agriculture Research Sub-committee approved technical research projects and more than 25 recommendations for farmers' and scientific community have been approved by the authorities. Two plan projects, funded by State government with five years duration, one each under Dept. of Veterinary Microbiology and Dept. of Veterinary Pharmacology are also going on since year 2013-14. Till date, the college has organized two National seminars in the year 2012 and 2014.

Navsari Agricultural University has a Livestock Research Station since more than three decades, in which students are presently imparted practical training. Livestock Research Station is well established with sufficient infrastructure and human resources. The well-known breed of Goat "Surti" and Buffalo "Surti" both having origins from Surat, are being maintained at the station. Apart from that, the station has also got a Kankrej and Kankrej x HF animals unit.

Faculty members of the college frequently participate in the various extension activities like *Pashu Arogya Mela*, *Krushi Mahotsav*, *Pashupalan Shibir*, *Mera Gaun Mera Gaurav* etc. organized by State / Central Government, Cooperative dairies or other organizations like NGOs, etc.

Student Amenities:

Boys' hostel has been built up with 70 rooms to accommodate about 210 boys, a reading room, TV room with satellite TV facility, internet connectivity with *wi-fi*, self-run mess facility, a playground, CCTV, solar heater, RO water cooler system etc. The hostel was inaugurated by erstwhile Hon. Min. Shri Mangubhai Patel (Minister, Forest and Environment) on 24th December, 2010. The hostel has been appropriately named as *Eklavya* Boys' Hostel.

A Student Representative Council is also instigated for various cultural and sports events. Students of the college participate in the various sports and cultural competitions at inter-collegiate, inter-university and national level. Every year, during the month of April, Annual day function is celebrated to encourage the talents from the students and bolster their extracurricular skills.

DEPARTMENT OF VETERINARY PHARMACOLOGY AND TOXICOLOGY COLLEGE OF VETERINARY SCIENCE & ANIMAL HUSBANDRY NAVSARI AGRICULTURAL UNIVERSITY, NAVSARI

Introduction

The purpose of an education is to assist the individual in becoming an independent person who can think. With fulfilling this idea, Veterinary Pharmacology and Toxicology is considered a bridge between basic and clinical Veterinary Science subjects. Veterinary Pharmacology and Toxicology provides understanding about action of drugs on body, how it is absorb and eliminated, its toxic effect, its clinical indications and contraindications, and its dosage in species of animals to be treated. Intellectual framework of pharmacologic and toxicologic principles is an art of rational therapeutics for potential risks, animal wellbeing and public health implications. In the new millennium, there has been great emphasis towards developing animal specific drugs for the unique indication and improving basic pharmacology knowledge. In this direction, the Department of Pharmacology and Toxicology was established in June 2010, as a part of College of Veterinary Science and Animal Husbandry, Navsari Agricultural University, Navsari, Gujarat.

Objective

a) Education for UG and PG students
b) Research work in Pharmacodynamics, Pharmacokinetics, Toxicodynamics, Toxicokinetics, Genotoxicity, Pharmacogenomics.
c) Extension of Ethnoveterinary medicine and its field application.

Education:

a) Under graduate Teaching (2008-2015)

Code	Course Name	Credit	Semester
VPT-311	General and Systemic Veterinary Pharmcology	2+1 = 3	V
VPT-321	Veterinary Neuropharmcology	2+1 = 3	VI
VPT-411	Veterinary Chemotherapy	2+0 = 2	VII
VPT-421	Veterinary Toxicology	2+0 = 2	VIII
VLD-421	Veterinary Laboratory Diagnosis (Toxicology Part)	0+1 = 1	VIII

Under graduate Teaching (2016 onwards)

Veterinary Pharmacology and Toxicology	4+1 = 5	Third Professional Year

b) Post graduate Teaching

Code	Course Name	Credit
VPT-601	General Pharmacology	2+0
VPT-602	Autonomic and Autacoid Pharmacology	2+1
VPT-603	CNS Pharmacology	2+1
VPT-604	Digestive and Respiratory Pharmacology	2+0
VPT-605	Cardiovascular and Renal Pharmacology	2+0
VPT-606	Endocrine and Reproductive Pharmacology	2+0

VPT-607	Chemotherapy	2+1
VPT-608	Toxicology of Xenobiotics	2+1
VPT-609	Toxicology of Plants and Toxins	2+0
VPT-610	Pharmacological Techniques	1+1
VPT-611	Techniques in Toxicology	1+1
VPT-612	Ethnopharmacology	2+0
VPT-691	Master's Seminar	1+0
VPT-699	Master's Research	20
VPT-701	Advances in Neuropharmacology	2+0
VPT-702	Autacoid Pharmacology	1+0
VPT-703	Pharmacology of Herbal Drugs	2+1
VPT-704	Drug Metabolism	2+0
VPT-705	Molecular Pharmacology	2+0
VPT-706	Pharmacokinetics	2+1
VPT-707	Pharmacogenomics	2+0
VPT-708	Immunopharmacology	1+0
VPT-709	Molecular Toxicology	2+0
VPT-710	Clinical Pharmacology	1+1
VPT-711	Clinical Toxicology	2+1
VPT-712	Ecotoxicology	2+0
VPT-713	Regulatory Toxicology	2+1
VPT-790	Special Problem	0+2
VPT-791	Doctoral Seminar I	1+0
VPT-792	Doctoral Seminar II	1+0
VPT-799	Doctoral Research	45

PG and Ph.D. Students completed:

Sr. No.	Degree	Name of Student	Thesis Title
1.	M.V.Sc.	Rikesh B. Patel (2013)	Studies on Pharmacokinetics and safety of Cefpirome in Cow Calves
2.	M.V.Sc.	Prahlad F. Solanki (2013)	Studies on Pharmacokinetics and safety of Cefpirome in Goats
3.	M.V.Sc.	Ritesh L. Patel (2014)	Studies on Pharmacokinetics and safety of Cefquinome in Cow Calves
4.	Ph.D.	Shireen Tiwari (2014)	Studies on effect of febrile condition and coadministration of meloxicam on pharmacokinetics of Cefquinome and its safety in Goats
5.	M.V.Sc.	Tamanna H. Solanki (2016)	*In vitro* release and pharmacokinetics of enrofloxacin PHBV microsphere in rats

Facilities available:

A) Laboratories:

1) Experimental Pharmacology & Pharmacy Laboratory

2) Toxicology Laboratory

3) Post graduate research Laboratory
4) Pharmacokinetic Laboratory
5) Ethnopharmacology Laboratory
6) Small laboratory animal house

B) Instruments:

- Semi-preparative & Analytical HPLC
- Laboratory grade water purification system (Milli Q)
- Refrigerated centrifuge
- Ultrasonic cleaner (Sonicator)
- Digital Ph meter
- Cyclomixer
- Microcentrifuge machine
- Deep freeze (-45°C)
- Rotatary vacuum evaporator
- Chilled water circulator
- Lyophilizer
- Vacuum oven
- Sterilization (Autoclave, Hot air oven)
- Biosafety cabinet (BSL-II)
- Small animal anesthesia system
- Digital Plethysmometer
- Digital Rotarod
- Double beam spectrophotometer
- Electronic stimulator
- Cooks pole climbing apparatus
- Student's organ bath assembly
- Photoactometer
- Centrifuge machine (14000 rpm)
- Magnetic stirrer with hot plate

Research Activities:

Sr. No.	Research Projects
1	Evaluation of Antibacterial Efficacy of *Ossimum bascilicum* (Damro) of South Gujarat
2	Evaluation of Antibacterial Efficacy of *Cassia fistula* (Garmalo) of South Gujarat
3	Studies on Pharmacokinetics and Pharmacodynamic relationship of Cefpirome in Cow Calves
4	Studies on Pharmacokinetics and Pharmacodynamic relationship of Cefpirome in Goats
5	Studies on Pharmacokinetics and Pharmacodynamic relationship of Cefquinome in Cow Calves
6	Studies on Pharmacokinetics and Pharmacodynamic relationship of Cefquinome in Goats
7	Evaluation of in vitro antimicrobial properties of endophytes isolated from medicinal plants
8	Evaluation and Validation of Antimicrobial and Anti-inflammatory Activity of Medicinal Plants Used by Vanbandhus of South Gujarat (Rs. 147.7 lakhs) (Sanctioned in June-2012)

Publications:

Research Articles: 29

Book: 02

Chapter in book: 04

Laboratory Manuals: 08

Lead Papers/Review articles: 02

Articles in Vernacular language: 07

Organization of State Level Seminar:

"Role of Veterinarians in containment of Antimicrobial Resistance" organized at Veterinary College, NAU, Navsari on World Veterinary Day -2012 on 28/04/2012

Manpower:

Sr. No.	Name of faculty	Designation
1.	Dr. Shailesh K. Bhavsar	Professor and Head
2.	Dr. Raseshkumar D. Varia	Assistant Professor
3.	Dr. Jatinkumar H. Patel	Assistant Professor
4.	Dr. Falguni D. Modi	Senior Research Assistant
5.	Mrs. Sejal Patel	Laboratory Technician
6.	Mrs. Lipsa Desai	Laboratory Technician
7.	Mrs. Priyanka Patel	Animal Attendent

Thrust areas of research at department:

- Ethnopharmacology
- Pharmacokinetics
- Toxicology
- Pharmacogenomics

CENTRAL ORGANIZING COMMITTEE

XVI Annual conference of Indian Society of Veterinary Pharmacology and Toxicology and National symposium on "Animal Health and Production: Challenges & Opportunities in Veterinary Pharmacology & Toxicology"

23-25 November 2016

Sr. No.	Name of Members	Designation
1.	Dr. C. J. Dangaria Vice- Chancellor, NAU, Navsari	Patron- in- Chief
2.	Dr. A. N. Sabalpara Director of Research, NAU, Navsari	Patron
3.	Dr. A. K. Srivastava Director, NDRI, Karnal	President - ISVPT
4.	Dr. (Mrs.) Mudasir Sultana Professor and Head (VPT) C.V.Sc. & A.H., Jammu	Vice-President - ISVPT
5.	Dr. Gopakumar Former Principal, C.V.Sc. & A.H., Wayanad, Kerala	General Secretary - ISVPT
6.	Dr. A. M. Thaker Principal, C.V.Sc. & A.H., A.A.U., Anand	Executive Secretary (Head Quarter) - ISVPT
7.	Dr. N. H. Kelawala Dean, C.V.Sc. & A.H., NAU, Navsari	Chairman
8.	Dr. Sunil R. Chaudhary Associate Director of Research, NAU, Navsari	Vice- Chairman
9.	Dr. S. K. Bhavsar Professor and Head (VPT)	Organizing Secretary
10.	Dr. R. D. Varia, Assistant Professor (VPT) Dr. J. H. Patel, Assistant Professor (VPT)	Co- Organizing Secretaries
11.	Dr. Falguni D. Modi, SRA (VPT)	Treasurer

LOCAL ORGANIZING COMMITTEE

Dr. N. H. Kelawala, Dean, College of Veterinary Science & Animal Husbandry, NAU, Navsari and In-charge Dean, College of Fisheries Science, NAU, Navsari
Dr. M. K. Arvadia, Principal, N.M. College of Agriculture, NAU, Navsari
Dr. B. N. Patel, Principal, ASPEE College of Horticulture and Forestry, NAU, Navsari
Dr. G. R. Patel, Director of Extension Education, N A U., Navsari
Dr. Sunil R. Chaudhary, Associate Director of Research, NAU, Navsari
Dr. H. R. Pandya, Principal, AABMI, NAU, Navsari
Dr. C. V. Savalia, Director – Students' Welfare, N A U., Navsari
Dr. G. G. Radadiya, Director of Information Technology, NAU, Navsari
Dr. P. K. Srivastava, Principal, College of Forestry, NAU, Navsari
Dr. V.A. Solanki, I/c Registrar, NAU, Navsari
Dr. D. T. Chaudhary, Comptroller, NAU, Navsari

Sr.No.	Name of the Committee	Name of Members
1.	INVITATION COMMITTEE	Dr. C. V. Savalia (Convener) Dr. M. D. Patel (Co-convener) Dr. Rajeev Kumar Dr. Niranjan Kumar Dr. Priti Vihol
2.	FOOD & REFRESHMENT COMMITTEE	Dr. J. B. Solanki (Convener) Dr. A. A. Vagh (Co-convener) Dr. N. S. Dangar Dr. S. V. Mavadiya Dr. S. B. Patel Dr. A. P. Raval
3.	STAGE AND INAUGURAL SESSION COMMITTEE	Dr. Sandhya Chaudhary (Convener) Dr. Gopal Puri (Co-Convener) Dr. V. K. Singh Dr. A. K. Sharma Dr. S. M. Parmar Dr. T. K. S. Rao Dr. R. Menaka Dr. Dipti Nayak
4.	REGISTRATION CUM KIT COMMITTEE	Dr. B. P. Brahmkshatri (Convener) Dr. R. R. Singh (Co-convener) Dr. U. V. Ramani Dr. D. R. Patel Dr. K. K. Verma Dr. Pushpa Rathod
5.	SCIENTIFIC CUM PUBLICATION COMMITTEE	Dr. S. K. Bhavsar (Convener) Dr. R. D. Varia Dr. J. H. Patel Dr. Falguni Modi
6.	SCIENTIFIC / TECHNICAL SESSIONS COMMITTEE	Dr. V. B. Kharadi (Convener) Venue-I Dr. C. T. Khasatiya (Co-convener) Dr. K. K. Tyagi Dr. S. K. Jhala
		Dr. N. B. Patel (Convener) Venue-II Dr. C. F. Chaudhari (Co-convener) Dr. S. Chaurasiya Dr. Surbhi Tyagi
		Dr. Gopal Puri (Convener) Venue-III Dr. U.V. Ramani Dr. Niranjankumar Dr. Rajeevkumar

7.	FINANCE AND FUND RAISING COMMITTEE	Dr. N. H. Kelawala (Convener) Dr. S. K. Bhavsar (Co-convener) Dr. Falguni Modi Dr. J. H. Patel Dr. R. D. Varia A.A.O.
8.	EXCURSION TOUR COMMITTEE	Dr. V. S. Dabas (Convener) Dr. D. N. Suthar (Co-convener) Dr. R. H. Bhatt Dr. J. A. Vala
9.	TRANSPORT AND ACCOMMODATION & COMMITTEE	Dr. I. H. Kalyani (Convener) Dr. V. R. Patel (Co-convener - Accommodation) Dr. G. P. Sabapara (Co-convener - Transport) Dr. L. M. Sorathiya Dr. N. F. Chaudhari Dr. J. M. Patel Dr. Y. D. Padheriya Dr. B. J. Trangadiya Dr. L. C. Modi Dr. D. C. Patel Dr. Swati Gupta Shri Sandip Shri V. P. Vejpara Shri R. D. Prajapati (Junior Engineer)
10.	AUDIO-VISUAL, PRESS – MEDIA AND POSTER SESSION COMMITTEE	Dr. J. N. Mistry (Convener) Dr. M. R. Bhatt (Co-convener) Dr. Durgga Rani Dr. R. S. Ghasura Dr. K. K. Sharma
11.	CULTURAL COMMITTEE	Dr. G. M. Pandya (Convener) Dr. S. A. Mehta (Co-convener) Dr. H. C. Sharma Dr. Mamta Janmeda Dr. Jigisha Ninama
12.	MEMENTO, AWARDS AND BANNER COMMITTEE	Dr. J. H. Patel (Convener) Dr. D. C. Moliya (Co-convener) Dr. M. Choubey
13.	VALEDICTORY FUNCTION COMMITTEE	Dr. Sandhya Chaudhary (Convener) Dr. V. K. Singh Dr. A. K. Sharma Dr. R. Menaka Dr. G.M.Pandya Dr. Sudhir Mehta

PROGRAM ISVPT 2016

Time	PROGRAM		
DAY-1 (23rd November 2016) Venue: University Auditorium, NAU, Navsari			
08.00 to 09.00	Breakfast & Registration		
09.00 to 11.15	Inauguration		
11.15 to 11.30	High tea		
11.30 to 12.30	Chellapa Memorial Oration Dairy nutraceuticals and functional dairy foods: Current status, issues and challenges Dr. A. K. Singh, Principal Scientist, Division of Dairy Technology, National Dairy Research Institute, Karnal, Haryana		
12.30 to 13.30	Dr. M. Sabir Oration Biomarker based translational research in new drug discovery: Significance of Veterinary Pharmacology Dr. R. K. Goyal, Vice Chancellor, Delhi Pharmaceutical Sciences and Research University, Delhi		
13.30 to 14.30	Lunch		
	Technical Session I : National Symposium Chairperson: Dr. V. V. Ranade Co-chairperson: Dr. N. Gopakumar Rapporteur: Dr. C. V. Savalia		
14.30 to 15.00	NS-01	Dr. J. K. Malik	Modulation of arsenic-induced apoptosis in immune cells by curcumin: An overview
15.00 to 15.30	NS-02	Dr. A.K. Srivastava	Nutritional Pharmacology: The imperative facet in therapy
15.30 to 16.00	NS-03	Dr. M. R. Jain	New drug discovery: Challenges & Opportunities
16.00 to 16.15	Tea		
16.15 to 16.45	NS-04	Dr. D. B. Patil	Challenges and opportunities in Veterinary Pharmacology and Toxicology
16.45 to 17.15	NS-05	Dr. A. M. Thaker	Non-biological contaminants: How safe is our food of animal origin?
17.15 to 17.45	NS-06	Dr. N. Punniamurthy	Ethnopharmacology: Challenges and Opportunities
17.45 to 18.15	NS-07	Dr. M. H. Parabia	Role of ethnobotanical studies in ethnopharmacological research
18.30 to 20.00	Cultural Evening		
20.00 to 21.30	Dinner		

DAY-2 (24th November 2016) Venue: Hall I, Hall II & Hall III Veterinary College, NAU, Navsari)		
08.00 to 09.00	Breakfast	
09.00 to 11.15	Award Sessions (Hall-I)	
	Technical Session II: Dr. Jayvir Anjaria Award Chairperson: Dr. Dheer Singh Co-chairperson: Dr. A. H. Ahmed Rapporteur: Dr. R. K. Sharma	
11.15 to 13.30	Technical Session III: Dr. R. Natrajan Award Chairperson: Dr. C. Nair Co-chairperson: Dr. S. P. Singh Rapporteur: Dr. Vinod Kumar	
13.30 to 14.30	Lunch	
	Technical Session IV (Ethnopharmacology) (Hall-I) Chairperson: Dr. N. Punniamurthy Co-chairperson: Dr. L. C. Lohan Rapporteur: Dr. S. P. S. Saini	
14.30 to 14.50	Dr. D. U. Bawankule	Integrated approach towards phyto-pharmaceutical research: An overview
14.50 to 15.10	Dr. C. C. Barua	Flavonoids: A potent source for anti cancer activity
15.10 to 19.30	Oral Presentation of Abstracts	
	Technical Session V : Antimicrobials and Antimicrobial Resistance (Hall-II) Chairperson: Dr. C. Varshneya Co-chairperson: Dr. M. M. Gatne Rapporteur: Dr. Binita Angom	
14.30 to 14.50	Dr. J. S. Sanganal	National policies to change the norms of antibiotic use
14.50 to 15.10	Dr. R. K. Sharma	Antibacterial resistance
15.10 to 15.30	Dr. S. K. Mody	Smart Veterinary Prescriptions addressing antimicrobial drug resistance
15.30 to 16.00	Oral Presentation of Abstracts	
	Technical Session VI : Food Safety / Xenobiotic Residue (Hall-II) Chairperson: Dr. J. S. Sanganal Co-chairperson: Dr. U. D. Patel Rapporteur: Dr. P. K. Verma	
16.00 to 16.20	Dr. Y. P. Sahni	Antibiotic residues: A global health hazard
16.20 to 16.40	Dr. Hitesh B. Patel	Metabolomics: A new frontier in food safety and quality
16.40 to 17.40	Oral Presentation of Abstracts	
	Technical Session VII : Pharmacokinetics / Toxicokinetics (Hall-II) Chairperson: Dr. S. K. Mody Co-chairperson: Dr. P. Sriram Rapporteur: Dr. Nety Shraddha	

17.40 to 18.00	Dr. Dheer Singh	Exosome nanoparticle: A novel drug delivery vehicle
18.00 to 18.30	Oral Presentation of Abstracts	
	Technical Session VIII: Education in Veterinary Pharmacology & Toxicology (Hall-II) Chairperson: Dr. S. Ramesh Co-chairperson: Dr. Satish Kumar Jain Rapporteur: Dr. Santwana Palai	
18.30 to 18.50	Dr. A. H. Ahmad	Teaching methodologies in Veterinary Pharmacology and Toxicology
18.50 to 19.10	Dr. M. M. Gatne	Teaching of Veterinary Pharmacology and Toxicology in Veterinary Colleges
19.10 to 19.30	Oral Presentation of Abstracts	
17.30 to 18.30	Poster Session I Venue: Library Hall	Poster Session II Venue: Museum
	Technical Session IX: Toxicology of Xenobiotics (Hall-III) Chairperson: Dr. A. M. Thaker Co-chairperson: Dr. Hitesh B. Patel Rapporteur: Dr. M. Usharani	
14.30 to 14.50	Dr. S. P. Singh	Pesticides induced immunotoxicity and its phytoremedy
14.50 to 15.10	Dr. N. B. Shridhar	Mycotoxicosis in livestock of Karnataka: An update
15.10 to 16.30	Oral Presentation of Abstracts	
	Technical Session X (Molecular & Neuropharmacology) (Hall-III) Chairperson: Dr. Chandana Barua Co-chairperson: Dr. Usha P.T.A. Rapporteur: Dr. Arpita Shrivastava	
16.30 to 16.50	Dr. Thakur Uttam Singh	Role of TRPV4 channels in pulmonary vasculature
16.50 to 17.30	Oral Presentation of Abstracts	
19.30 to 20.00	General Body Meeting (Hall-I)	
20.00 to 21.30	Dinner	

DAY-3 (25th November 2016) Venue: Hall I & Hall II (Veterinary College, NAU, Navsari)		
08.00 to 09.00	Breakfast	
	Technical Session XI (Animal Welfare and Good Laboratory practices) (Hall-I) Chairperson: Dr. N. Prakash Co-chairperson: Dr. Sudhirkumar Tiwari Rapporteur: Dr. Neetu Rajput	
09.00 to 09.20	Dr. S. Ramesh	Ethics and animal welfare in animal experimentation
09.20 to 09.40	Dr. S. D. Patel	Laboratory animal welfare: It's influence and assessment
09.40 to 10.00	Dr. R. Date	Skin sensitisation alternative test methods and approaches
10.00 to 10.20	Oral Presentation of Abstracts	
	Technical Session XII (Clinical Regulatory Pharmacology & Toxicology/ Nutritional Pharmacology) (Hall-II) Chairperson: Dr. S. C. Parija Co-chairperson: Dr. Thakur Uttam Singh Rapporteur: Dr. R. D. Singh	
09.00 to 09.20	Dr. Milind Deore	Alternative methods to animal testing and cosmetic products' safety -An overview
09.20 to 09.40	Dr. D. G. Ujawane	Overview of endocrine disruptor screening programme and its key element
09.40 to 10.00	Dr. Satish Panchal	Reproductive toxicology in non-clinical safety
10.00 to 10.20	Dr. U. D. Patel	Quercetin: A nutraceutical ingredient or drug?
10.20 to 10.40	Dr. J. H. Patel	Andrographolide: Pharmacological and toxicological profile
10.40 to 11.00	Oral Presentation of Abstracts	
11:00 to 13:00	Plenary Session and Valedictory function Venue: Vivekanand Hall, ASPEE College of Horticulture & Forestry, NAU, Navsari	
13.00 to 14.30	Lunch	

CONTENTS

ISVPT-2016

Orations

Chellapa Memorial Oration

Dairy Nutraceuticals and Functional Dairy Foods:
Current Status, Issues and Challenges

Dr. A. K. Singh
Principal Scientist, Division of Biochemistry
ICAR - National Dairy Research Institute, Karnal, Haryana

Dr. M. Sabir Oration

Biomarker Based Translational Research in New Drug Discovery:
Significance of Veterinary Pharmacology

Dr. R. K. Goyal
Vice Chancellor
Delhi Pharmaceutical Sciences & Research University, Delhi

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VANBANDHU VETERINARY COLLEGE, NAU, NAVSARI
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DAIRY NUTRACEUTICALS AND FUNCTIONAL DAIRY FOODS: CURRENT STATUS, ISSUES AND CHALLENGES

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Introduction

Nutraceuticals are becoming the most essential components of rapid growing health movement across the globe. Milk and milk constituents have gained prominence because of increasing scientific evidence pertaining to their health promoting and disease alleviating virtues. Significance of nutraceuticals assume altogether different dimension in our country where rapid rise in malnutrition and incidences of non-communicable diseases is posing newer challenges. It is costing not only 1-2% to National GDP, but adversely affecting the quality human resource as well. Still India is lagging behind on global malnutrition arena because of prevalence of stunting, anaemia, protein-energy malnutrition (PEM), osteoporosis, and vitamin A deficiency syndromes among children and women. On another front, we are facing issues related to imbalanced nutrition that has enhanced the burden of diabetes and cardiovascular diseases (CVDs) with estimation of 30 and 32 million patients respectively. India has a meager share (only 1%) of global nutraceutical market; however with increasing consumer awareness and disease burdens it is destined to become the major role player. Among the functional foods dairy based products occupy an important place, probably because of the well perceived health benefits associated with consumption of milk and milk nutrients. Milk, dahi and ghee are the three dairy products which has been part of our all religious ceremonies and have been mentioned for their disease preventing abilities in ancient literatures.

Mother's Milk: Natures Perfect Functional Foods

Breast milk or mother's milk is probably the first and most diverse kind of functional food which a new born consumes. It is designed by nature to provide all essential nutrients and therapeutic components in desired amount and also in best bio-available form. The bioactive components present in colostrum and mature milk include nutrients, minerals, trace elements and pre-vitamins as well non-nutrients (mostly bioactive) such as immunoglobulin, hormones, growth factors (Insulin-like growth factors), cytokines, prostaglandins, enzymes, lactoferrin, transferrin, nucleotides, polyamines and human milk oligosaccharides (HMO) (Blum and Baumrucker, 2008).

Breastfeeding continues to offer health benefits into and after toddlerhood. These benefits include; lowered risk of Sudden Infant Death Syndrome (SIDS), faster mental development, lowered incidences of cold & flu, lowered risk of asthma and eczema, decreased risk of obesity later in life, and decreased risk of developing psychological disorder. Breast milk provides a wide variety of proteins that have unique compositional and physico-chemical characteristics that is highly suitable for neonates. In addition to these, they also exhibit several extra-nutritional roles to promote the development and well being of infants.

The exact integrated properties of breast milk are not entirely understood and everyday new scientific evidence is emerging that make the task of infant food formulators more tedious. However, mimicking the composition and functionality of mother's milk is quite a daunting task and efforts have so far done are

concentrated towards balancing the nutritional content of cow or buffalo milk for infant feeding. Emerging trends in infant formula are: incorporation of long chain poly-unsaturated fatty acids (LUFA), fortification with prebiotics, trace elements (iron, zinc), addition of milk /soy protein hydrolysates and nucleotides (Alles *et al.*, 2004; Thompkinson and Kharb, 2007). But the type of bioactive molecules to be added in infant formula and their concentration still need extensive investigations. Furthermore, a strong need is felt to develop infant formula, for pre-term and neonates suffering with specific metabolic disorders.

Role of non-essential nutrients present in mother's milk is need to be evaluated in terms of infant and maternal health. Scanty information is available for few of the molecules present in human milk, however research strategies to elucidate their mechanisms of action would be different from the essential nutrients.

Milk Nutrients for General Well Being

With changing life-style, there has been increase in the number of chronic diseases at alarming rate. Despite the top most producer of milk globally, the per capita availability of milk is quite variable across the length and breadth of nation. India has attained the first rank in numbers of persons suffering or prone to diabetes, cardiovascular diseases (CVDs) and cancer. Moreover, incidences of infectious diseases are also on rise. One of the common reasons for these diseases could be attributed to impaired or weak immune system. Role of milk nutrients specially the minor milk proteins such as β-Lactoglobulin, α-Lactalbumin and lactoferrin, in modulating the immune system is well documented. Better availability of added nutrients in milk and milk nutrients has offered newer opportunities for the fortification of bioactive such as essential fatty acids, micronutrients and therapeutic amino-acids. Recent findings related to anti-obesity and anti-carcinogenic role of conjugated linoleic acids (CLA) in animal models have suggested the enrichment of CLA content in milk and milk products. Enhancement in CLA level through dietary manipulation or processing mediated interventions would appears promising. Milk mining through advanced technological interventions (separation technologies) has enabled us to isolate the wide array of components present in milk and so far more than 500 compounds have been identified so far. Recent developments in clinical sciences also contributed significantly in elucidating the mechanisms associated with therapeutic virtues of these molecules.

Milk Nutrients as Precursor for Bioactive Components

Casein, lactose and milk lipids the major milk nutrients often serve as base materials for the production metabolites having positive influence of physiological system and can be termed as nutraceuticals. Richness of milk protein particularly whey proteins, in sulphur containing amino-acids like cysteine and methionine assist in enhancing the level on natural antioxidant i.e. glutathione. Likewise, abundance of branched chain amino-acids facilitates the effective energy balance during exercises. Serotonin, a biomolecule production is also mediated by milk protein amino-acids. Bioactive lipids mediated compounds including prostaglandins; leucotrienes and thromboxane are produced in requisite amounts. Galactose, the hydrolytic product of lactose is essential for the development of vital organs including retina and brain. GMP, a by-product present in cheese whey modulates the bio-synthesis of cholecysotkinin, the satiety hormone. Milk phospholipids have attracted the attention of researchers because of their effect on brain health.

Designing of Novel Dairy Foods with Non-Dairy Bioactive and Ingredients

Fusion trend has also influenced the dairy food formulations and blending of raw materials form different food

groups wither for better nutritional status or for the improvement of quality of resultant product has gained momentum in last few decades. Development of low calorie and / or no fat products required substantial alteration in formulations and removal of milk fat and sugars or salt have numerous undesirable consequences on quality attributes of finished products. Search for fat, sugar and salt replacers have resulted in availability of various alternatives, which could be effective in minimizing or completely eliminating these macromolecules. Artificial sweeteners including aspartame, acesulfame-k, sucralose, saccharin etc., have also been permitted by the regulatory agencies in wide range of dairy products. Studies conducted at NDRI revealed that it is possible to incorporate these intense sweeteners in combination with bulking agents and fat replacers in traditional dairy products without posing any safety threat. Inulin, Fructooligosaccharides (FOS), Simplesse (modified whey protein), Oatrim (oat based fat replacer) and certain modified starches are fast becoming the essential ingredients in functional dairy products such as yoghurt, yoghurt drinks, ice creams, cheeses, spreads etc. Availability of safety and toxicity data related to these ingredients also enhance consumer faith in products based on these ingredients. Inulin and other non-digestible polysaccharides also have well documented health benefits, acting as prebiotic by assisting the proliferation of bifidobacteria and lactobacilli and improving the overall gastrointestinal health (Roberfroid *et al*, 1993). Other claimed benefits include increased calcium absorption with positive effects for bone health, a lowering of serum lipids with relevance for heart health, a positive effect on feeling of satiety with potential positive consequences for weight management, a potential effect to enhance resistance to infections and to stimulate the immune system. Phytochemicals, novel plant metabolites could be an ideal substrate for the manufacture of functional dairy foods. Among more than 1000 phytochemicals few such as carotenoids, flavonoids, phytosterols, phytoestrogens, glucosinolate and soluble fibres have been utilized in certain dairy products. These phytochemicals primarily act as antioxidants and perform putative functions mainly in life-style associated mortality and morbidity including CVD, diabetes and cancer.

Phytosterols exhibit anti-inflammatory, anti-neoplastic, anti-pyretic and immune-modulating activity. In the body, phytosterols can compete with cholesterol in the intestine for uptake, and aid in the elimination of cholesterol from the body. Saturated phytosterols appear to be more effective than unsaturated ones in decreasing cholesterol concentrations in the body. These actions reduce serum or plasma total cholesterol and low-density lipoprotein (LDL) cholesterol. In mammals, concentrations of plasma phytosterol are low because of their poor absorption from the intestine and their faster excretion from liver, and metabolism to bile acids, compared to cholesterol. Phytosterols have been successfully incorporated in yoghurt, cheese, dairy spreads and milk beverages.

Probiotic Dairy Foods:

The major focus in development of milk based therapeutic products has been towards the incorporation of probiotic microorganisms that harbour our gastro-intestinal (GI) tract and are frequently associated with health promoting attributes. Probiotic foods contain viable probiotic microorganisms in requisite number in suitable matrix and their viability & metabolic activity should be maintained through processing, packaging, storage till it is consumed. The global probiotic products market generated $15.9 billion in 2008. More than 500 probiotic F&B products have been introduced in the past decade. These products have received varying

levels of success, mostly in congruence with their overall health benefits. A number of scientific publications are emerging on selection, incorporation of probiotic cultures in dairy products and impact of unit operations on their viability during processing. The survivability of probiotics in complex GI tract and demonstrated health benefits in consumers is of great concern among researchers and processors (Mercenier *et al.,*2008) Several factors have been reported to influence the viability of probiotics in dairy foods and their subsequent implantation in host intestine. Certain processing and formulation interventions have been found to be effective in enhancing the viability of probiotics.

Through In-vitro and In-vivo trials the possible mechanisms for therapeutic aspects of probiotics have been revealed. These mechanisms are mainly related to anti-microbial activity, anti-mutagenic & anti-carcinogenic effect, modulation of immune response, anti-diarrheal and anti-allergenic reactions (Sandholm *et al*. 2002). However, variations exist in outcome of such investigations under different approaches that have been adopted to evaluate the functionality. The establishment of associated health benefits by consuming a certain probiotic dairy products through in-vivo assays is critical for the further success of this segment of functional foods. It has prompted newer initiatives at various forums to develop a guideline for efficacious investigations of probiotics for bringing the synergy among agencies involved and create confidence among consumers. The aim of the present chapter is to review the important group of probiotic microorganisms that have potential to be utilized for development of novel dairy foods including fermented milks, yoghurt, cheese, ice cream, composite dairy foods etc. The innovations that have been attempted to enhance the survivability probiotics across the value chain is dealt in depth. The review will also focus on mechanisms that are associated with therapeutic effects of probiotics with special reference to dairy products and their validation through In-vivo investigations.

Processing Mediated Intermediates: Boon or Curse?

However, how different processing interventions affect the nutritional and therapeutic virtues of milk nutrients is a matter of thorough investigations. Thermal treatment not only effective in improving the digestibility of milk proteins, but heating of milk is also known to produce various intermediates as Maillard reaction products. Many of these maillard reaction products have been identified with anti-oxidant potential; on the other hand these also have been implicated in allergic responses and carcinogenesis. Therefore, research investigations pertaining to processing induced changes on nutritional and therapeutic potential of various categories of processed dairy products should be initiated.

A great amount of work has been dedicated to these health promoting components. Biologically active peptides are of particular interest for food and pharma industry because they have been shown to play different physiological roles, including opioid like activity, antimicrobial, immunomodulatory and antihypertensive. These peptides could be generated during hydrolysis by digestive or microbial enzymes. Microbial enzymes from lactic acid bacteria (LAB) have demonstrated to be able to liberate theses peptides from milk proteins, in various fermented milk products (Korhonen and Pihlanto, 2007). Upon oral administration bioactive peptides may affect the major body systems- namely the cardiovascular, digestive, immune and nervous systems. The potential of certain peptides sequences to reduce the risk of chronic diseases or boost natural immune protection has aroused a lot of scientific interest over the past few years.

These beneficial health effects may be attributed to known peptide sequences exhibiting, e.g., antimicrobial, antioxidative, antithrombotic, antihypertensive and immunomodulatory activities (Sasaki and Kume, 2007).

Lactulose is an isomer of lactose, which is formed during heating of milk in small amounts. Lactulose plays a role in proliferation of *Bifidobacterium spp*. that has a positive relationship with human health. Investigation regarding the effect of incorporation of lactulose in infant formula on the intestinal *bifidobacterial* flora in rats indicated that 0.5 - 1.0% lactulose content in formula had no adverse effect on the absorption and retention of nitrogen, calcium, phosphorus and iron from the formula by *bifidobacterial* flora. Health benefit associated with lactulose among elderly is its ability to act as mild purgative, thus it helps in reducing the growth of ammonia producing organism. This particular property of lactulose has successfully utilized by medical practitioners in the treatment of portal systemic encephalopathy and chronic constipation. Human milk contains various types of oligosaccharides and most predominant among them is galacto-oligosaccharides (GOS). The presence of GOS is breast milk is linked with higher *bifidobacterial* count in infants (Sangwan *et al*., 2011). GOS are produced during the lactose hydrolysis via glycosyl transferase mediated activity of certain microbial strains. These galacto-oligosaccharides were earlier considered as unwanted products but now they are considered as prebiotics because they function as bifidobacteria growth promoting factors, reduce risk of colon cancer, prevent bone loss and lower serum cholesterol concentration.

Issues Related to Technological Aspect of Novel Dairy Foods

Designing of suitable diet with desired nutrients and pharmacologically-active components to meet the diverse needs of consumers is quite a daunting task. The healing power of milk nutrients is known for centuries and recent scientific investigations have proved the disease preventing or alleviating properties of milk nutrients. Several species of Lactic acid bacteria (LAB) assist in maintenance and improvement of gut health besides providing several other health benefits. It has been exploited all over the world for the development of probiotic dairy foods. Now the time has come when characterized indigenous probiotic microflora with proven technological and therapeutic attributes should be made available for the manufacture of novel probiotic dairy product. Although, probiotics have already started cementing their place in global dairy market, but many mysteries and health claims associated with probiotics needs to be addressed carefully.

Further, milk mining for the isolation of such bioactive molecules through appropriate technological interventions has gathered momentum in recent past. Newer ingredients and processes like membrane processing, high pressure processing (HPP) and supercritical fluid extraction (SCE), offer newer opportunities in delivering “wholesome” dairy products. Delivery of bioactive components in dairy products and its stability during the entire value chain is another major challenge. Various interventions including micro-encapsulation and nanotechnological could be the next important research area in coming days. Consumer acceptability of functional dairy foods will largely depends on their excellent sensory profile, validated heath benefits and also their cost effectiveness. The R&D efforts in these areas will help the Indian food industry to deliver nutritional and therapeutic products to consumers and also diversify their product profile to sustain.

Validation and Safety Issues

Appropriate validation studies through *in-vitro, in-vivo* or clinical trials have always been a great concern in investigating the mechanisms associated with functional food consumption and also determining the safety

and toxicity. The optimal levels of the majority of the biologically active components currently under investigation have yet to be determined. Designing of suitable animal and clinical investigations require multidisciplinary approaches including experts from diverse fields. The benefits and risks to individuals and populations as a whole must be weighed carefully when considering the widespread use of physiologically-active functional foods. Knowledge of toxicity of functional food components is crucial to decrease the risk: benefit ratio.

Conclusion

Milk has been considered as nature's perfect food and universally accepted a food with ability to module body's functions. However, research is revealing an over-accumulating range of physiological benefits associated with milk constituents or metabolites, emphasizing a positive role in programming the human health. Milk nutrients and their metabolites have well defied role in influencing the immune and vital systems. Continuous milk mining is going on to discover new milk molecule with certain positive health impact. At the same time, processing induced intermediates are posing threats and could be potentially toxic. Excess consumption of milk nutraceuticals related effect is not available for majority of compounds. Moreover, the effective delivery system to have a site specific availability is crucial with certain bioactive molecules. Probably, these are the areas that desire active collaboration with pharmacologists. A close association with them would be essential to understand the mechanisms of action, their effective dose regimen, kinetics parameters and also potent toxicity.

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BIOMARKER BASED TRANSLATIONAL RESEARCH IN NEW DRUG DISCOVERY: SIGNIFICANCE OF VETERINARY PHARMACOLOGY

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Indian pharmaceutical industry, although ranked first five in the world in terms of volume, has not witnessed impressive success in discovery of new drugs. Indian companies have an advantage of having established low cost R&D facilities and the new patent regime provides them an economic incentive to invest in new drug discovery projects. There have been several paradigms shifts with respect to methodologies as well as strategies in new drug discovery from time to time. Especially in last three decades major there have been major changes in the approach. While on one sides several new technologies got developed, there have been great advancements in immunology, molecular biology as well as genomics. Translational research using biomarkers is one of the main strategies being evolved by the industry in the drug discovery. It aims to integrate both clinical and molecular information and provides biomarkers to better understand the biological basis of disease and therefore select them as better disease targets.

In past 20 years, world over has been equipped with a plethora of molecular targets, identified from the sequencing of the human genome and advances in combinatorial chemistry. However, identification of biologically active small molecules for further optimization into candidate drugs has been challenging because of ethnic variations and hence could not capture full diversity of regulation seen in native cells. Biomarkers commonly utilized earlier in drug development and drug discovery included biochemical surrogate markers, enzymes, receptors and genes. Of late, application of stem cell research, novel biomarkers like microRNAs, extracellular RNAs, circulating tumour cells have emerged for non-invasive diagnostics as well as target identification in drug discovery.

The role of veterinary pharmacology is very important when it comes to animal research. Further, biomarkers are not limited to human application in diagnostics and therapeutics. Their presence can be seen in pet animals like dogs and cats. Similar to human medicine, a correlation between the cardiac biomarkers and the prognosis of cardiac disease has been found in animals. The recent literature describes the utility of these peptides for distinguishing cardiac from non-cardiac diseases in small animals. These markers have also found their way in equine cardiology for assessing the severity of heart valve diseases

India is fast becoming the preferred destination for high-end pathological and diagnostic services. The diagnostics and pathology labs market in India is projected to be US$ 3.4 billion. The Indian diagnostic services market is expected to grow at a compound annual growth rate (CAGR) of around 26 per cent during 2012-2015. The biomarkers represent a new step in both, veterinary and human medicine diagnostic methods, with the major advantages of precision and non-invasiveness. These new tests can help the clinician to formulate a correct diagnosis. For example, one of the most revolutionary discovery in cardiac biomarkers was the BNP capacity to distinguish between dyspnea due to cardiac and non-cardiac pathology. Thus, biomarker research can provide an opportunity for the innovative new drug discovery and diagnostics in India.

ISVPT-2016

TECHNICAL SESSION - I

NATIONAL SYMPOSIUM ON ANIMAL HEALTH AND PRODUCTION CHALLENGES AND OPPORTUNITIES IN VETERINARY PHARMACOLOGY AND TOXICOLOGY

Chairperson : Dr. V. V. Ranade

Co-chairperson : Dr. N. Gopakumar

Rapporteur : Dr. C. V. Savalia

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NS-01

MODULATION OF ARSENIC-INDUCED APOPTOSIS IN IMMUNE CELLS BY CURCUMIN: AN OVERVIEW

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It is becoming increasingly evident that environmental exposure in humans and animals to toxic metals is a widespread problem. Human beings, livestock and wildlife come in contact with these toxic metals through inhalation of air and food and water intake. Arsenic is a well documented environmental pollutant that is extremely toxic and is found in soil, water and air. Elevated levels of arsenic have been reported in groundwater in several countries. Bangladesh and West Bengal (India) are worst affected countries. In Bangladesh, an estimated 33 million people are being exposed to potentially dangerous levels of arsenic in their drinking water. Obviously, human beings and animals are being exposed to arsenic concentrations in excess of 10 ppb, the WHO Standard. Arsenic is reported to cause immunotoxicity, reproductive disorders, diabetes, cardiovascular diseases, embryotoxicity, tumors of skin, bladder, liver and lung, oxidative stress, and mutagenicity. It has been suggested that As III exerts its toxicity by generating reactive oxygen species (ROS) and thereby oxidative stress and ultimately apoptosis.

In general, programmed cell death is divided into apoptosis (PCDI), autophagy (PCDII), and necroptosis (PCDIII). Apoptosis is characterized by a pattern of molecular and morphological alterations that result in the packaging and removal of the dying cell. Apoptosis is known to be involved in varied physiological and pathological processes. Apoptosis occurs through the activation of specific signaling pathways and important regulatory mechanisms which include death receptors, mitochondrial dysfunction, caspases, ceramide, Bcl-2, tumor-suppressor genes and consumption of ATP leading to DNA fragmentation. Two major pathways are implicated in apoptosis namely the death receptor pathway and the mitochondrial pathway. The distinct pathways activated are dependent upon the cell type and the initiating factor. While intrinsic pathway is mediated by disruption of cellular homeostasis and is initiated intracellularly, extrinsic pathway is mediated via Fas or tumor necrosis factor (TNF) receptors.

Oxidative stress and apoptosis have both been associated with chemical exposures and toxicity. Certain chemical exposures can result in the alteration of secondary messengers, such as free radicals or ROS and these alterations have been linked to the induction of apoptosis in immune cells. Oxidative stress may trigger the mitochondrial pathway. In addition to the source of ROS, the mitochondria are also a target of excessive ROS generation. The enhanced ROS production increases the mitochondrial membrane permeability leading to the release of cytochrome c from the mitochondria, which triggers the process of apoptosis. The release of cytochrome c into the cytoplasm is followed by the activation of different caspases such as caspase-9 and caspase-3. Cytochrome c binds to the adapter protein apoptotic protease-activating factor (Apaf-1); in presence of ATP and then unites with procaspase-9 to form an apoptosome. This results in the autolytic activation of procaspase-9 to active caspase-9, which in turn cleaves and activates downstream procaspase-3 to active caspase-3. At this stage, several different signaling pathways come together leading to cleavage of

multiple downstream substrates which ultimately result in numerous morphological and biochemical changes leading to cell apoptosis.

Curcumin or diferuloylmethane (1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione) is a naturally occurring hydrophobic polyphenol compound, isolated from the rhizomes of the plant *Curcuma longa* (Linn), which has been used in both Oriental and Ayurvedic medicine since ancient times. Curcumin has been shown to possess broad spectrum of pharmacological properties including antioxidant, chemopreventive, chemotherapeutic, antineoplastic, antimutagenic and anti-inflammatory activities. Curcumin is a potent scavenger of a variety of ROS including superoxide anion radicals, hydroxyl radicals and inhibits lipid peroxidation and effectively blocks thiol depletion. In view of its ability to modulate different molecular pathways, curcumin has been suggested as a promising therapeutic and nutraceutical compound that could be used for treatment or prevention of many diseases.

We investigated the apoptogenic potential of arsenic in murine splenocytes and thymocytes and its modulation by curcumin. To examine the modulatory effect of curcumin on arsenic-induced apoptosis, the apoptotic DNA, apoptotic cells, ROS generation and mitochondrial transmembrane potential (MTP) were determined by flow cytometry using propidium iodide, annexin V-FITC, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) and 3,3'-dihexyloxacarbocyanine iodide ($DiOC_6$) dyes, respectively. DNA fragmentation patterns (DNA ladders) were examined by agarose gel electrophoresis. Murine splenocytes were exposed to sodium arsenite (5 µM) with and without curcumin (5 and 10 µg/ml) and incubated at 37°C for 12 h. Exposure of cells to sodium arsenite alone resulted in induction of apoptosis concomitantly with increases in the number of cells with ROS generation, loss of MTP, an increase in the frequency of cells with sub-G_1 DNA content and DNA fragmentation. Co-treatment with curcumin significantly decreased the percentage of cells displaying ROS generation and resulted in significant decreases in cells that lost MTP. Flow cytometric data revealed that curcumin co-exposure reduced the relative levels of cells in the sub-G_1 fraction from the levels in the arsenic-treated cells. Annexin-V FITC/PI staining revealed that curcumin co-treatment caused decrease in the number of arsenic-induced apoptotic cells. Furthermore, the DNA ladder induced by arsenic following 12-h exposure was diminished by curcumin co-treatment.

Similar to splenocytes, murine thymocytes were exposed to arsenic alone and arsenic plus curcumin (5 and 10 µg/ml) for 12 h. Arsenic produced a concentration-dependent increase in the percentage of apoptotic cells with maximum apoptosis produced at 10 µM concentration. Arsenic (5 µM) produced a characteristic internucleosomal fragmentation in thymocytes. Simultaneous exposure of thymocytes to arsenic (5 µM) and curcumin (5 and 10 µg/ml) was found to decrease the arsenic-induced DNA fragmentation. Apoptosis induced by arsenic (5 µM) was significantly decreased by curcumin in a concentration-dependent manner. The maximum inhibitory effect was observed at a concentration of 10 µg/ml of curcumin. In arsenic (5 µM)-exposed thymocytes, there was a significant increase in cell population with loss of MTP. Curcumin decreased the number of cells with loss of MTP caused by arsenic in a concentration- dependent manner.

The generation of ROS and decrease in MTP appear to be the major contributory factors in arsenic-induced apoptosis in murine immunocytes. Apoptosis induced by arsenic is likely to contribute to its immunotoxic effects. Curcumin is effective in counteracting arsenic-induced apoptosis in murine splenocytes and thymocytes and this effect may be mediated in part through inhibition of induced generation of ROS. This presentation will provide a comprehensive overview of recent studies conducted on curcumin with respect to its modulatory effect on arsenic-induced apoptosis in immune cells.

NS-02

NUTRITIONAL PHARMACOLOGY: THE IMPERATIVE FACET IN THERAPY

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Nutritional pharmacology is the study of pharmacological effects of nutrients and their interactions with the drugs. The nutrients and drugs modulate their actions because of their common kinetic paths in the body, such as absorption, distribution, metabolism and excretion. Specially, some nutrients like arginine and PUFA have therapeutic effects in certain diseases, such as hypertension. Nutrients regulate the metabolism of drugs directly by acting as cofactors of biotransformation enzymes or indirectly by affecting the gene expression. However, the genetic differences among individuals can influence the drug-nutrient interactions. Hence, the effect of nutrients on pharmacology of drugs needs extensive studies with cautious consideration of pharmacogenetics.

Nutritional Pharmacology:"The study of those substances which are found in food, e.g. vitamins, minerals and phytochemicals, that might have a desirable pharmacological effect when fed to an individual in quantities in excess of the amounts needed to prevent nutritional deficiency is known as nutritional pharmacology".

Role of nutrients in pharmacokinetics: The key events in pharmacokinetics are absorption, distribution, metabolism and excretion. Nutrients and drugs share these process, hence nutrient and drug interaction is utmost important for the effect of drug on body (Figure 1). The following mechanisms can explain the drug-nutrient interactions (Raiten, 2011).

a. Ingestion: Disease and drugs affect the appetite and thus decrease food intake, resulting in malnutrition, which further effect the efficiency of drugs.
b. Absorption: Food or nutrients modulate the drug absorption. For example, the nutrients in stomach at acidic pH affect the drug absorption. Similarly, the drugs affect the nutrient absorption. For instance, the drugs increasing the gastrointestinal motility decreases the absorption of nutrients. Specifically, the transport of drugs depends on the factors such as lipid solubility and competition with amino acid transporter system.
c. Distribution: The distribution of nutrients and drugs mainly depends on the body composition, especially the availability and functional integrity of transport systems, receptor integrity and intracellular metabolic machinery. All of these requirements are according to the nutritional and disease status conditions.
d. Metabolism: The metabolism of drugs are mainly caused by biotransformation enzymes through either oxidation or reduction. The enzymes involved in these processes require cofactors which are supplied by nutrients. On the other hand, certain drugs induce these biotransformation enzymes to convert the inactive nutrients into active nutritional components. Similarly, certain non-nutrient components in food or food supplements affect the activity of the enzymes involved in the drug metabolism. Overall, drug metabolism is affected by the nutrients and nutritional status of the human/animal. e.g.: Excess protein, PUFA, Vitamin C increases the rate of oxidation in drug metabolism.

Excess carbohydrate decreases the rate of oxidation in drug metabolism.

e. Elimination: The excretion of drugs and nutrients is affected vice-versa. For examples, the phase II biotransformation process involves the attachment of functional groups to the drugs for making them more water soluble for easy excretion. Nutrients act as cofactors for those enzymes involved in biotransformation process.

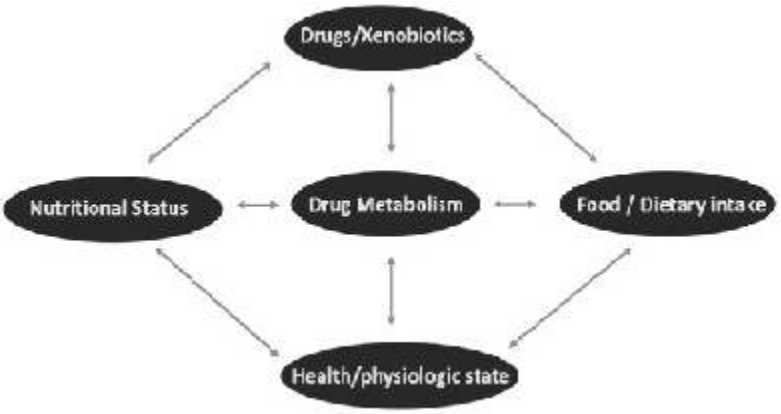

Figure 1. Drug nutrient interaction

Role of nutrients in disease and therapy: As it is a well-established fact that nutrients affect the metabolic regulation of the cells, the studies in nutritional pharmacology are imperative in therapeutic purposes. Several epidemiological studies related the differences in diet with the differences in disease incidence. For instance, saturated fat intake and cardiovascular diseases and vitamin D deficiency and osteomalcia. In fact, cardiovascular disease is an epidemic in India with 272 patients out of 100,000 people, which is greater than the global average of 235 per 10000 people. One of the main reason for such an epidemic is due to reversal of socioeconomic gradients, tobacco intake and low intake of fruits and vegetables, especially in lower socioeconomic sectors (Prabhakaran *et al*., 2016).

It has been only during the last two decades that the concept of nutritional pharmacology has been recognized. Some of the nutrients that have been studied for drug like effects include arginine, glutamine, glycine, taurine, long-chainfatty acids etc (Table 1). Arginine is known to have most diverse pharmacological effects. In high concentrations, it can induce the secretion of numerous hormones including pituitary growth hormone, insulin-likegrowth factor (IGF-1), insulin, vasopressin, adrenal catecholamines etc. Both laboratory and clinical studies have shown that excess arginine can improve wound healing, survival in infection and reduce blood pressure in hypertensive patients (Alexander 2002). Glutamine is the most abundant amino acid found in the body and to a matter of surprise, it has been observed that giving excess glutamine have pronounced strong pharmacologic effects. It improves resistance to infection by improving neutrophil and lymphocyte functioning (Abouwer *et al*., 1996).

Long chain fatty acids are important for maintaining the proper functioning of cell membranes. They act as important intracellular messengers, regulating the activities of several kinases, adenylate cyclase, phosphorylated proteins, calmodulin, intracellular calcium flux, cyclic AMP etc (Alexander, 1998). The most important of these are polyunsaturated fatty acids (PUFAs) especially n-3 and n-6 which are not synthesized in

sufficient quantities by human bodies. Both of these PUFAs are known to be precursors for eicosanoids, including prostaglandins, prostacyclins, thromboxanes, lipoxins andleukotrienes. Vitamins like folic acid have a therapeutic role in the prevention of cervical dysplasia, spina bifida and vascular diseases, ascorbic acid for immune dysfunctions prevention (Bland, 2008). It has been previously reported through various studies that two or more pharmaconutrients in diet of different category of patients resulted in better and faster recovery rates (Weimann *et al*., 1998; Kudsk *et al*., 1996; Gottschlich *et al*., 1990), indicating that combination of nutrients could work better.

Table 1. Nutrients having drug like effects

S.No.	Nutrient	Drug like effect
1	Arginine	Induce the secretion of numerous hormones e.g., GH, IGF1, insulinvasopressin Wound healing Survival during infection Decrease blood pressure
2	Glutamine	Improves resistance to infection
3	PUFA	Precursors for eicosanoids e.g., Prostaglandins
4	Folic acid	Prevention of cervical dysplasia, spinabifida and vascular diseases
5	Vitamin C	Promotes immune functions.

Nutrients and genes: Nutrients can regulate the gene expression by both direct and indirect mechanisms. Macronutrients, including saccharides, amino acids, fatty acids and their metabolites, and micronutrients, such as vitamins and minerals, interact directly with transcription factors and control the expression of specific genes. Another indirect way by which most of the nutrients influence genes is by modulating the secretion or action of one or more hormones, which, in turn, alters the phenotypic responses and hence the expression profile of specific genes (De Caterina and Madonna, 2004).This can be utilized to ameliorate diseases like cancer, diabetes and other cardiovascular disorders. Nutrigenomics is one such branch which helps to formulate novel functional foods and study their safety profiles. Nowadays, pharmaceutical and food companies are capitalizing on research in nutrigenomics and biotechnology to synthesize commercial products to compensate for nutritional deficits.

Genes are thought to play a key role in maintaining the health status of the individual in response to the dietary variability of nutrients. Ingestion of nutrients activates several neural and endocrinal circuits via gene expression which is reflected by the phenotype. The last decade has provided many evidences which indicate that major (glucose, fatty acids, amino acids) or minor (iron, vitamin, etc.) dietary constituents regulated gene expression in a hormonal-independent manner. Recently various studies have elucidated the relationship between nutrient factor and expression of specific gene. E.g., Conjugated linoleic acid (CLA) is a naturally occurring common fatty acid having importance in many biological functions especially reproductive benefits. If fed to cows, CLA modulates follicle dominance and the stage of estrous cycle. Specially, CLA regulates the proliferation and steroidogenesis of the granulosa cells in buffaloes (Sharma and Singh, 2012). When fed to early lactating dairy cows i.e. in postpartum period, CLA decreases fat excretion in milk thus protecting them from negative energy balance (Von Soosten *et al*. 2011).

The fatty acids are known to reduce the risk factors associated with several diseases such as cardiovascular diseases and cancer. For example, Omega (n-3) fatty acids regulate two groups of transcription factors i.e. sterol regulatory-element binding proteins (SREBP) and peroxisome proliferator activated receptors (PPAR), both of which are critical for modulating the expression of genes controlling both systemic and tissue-specific lipid homeostasis (Deckelbaum *et al*., 2006).

Diets high in simple carbohydrates lead to the induction of a set of enzymes in the mammalian liver which are involved in lipogenesis, through transcriptional mechanisms that lead to elevated levels of the mRNA for these enzymes. Carbohydrate response element-binding protein (ChREBP) is activated in response to high glucose concentrations in liver independent to insulin. ChREBP binds to the carbohydrate response element of liver pyruvate kinase (LPK) gene and this gene in turn is responsible for regulation of two key liver lipogenic enzymes, acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) (Ishii *et al*., 2004).

Nutrients, pharmacogenetics and pharmacogenomics: Pharmacogenetics is the study of genetic factors on the drug actions. Pharmacogenomics is the study of genetic variations of individuals to the response of drugs. The genetic differences among individuals or animals in those genes essential for drug metabolism affect the drug action differently in different individuals. Accordingly, the nutrient-drug interactions may differ based on the genetic background (Figure 2). Therefore, we cannot make a definitive conclusion on therapeutic effects of nutrients that can vary from one person to another based on differing pharmacogenetics. In such a situation, the specific genotype of the individual might give the information about the need for specific nutrient pharmacology,clearly indicating the need of clinical trials to test the uniqueness related to the nutrient demands of the participating patients. This may prevent the clarification of the nutrient pharmacology question in the future and result in only those nutrients that are effective at highlevels. The concept that a specific nutrient is having a pharmacological benefit will be found to be correct when nutritional pharmacology is applied to the right patient with the right dose of the right nutrient.

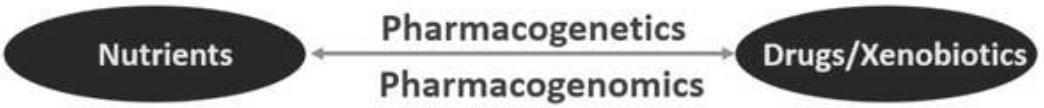

Figure 2: Involvement of pharmacogetics and pharmacogenomics on nutrient-drug interactions

Conclusion: Nutritional pharmacology is the study of nutrients that can act as drugs at higher concentration and also to study the nutrient and drug interactions. It is obvious that nutrients and drugs follow similar pharmacokinetic ADME principles and hence, they modulate their effects vice-versa. Certain nutrients, like arginine and PUFA have therapeutic effects in certain diseases, such as hypertension. In addition, nutrients regulate the gene expression thereby affecting the drug action. However, the genetic differences among individuals can influence the drug-nutrient interactions (Figure 3), hence the effect of nutrients on pharmacology of drugs needs extensive studies with cautious consideration of pharmacogenetics.

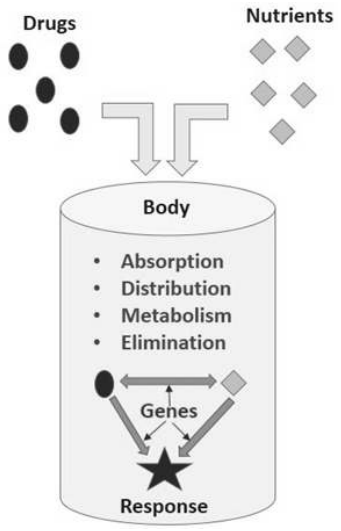

Figure 3: Drug response by nutrients, drugs and their interactions with genes

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NS-03

NEW DRUG DISCOVERY: CHALLENGES & OPPORTUNITIES

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Over the last few decades there has been a rapid progress in the discovery and development of novel medicines globally. The evolution of newer techniques across a spectrum of scientific disciplines have contributed significantly to the overall understanding of the mechanistic complexity of a plethora of medical conditions, and thereby opened up interesting possibilities of treatment specific to nature of the ailment. However, at the same time the newer medicines are subjected to extensive investigation for safety, efficacy, and suitability before approved for the intended use. This entire process is highly orchestrated, challenging and time-consuming. The ratio of newer therapies approved versus the overall pharmaceutical R & D spending is deteriorating owing to the increased regulatory pressures, expensive research and global competitive scenario, whilst the underlying opportunities are tremendous.

Discovery of a novel drug and pushing it through the pipeline, can take anything between 10 to 15 years or even more, making it a long term high-risk-high-budget endeavour with reduced success rates. A typical project kicks-off with just the idea of providing superior and convenient therapy for the identified medical condition. Based on the symptomatic attributes, various molecular targets participating directly or indirectly in the disease manifestation are sought after; and thus begin the journey of a new drug. The target can be any molecular component which is "druggable", which can be presumed to be fiddled by the concept drug, and eventually enable alleviating the condition. The challenge now is to identify a molecule which is able to meet these requirements. Theoretically, there are infinite possibilities to combine various elements into compounds. But here, we are seeking something that is exclusively specific. Therefore, numerous rational approaches are preferred for identifying the promising lead candidate. Practically, the numbers to begin with can be in tens-of-thousands. So many compounds are screened using a battery of *in silico, in vitro and in vivo* tests & models. At every stage only the bonafide candidates that meet specified criteria move ahead in the pipeline, which are only a handful. The lead compounds compete in terms of chemical stability, bioavailability, efficacy and safety. The lead compounds are also groomed further, through a process called lead optimization. Having gone through all these, the lead candidate goes through the trials in small to large animals, and after that, an IND is filed, which is a major milestone in drug development process as it facilitates the entry into clinical development.

One of the instrumental steps in the drug development process undoubtedly is animal testing, which also extensively supports the program through the clinical stage. Animal research is primarily carried out for eliminating the plausible risks associated with exposing humans to a test drug. Therefore in most cases, animal studies play a pivotal role in determining the fate of the drug, amidst the hovering translational challenges which are difficult to bridge even today. Since a particular drug has gone through extensive animal testing during its development, it is easy to imply that the drug may be suitable for use in animals also. However, this is

not always the case. The process for developing a drug for veterinary use poses its own sets of challenges and opportunities. Numerous examples can be cited where a drug intended for human use is not found suitable for veterinary use. Therefore it is important that the drug development for veterinary use is looked at from a systematic and dedicated approach and also labelled accordingly.

The US-FDA and European Union have embarked upon the active initiative to promote veterinary medicines and a highly structured framework is already set up, which provides a clear roadmap for development and approval of medicines intended for veterinary application. This is of particular importance as there are increasing numbers of human drugs used for veterinary purpose. Although there are clear guidelines available in these regions for the off-label use of human drugs for animals, it is practically difficult to observe such control. The best way we will be able to cope with this situation is by promoting dedicated drugs for veterinary use. Such an initiative would require considerable support from the governing bodies, as particularly in India, where there are currently no set guidelines and prominent distinction in the development process of drugs intended for human and animal use. Although, the Drugs and Cosmetics Rules, 1940, recognizes the evaluation of drug approval package for veterinary medicine only by expert veterinarian, still a considerable work needs to be accomplished in order to leverage the underlying potential of veterinary medicine.

NS-04

CHALLENGES AND OPPORTUNITIES IN VETERINARY PHARMACOLOGY AND TOXICOLOGY

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Background

From the Egyptian period to the age of enlightenment, veterinary pharmacology and toxicology has evolved by contributing Materia Medica to current modern veterinary therapeutics. Veterinary pharmacology and toxicology has a unique and strong impact not only on animal but also on human health. Veterinary pharmacology and toxicology has enhanced quality of life in a number of way which includes improvement in health status, prevention from zoonotic diseases, cross kingdom life saving drugs, protection of environment from manmade contaminants, preservation of genetic resources.

According to World Health Organization, 60% of human pathogens are of animal origin and 75% of the new diseases that have affected humans over the past 10 years have been caused by pathogens originating from an animal or from products of animal origin. A collaborative approach has been started known as "One Health" (formerly called "One Medicine") is dedicated to improving the lives of all species human and animal through the integration of human medicine, veterinary medicine and environmental science. FAO, OIE and WHO have recognized a joint responsibility for addressing zoonotic and other high socio-economic impact diseases. They have together developed a Tripartite Concept Note that sets a strategic direction and proposes a long term basis for international collaboration aimed at coordinating global activities to address health risks at the human-animal-ecosystems interfaces (WHO, 2015).

To curb all such possible challenges and fetch new opportunities in veterinary pharmacology and toxicology, nationally a number of organizations (Indian Pharmacological Society, Indian Society for Rational Pharmacotherapeutics, Association of Toxicology, Indian Society for Pharmacology and Toxicology, Indian Institute of Toxicology, Society of Toxicologic Pathology, Indian Pharmaceutical Association, Lab Animal Scientist Association etc. and many more) are working with one medicine goal, whereas among many global organization like OIE, WHO, the International Pharmaceutical Federation (FIP) has been working in the same direction. FIP has more than two million pharmacists and pharmaceutical scientists, with 137 member groups, including the American Association of Pharmaceutical Scientists. It sets standards through professional and scientific guidelines, policy statements and declarations, as well as by collaboration with other international organizations, including the World Health Organization.

Challenges in Animal Health

Efficient prevention and control of animal diseases relies on appropriate legislation, animal disease early detection and rapid response mechanisms. This is part of Good Veterinary Governance (WHO, 2015). However various countries has their issues either directly or indirectly related to veterinary pharma and

toxicology industry.

In China, the lack of access to anaesthetics (ketamine) and analgesics (opioids) created worry because it is an essential part of a veterinarian's arsenal. In theory, drugs not considered at risk of abuse can be registered with the appropriate Government authority and imported, but Western drug companies typically find the bureaucratic jungle of drug registry is a nightmare that results in drug registration delays of three to five or more years (James and Kelly, 2012).

Currently, in Indian scenario, the training programs are deficient in quality (lack of practical training), quantity (too few for e.g., India, a country of 1.2 billion population), coverage (inadequate in some geographical areas), and in training clinician investigators and other professionals. The existing programs has a number of deficiencies. At present, less clinical research is undertaken and fewer clinical trials are done by clinical pharmacologist than the expected (Kshirsagar *et al*., 2013).

U.S. has started a churning thought wheel in the field of pharmacology so that Veterinarians, producers and inspectors are up to date on the latest regulations and federal programs. As a part of it, developing educational materials and holding meetings across the state. A large number of inspectors either recruited or hired on a contract basis to meet the challenges in monitoring food and health. The concept of veterinary diagnostic lab in the U.S. that offers clinical pharmacology services which has attracted clients across the country (Martinez and Soback, 2005).

Existing drug practice in farm-animal faces a number of challenges, including increased competition, a lack of marketing expertise, inadequately trained staff, the remoteness of rural locations and the perceived unattractiveness of farm-animal work to women. Changes in government policy, the impact of disease outbreaks, the recession and increased competition all suggest that the needs of farmers remaining in the agricultural and animal husbandry sector have changed significantly and now require a greater attention.

I. Antimicrobial resistance (AMR)

The discovery and introduction of antimicrobial agents to clinical medicine was one of the greatest medical triumph of the 20th century that revolutionized the treatment of bacterial infections. However, the gradual emergence of populations of antimicrobial-resistant pathogenic bacteria, resulting from use, misuse, and abuse of antimicrobials has today become a major global health concern. Antimicrobial resistance (AMR) genes have been suggested to originate from environmental bacteria, as clinically relevant resistance genes have been detected on the chromosome of environmental bacteria. As only a few new antimicrobials have been developed in the last decade, the further evolution of resistance poses a serious threat to public health. Urgent measures are required not only to minimize the use of antimicrobials for prophylactic and therapeutic purposes but also to look for alternative strategies for the control of bacterial infections (Cantas *et al*., 2013).

World Bank has warned recently that drug-resistant infections have the potential to cause serious economic damage, much more than that caused by the 2008 financial crisis. This will have an impact on global incomes, poverty, trade and healthcare and will primarily hit the low-income countries. Countries could lose more than 5% of their GDP in drugs for antimicrobial resistance (AMR) bugs. Global livestock production will have a decline (estimated) between 2.6% and 7.5% per year by year 2050 (World Bank, 2016).

The risk of transfer of antimicrobial resistance from animals to humans could be much reduced if transfer of bacteria could be minimised. Stringent hygiene in markets, abattoirs, and food processing plants,

pasteurisation, irradiation, effective cooking reduces the risk. Clearly antimicrobial resistance would not develop in animals, if antimicrobials were not used in animals however, a decision to prohibit their use in animals would devastate the livestock industry and will have a catastrophic effect on animal welfare.

Antibiotics fed at low, generally subtherapeutic concentrations are known to improve feed conversion efficiency and thus performance in food producing animals. The improvement may reflect a reduction in subclinical disease, although this is probably not the whole reason. Prophylactic use of antimicrobials, group medication is the thrust areas of AMR. The antimicrobials are administered at therapeutic dosages, which clearly differentiates this strategy from that used to enhance production. Newly targeted therapeutics presents a rational and justifiable use where the antimicrobial should be selected on the basis of the sensitivity of the infecting organism and the pharmacokinetics of the drug, ensuring attainment of appropriate concentrations at the site of infection. Narrow spectrum agents which affect the fewest commensal bacteria should be used and the drug administered in the most effective dosage.

Currently little information exists on optimal administration strategies for antimicrobials in animals or humans. Subtherapeutic concentrations of antimicrobials, pharmacokinetic-pharmacodynamic relations for antimicrobials, AMR through chromosomal mutation are the key areas of discussion and research. Another interesting thought in debate is development of genetic material coding for resistance in commensal organisms may thus be selected and transferred to humans and then to human pathogenic organisms. Antimicrobials have prudent use in animals and will continue to provide benefits to society and will help ensure high standards of welfare for those animals in our care (McKellar, 1998).

II. Ophthalmic drug delivery

Delivery of drugs to the posterior eye is challenging, owing to anatomical and physiological constraints of the eye. New therapeutic entities (e.g. Oligonucleotides, aptamers and antibodies) are tested however currently, the intravitreal route is widely used to deliver therapeutic entities to the retina. Unfortunately, frequent administration of drugs via this route can lead to retinal detachment, endophthalmitis and increased intraocular pressure. Various controlled delivery systems, such as biodegradable and non-biodegradable implants, liposomes and nanoparticles, have been developed to overcome such adverse effects, with some success. The periocular route is a promising alternative, owing to the large surface area and the relatively high permeability of the sclera. However, the blood-retinal barrier and efflux transporters hamper the transport of therapeutic entities to the retina.

III. Acaricide and Anthelmintic Resistance

Rhipicephalus (Boophilus) microplus and Hyalomma anatolicum collected from Haryana and Rajasthan states of India shown acaricide resistance. There is a need for strategic use of available acaricides to overcome the development of acaricide resistance in ticks (Gaur *et al*., 2016).

Anthelmintic resistance in parasitic nematodes of cattle is common. Farmers raising herds need to be aware of the risks posed by anthelmintic resistance. Routine standardised faecal nematode egg count reduction testing is recommended to ensure optimal productivity and to guide decision-making when purchasing anthelmintics to be used on-farm (Waghorn *et al*., 2006).

Opportunities in Animal Health

Drug delivery modalities uses both biological and synthetic agents for diagnosis and treatment of diseases.

Pharmaceutical agents such as nanospheres, liposomes, and immunoglobulins for example have been developed which can alter the pharmacodynamics of a compound, act as carriers for sustained or controlled drug release, and for immunization therapy.

I. Imaging modalities in drug discovery

Now the non-invasive real-time assessment of biological and biochemical processes in living subjects is possible through technologies like micro PET, micro CT-Scan, SPECT, Micro MRI, Radioisotopes, Optical imaging. This allows to trace molecules in cellular level and molecular level which eventually helps in studying pharmacodynamics and pharmacokinetics more efficiently. The data obtained from such technologies assist in developing new drugs more effectively and leads to a cost effective drug discovery. Use of such technologies definitely need collaboration with experts other fields (interdisciplinary approach) possibly from the field of Veterinary Surgery and Radiology.

II. Trans-tympanic drug delivery

Systemic drug application for the treatment of inner ear diseases is restricted because of the blood-labyrinthine barrier and the limited blood supply to the inner ear. Local drug delivery to the inner ear can be an alternative method to overcome the problems with systemic application. Microendoscope is used for examining such transtympanic drug delivery to the round window membrane (Mood and Daniel, 2012).

III. Hyaluronan-Based Hydrogel (Thiol-Modified)

Corneal wounds currently tested to treat using hyaluronan-based crosslinked hydrogel which has wound-healing properties, support cell delivery, and can deliver drugs locally (Wirostko *et al*., 2014).

IV. Nanoparticles

a. Drug delivery and function assays

Delivering therapeutic compound to the target site is a major problem in treatment of many diseases. A conventional application of drugs is characterized by limited effectiveness, poor biodistribution, and lack of selectivity. These limitations and drawbacks can be overcome by controlling drug delivery. This helps to transport drugs to the place of action, influences vital tissues with minimal undesirable side effects. In addition, it protects the drug from rapid degradation or clearance and enhances drug concentration in target tissues. Due to their small sizes, the nanostructures exhibit unique physicochemical and biological properties which enables cell-specific targeting that make them a favorable material for biomedical applications.

Magnetic nanoparticles (MNPs) are a type of core/shell nanoparticle structure that consists of a magnetic core encapsulated in an organic or a polymeric coating. Thus they can be utilized in a variety of applications, ranging from storage media for magnetic memory devices to probes and vectors in the biomedical sciences (Akbarzadeh *et al*., 2012)

b. Injectable scaffolds

Regenerative stimuli-responsive gels can be delivered in a less invasive manner which has been developed as a novel scaffold containing hydroxyapatite and carbon nanotubes as nanofillers. The inclusion is thermosensitive gel which is injectable composite and has improved mechanical properties, good bioactivity, and prolonged drug release (Yasmeen *et al*., 2014)

c. Corneal diseases and nanotechnology

Topical nonsteroidal anti-inflammatory drugs (NSAIDs), steroids, antibiotics and tissue transplantation are currently used to treat corneal pathological conditions. However, barrier properties of the ocular surface

necessitate high concentration of the drugs applied in the eye repeatedly. This often results in poor efficacy and several side-effects. Nanoparticle-based molecular medicine seeks to overcome these limitations by enhancing the permeability and pharmacological properties of the drugs. Numerous polymeric, metallic and hybrid nanoparticles capable of transporting genes into desired corneal cells to intercept pathologic pathways and processes leading to blindness have been identified (Chourasia *et al*., 2016).

d. Shaping Magnetic Fields

Magnetic fields have the potential to noninvasively direct and focus therapy to disease targets. External magnets can apply forces on drug-coated magnetic nanoparticles, or on living cells that contain particles, and can be used to manipulate them in vivo. Ear and eye targets are too deep and complex to be targeted by a single external magnet, but they are shallow enough that a combination of magnets may be able to direct therapy to them. In near future, magnetic fields could be shaped (in space and time) to direct magnetic constructs to ear and eye targets. (Shapiro *et al*., 2014)

Challenges in Animal Production

Humankind relies on agriculture and animal husbandry for food. Still, today over 20% of animal production losses are linked to animal diseases (WHO, 2015).

I. Feed drug residue

Veterinary drugs and pesticides are used routinely in animal production to manage diseases and control parasites, and crop protection chemicals are used in production of animal feeds. It is possible, therefore, for foodstuffs of animal origin to be adulterated with residues of veterinary drugs and pesticides, and for animal fibers to be contaminated with residues of ectoparasiticides. Veterinarians must consider the implications of both possibilities when providing for the health and welfare of animals. First, animals and animal products destined for human consumption must not contain residues of drugs or pesticides that exceed legally permitted concentrations. Second, pesticide residues in fiber have potential implications for public health, occupational health and safety, and environmental safety (Reeves, 2015).

II. Toxins in feed

Fusarium and Aspergillus mycotoxins affect dairy cow health, performance and the efficacy of anti-mycotoxin Additive. Feeds naturally contaminated with low concentration of mycotoxins produced by Fusarium spp. and Aspergillus spp. in a diet of dairy cows can have a negative influence on somatic cell count, blood parameters and immunity. The addition of an anti-mycotoxin Additive to diet of dairy cows can prevent many of these effects. The toxic effect of aflatoxins alone or combined with zearalenone had a continuous toxic effect on laying performance in the recovery phase. Addition of Bacillus subtilis biodegradation product has a protective effect on layers fed contaminated diets (Jia *et al*., 2016). Diagnostic kits for toxin detection and specific neutralizing additives is a field of demand where new bio-entrepreneurs should be encouraged.

Opportunities in Animal Production

Agrochemicals, veterinary drugs, antibiotics and improved feeds can increase the food supply while minimising production costs in various livestock production systems around the world. However, these days, quality-conscious consumers are increasingly seeking environmentally safe, chemical-residue free healthy foods, along with product traceability and a high standard of animal welfare, which organic production methods are said to ensure. Organic production is not only a challenge for producers in developing countries, it

offers new export opportunities as well. Organic agriculture is practised by 1.8 million producers in 160 countries, and production of organically grown food continues to increase steadily by 15% per year. Most tropical countries are now exporting organic agricultural products but, apart from organic beef from Brazil and Argentina, organic livestock products are yet to take off. Most trade in organic livestock products is restricted to the European Union and other developed nations. Nevertheless, tropical countries cannot afford to neglect this emerging system of animal production. Organic livestock farming is still evolving, and further research is needed to make it sustainable (Chander *et al*., 2011). A pharma industry has a pivotal role in bringing this dream to a reality through incubator centers to develop novel organic products.

I. Nutraceuticals

"Nutraceuticals" are food-derived products largely used for their presumed health-promoting or disease-preventing effects. In recent years, many efforts have been aimed at assessing nutraceutical efficacy and safety, but these factors are difficult to address because of the complex chemical compositions and multiple mode of actions. Thus, the study of nutraceutical ingredients poses several challenges for the medicinal chemistry field, some of which are related to extraction and chemical characterization, some to in vitro and in vivo bioactivity evaluation, and some to the bioavailability and interaction of these natural mixtures with organs and microbiota. Furthermore, because of their nature as medicinal and food products, these nutraceuticals can also be considered as a valuable source of new "lead compounds", creating the opportunity to discover new classes of therapeutic agents (Sut, *et al*., 2016). There is a wide opportunity for veterinary pharma industry to develop and progress in the field of nutraceutics as a bio-entrepreneurs.

II. Veterinary Psychopharmacology

Laboratory animal research benefit human behavioral medicine whereas veterinary community benefits from the human clinical assessment of medications for behavioral issues in animals, since most psychoactive drugs are used extra-label in animals. Medications are evaluated in laboratory animal species for use in human medicine for both behavioral and psychological disorders, but their clinical use is not typically evaluated in companion or domestic animals. Veterinarians and animal behaviorists have the opportunity to apply human clinical evidence of these psycho-pharmaceuticals to certain anthropomorphized behavioral problems of animals. Among many challenges, diagnostic testing in animals using species-specific pharmaceutical and biopharmaceutical agents is one the difficult area (Lane *et al*., 2007).

III. Meat adulteration test

Several methods exist for determination of the origin of animal species in meat products. Recently, the DNA based analysis is superior in terms of specificity, accuracy, reliability and legal acceptability compared with electrophoretic, immunological and thermostable antigen methods. Compared to use of nuclear DNA, the detection method based on mtDNA improved the sensitivity further because of their high copy number (about 2,500 copies) of mtDNA against just few copies of genomic DNA per cell. Therefore, mtDNA can be more efficiently used to detect species-specific DNA (Bhat *et al*., 2016).

IV. Milk adulteration tests

Food adulteration is an act of intentionally debasing the quality of food offered for sale either by the admixture or substitution of inferior substances or by removal of valuable ingredient. Bioavailability of such substances

and its pharmacodynamic properties are altered resulting in poor absorption or assimilation. There is a strong need of developing a rapid method which is nanotechnology based test and which detect adulterants at source.

Conclusion

Antibiotic use plays a major role in the emerging public health crisis of antibiotic resistance. Although the majority of antibiotic use occurs in agricultural settings, relatively little attention has been paid to how antibiotic use in farm animals contributes to the overall problem of antibiotic resistance. New cloud based analysis of genomics data using high end techniques from portable equipment will provide new solutions to AMR issues.

Correlation of big data using computational analysis for genes has changed the use of old drugs such as Topiramate to a newer purpose of treating (earlier it was used as anticonvulsant now shown prominent effect in treating crohn's disease) other ailments. (Dudley et al., 2011). Bioinformatics is redefining drug usage and dosage.

Unfortunately, most public policy are based only on expert opinion and consensus (Landers, et al., 2012). Veterinary techno-entrepreneur is the need of an hour and hopefully new strategies around that will address the current issues and fill the gaps between techniques and technology. In a nutshell, challenges and opportunities in veterinary pharmacology and toxicology is multifaceted and which can be seen in adjacent diagrams (Fig: 1 & 2).

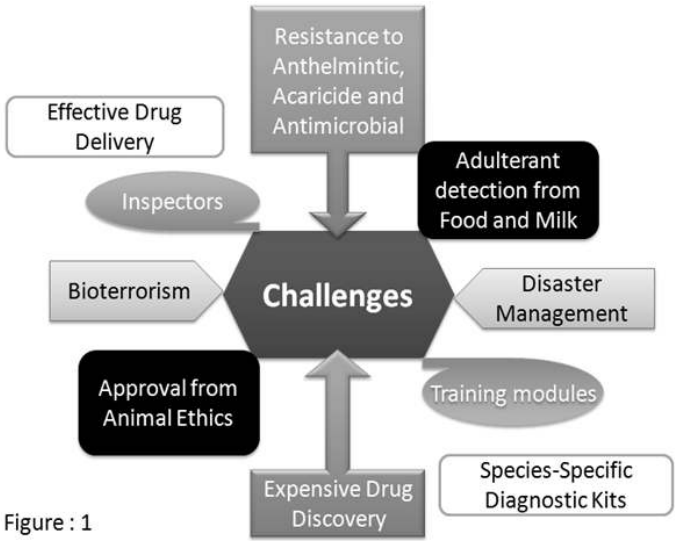

Figure : 1

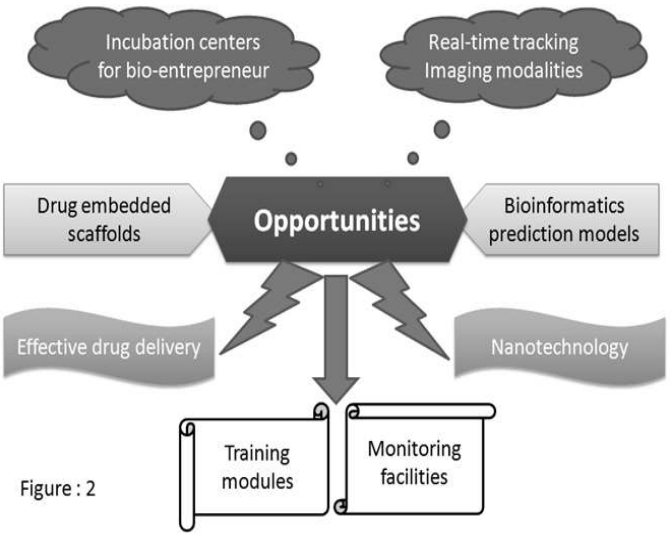

Figure : 2

Concept of a clean milk and cleaned milk is yet to be penetrated in Indian milk market but drug residues in milk which is another important issue for those who prefer cold or unheated milk for drinking. A technology driven innovation in pharmacology is need to be established to resolve such issues. Derived issues like antibiotic resistance, inadequate dosage regimen in various systems including ophthalmology needs assistance from pharmacology due to the practical hurdles in implementation.

Tailor-made teaching programs with interdisciplinary approach. Dual appointments in clinical disciplines, public health, industry, and clinical pharmacology would help in providing the links and meeting with the current and future demands on Clinical Pharmacology training (Kshirsagar *et al.,* 2013).

It is evident that change is incremental in veterinary pharma industry unless a transforming discovery occurs which dramatically changes medicine and pharmacology. Nowadays few of such transforming technologies (computer aided technology, microfluidics, nanotechnology, high-throughput screening, control and targeted drug delivery, pharmacogenomics, etc.) in veterinary therapeutics are expected to gain momentum. As a result more efficacious and safer drugs will be discovered and developed. A balance of economic and regulatory constraints in new drug discovery is possible through adoption and fusion of innovative techniques, integration and indigenous approach in new technologies and bioinformatics (Riviere, 2007). This very idea might help to treat ailments, eradicate poverty and resolve issues of challenges with compassion, knowledge and diligence.

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NS-05

NON-BIOLOGICAL CONTAMINANTS: HOW SAFE IS OUR FOOD OF ANIMAL ORIGIN?

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Introduction

Contaminants of non-biological origin comprise chemical and physical hazards which may be introduced during animal production, slaughter and processing or packaging. In broader sense, it can be classified as Xenobiotics or else specifically residues of veterinary drugs, industrial chemicals, heavy metals and pesticides which may be introduced during animal production. The most contentious residues which occur in meat, milk and eggs are antibacterial drugs, hormonal growth promoters and certain pesticides, heavy metals and industrial chemicals (McEwen and McNab, 1997)

The fact that food and optimal health are closely correlated is not a novel concept. About 2500 years ago, Hippocrates (460-377 BC), the renowned father of modern medicine, conceptualized the relationship between the use of appropriate health foods and their therapeutic benefits and quoted, "Let food be thy medicine, and medicine be thy food". Understanding of the relationships between foods, physiological function and disease have been progressed in recent years. Thus physical, physiological and pathological interactions between diet and xenobiotics influence nutritional processes and alter drug/xenobiotic metabolism, disposition, potency and toxicity. International society for the study of xenobiotics, a premier international scientific organization states that the term "xenobiotics" study includes medicinal drugs, agricultural chemicals, industrial chemicals, environmental contaminants and exogenous substances encompassing bioavailability, distribution, biotransformation, interactions and elimination with biological systems. The study also includes the rates and extent of the processes and their biological consequences.

Role of residues, toxicants and/or contaminants and their adverse health effects.

Food Safety And Standards (Contaminants, Toxins and Residues) Regulations, 2011 enlists "Residues" in food articles which includes crop contaminants (Aflatoxin B, Aflatoxin M, Patulin and Ochratoxin A), naturally occurring toxic substances (Agaric Acid, Hydrocyanic Acid, Hypericine And Saffrole), pesticide/insecticide residues (total of 149 insecticides *viz.,* Cardaryl, DDT, Endosulfan A and B, Melathion etc.), metal contaminants (Arsenic, Tin, Zinc, Cadmium, Mercury, Methyl Mercury, Chromium and Nickel), antibiotics (Tetracycline, Oxytetracycline, Trimethoprim and Oxolinic Acid) and pharmacologically active chemicals (Nitofurans Like Furaltadone, Furazolidone, Chloramphenicol and Nalidixic Acid).

As per definition laid in Food Safety and Standards Act, 2006, "Contaminant" means any substance, whether

or not added to food, but which is present in such food as a result of the production (including operations carried out in crop husbandry, animal husbandry or veterinary medicines), manufacture, processing, preparation, treatment, packaging, transport or holding of such food or as a result of environmental contamination and does not include insect fragments, rodent hairs and other extraneous matters. Crop contaminant refers to any substance not intentionally added to food, but which gets added to articles of food in the process of their production (including operations carried out in crop husbandry, animal husbandry and veterinary medicine), manufacture, processing and preparation, treatment, packaging transport or holding of articles of such food as a result of environmental contamination.

The toxicants may be naturally occurring toxin (inorganic substances, toxins of microbial origin, plant products or organic compounds) or food additives such as preservatives, colours, sweeteners, flavouring agents etc. In addition, food contaminants such as pesticide and herbicide residues, water and other pollutants are further sources of toxicants. Drugs or pharmaceutical products, which are used to cure diseases are also xenobiotics with both therapeutic toxic potentials (Kamala Krishnaswamy, 1987).

Generally, all pharmaceutical preparations administered to animals producing foodstuffs can give rise to residues in edible tissue, milk or eggs. In addition to drug dose, residue levels depend on withdrawal time. In spite of most drugs representing a relatively low risk for the general public, when used responsibly and in line with instructions approved by the laboratories making veterinary drugs, adverse reactions have been frequently reported for some compounds; these would include antibacterial, antihelminthic, anticoccidial and antiprotozoal drugs and growth promoters.

Dose response information is essential for quantifying an adverse health effect. This may be graphically presented as being the relationship between the increase of a dose and the increase of a pertinent biological response. Such dose response curve is essential for identifying a non-active dose taken as being the no observed adverse effect level (NOAEL), the highest dose of a substance which causes no detectable adverse alteration in line with defined treatment conditions. Interspecies differences should be taken into account as well as the fact that humans may exhibit substantial differences in their sensitivity to certain toxins due to differences regarding metabolic pathways and other factors. Uncertainty factors are thus applied when extrapolating from the toxicity observed in laboratory animals to health risks in humans, this usually being a factor of 10 for interspecies difference and a factor of up to 10 for human variability (depending on the extent and quality of available human data).

Health authorities recommend maximum acceptable or tolerable levels for chemicals which are neither genotoxic nor carcinogenic, such as acceptable daily intake (ADI), reference dose (RfD), especially for pesticides, tolerable daily intake (TDI) and provisional tolerable weekly intake (PTWI) for contaminants which may accumulate in the body. The responsible agencies conduct risk assessment to determine such levels; this consists of hazard identification and characterization, exposure assessment and subsequent risk characterization.

Hazards are identified and characterized from human epidemiological observations and animal-based toxicity testing supported by in vitro mechanistic studies which can make extrapolation from animals to humans become more realistic. Structureactivity relationships-based indications and the increased use of novel molecular biology techniques are also very valuable.

Food and Agricultural Organization (2000) defined "Pesticide" as any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies.

The term includes substances intended for use as a plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit. Also used as substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport. Subclasses of pesticides include: herbicides, insecticides, fungicides, rodenticides, pediculicides, biocides, molluscicides, nematicides, plant growth regulators and others. (Aktar *et al*., 2009).

Indiscriminate use of these pesticides has resulted in contamination of almost every part of our environment. Pesticide residues are found in soil, air, ground water and surface water, posing significant threat to target and non-target species including man (Gilden *et al,* 2010).

Residues of pesticides may remain in treated products and get into human food chain. These residues should not exceed a limit above which they may pose risks to human health. The concepts of Maximum Residue Limits (MRLs), Acceptable Daily Intake (ADI) and Theoretical Maximum Daily Intake (TMDI) for pesticides have been devised to keep a check on the pesticides' residues in food chain and keep them within safe limits.

The FDA stresses that pesticides pose much less of a safety hazard than other food contaminants, such as food poisoning microorganisms that cause everything from diarrhea to deadly botulism. The FDA also emphasizes that cancer- causing compounds that occur naturally in the food supply are a much greater threat than are synthetic carcinogens. In some instances, the chemicals applied to agricultural commodities can in fact safeguard from naturally occurring health threats. Thus natural does not always mean better, and chemicals do not always mean bad.

A brief note on residues and Pharmacologically Active Substances

The presence of residues of banned substances/ substances permitted but exceeding the prescribed limits by the regulatory authorities in case of veterinary drugs, pharmaceutical products and pharmaceutically active substances in products of animal origin (milk, eggs, muscles, liver, kidney, fish-flesh and honey) and from various species *viz.,* bovine, ovine, porcine, caprine, poultry, rabbit, farmed fish etc. is a matter of public health concern. The presence of these substances may lead to allergies, suspected to be carcinogens, mutagens or may lead to emergence of resistant microbes. As a consequence, national food safety authorities and regulatory authorities of India have banned the use/ strictly regulated its use in veterinary practice and established legal guidance to ensure proper use of drugs, pharmaceutical products and pharmacologically active substances. The FSSAI has laid down standard protocols to detect various xenobiotic residues using analytical techniques like immunoassay for screening and liquid chromatography with ultra-violet/ fluorescence detection, mass spectrometry to determine and identify the commercially available drugs, pharmacologically active substances in products of animal origin.

The Pharmacologically Active Substances prohibited in unit processing sea foods including shrimps, prawns or any other variety of fish and fishery products include all Nitrofurans (Furaltadone, Furazolidone,

Furylfuramide, Nifuratel, Nifuroxime, Nifurprazine, Nitrofurnatoin, Nitrofurazone), Chloramphenicol, Neomycin, Nalidixic acid, Sulphamethoxazole, Aristolochia spp and preparations thereof, Chloroform, Chloropromazine, Cholchicine, Dapsone, Dimetridazole, Metronidazole, Ronidazole, Ipronidazole, Other nitromidazoles, Clenbuterol, Diethylstibestrol (DES), Sulfanoamide drugs (except approved Sulfadimethoxine, Sulfabromomethazine and Sulfaethoxypyridazine), Fluoroquinolones and Glycopeptides.

Conclusion

There can never be an absolute guarantee that our food is safe; it is simply impossible to test every contaminant. Every country has an agency which oversees food safety; this is defined as being the, "reasonable certainty of no harm," and the aforementioned agencies regulate which additives are allowed in food and what levels of unavoidable contaminants are acceptable. The US Department of Agriculture's (USDA) Food Safety Inspection Service (FSIS) is responsible for the safety of meat, poultry, and egg products in the USA (Lodovico et al., 2008). The European Food Safety Authority (EFSA) is the keystone of the European Union's (EU) risk assessment regarding food and animal feed safety. The Codex Alimentarius Commission (created by the FAO and WHO) develops food standards, guidelines and related texts such as codes of practice under the Joint FAO/WHO Food Standards Programme (JECFA). The main purposes of this programme are protecting consumers' health, ensuring fair trade practices in the food trade and promoting the coordination of all food standards' work undertaken by international governmental and non-governmental organizations.

Interest in diseases caused by food has mainly been orientated towards the acute presentation principally produced by microbiological agents; however, consuming food contaminated by chemical substances could lead to chronic exposure leading to the presentation of diseases lacking an apparent cause and being difficult to diagnose. Foods of animal origin presuppose the risk of contamination, whether from drugs and growth promoters used for optimizing livestock production systems, or with biological toxins present in food ingested by animals. It is thus necessary to control these substances in foods, thereby supposing technological and institutional efforts. Sanitary authorities must thus promulgate and ensure compliance with standards and guidelines concerning the production of harmless foodstuffs. Achieving such objective represents a great challenge for underdeveloped and developing countries due to institutional difficulties and the limited availability of equipment and qualified personnel. All nations must make it a priority to try to ensure the safe consumption of foodstuffs by their populations, exercising strict sanitary control aimed to avoid problems of health in the population and preventing the appearance of new problems affecting the development of the agro-food industry and global trade in foodstuffs.

Essential elements for effective food safety programs: (Paul Sutmoller for OIE, 1997)

- Sound, transparent, science-based import/export regulations
- Up-to-date active disease surveillance and information systems
- Efficiently functioning veterinary services
- Alert field veterinarians, public health officials able to detect food-borne illnesses
- Fully participating and cooperating animal industries.

A food production chain using science-based and transparent pre-harvest and post-harvest food safety programs is much more likely to satisfy consumer's food safety concerns than present meat inspection

procedures and end-point sampling. Such overall food safety programs also will protect an exporting country against the unfair or unjustified use of food safety concerns as non-tariff trade barriers. Pre-harvest programs must be based on "good management practices" (GMP) and post-harvest programs must apply both GMP and Hazard Analysis and Critical Control Point (HACCP) principles. Surveillance and information systems, GMP and HACCP and import/export regulations all require a basic understanding of risk analysis elements: risk assessment, risk management and risk communication.

Although there have been many concerns in the past several decades regarding the presence of chemical residues in meat, milk and eggs, considerable progress has been achieved in reducing the probability of occurrence of these residues.

Summary

In general, chemical contaminants in foods from animals are infrequently found at concentrations which could be hazardous to the consumer, and there is a temptation to conclude that these are not very significant from the public health standpoint. Nevertheless, such contaminants remain very significant from the perspective of consumer confidence and international trade. As tariffs are removed and goods flow freely between countries, importing countries must be confident that the goods available for purchase are safe, and in addition to this, there is, from time to time, pressure to use chemical residues as non-tariff barriers to importation. Continued vigilance is required to ensure that hazardous residues do not contaminate the international food supply.

State and private animal and human health services must be well organized and coordinated to ensure high food-safety standards throughout the food chain from farm production to household consumption. It serves as a timely reminder to governments of the acknowledged fact that the health of food producing livestock and the safety of food derived from it must remain a priority of state veterinary services, livestock producers and others who contribute to farming.

High general awareness and standards of food safety increase opportunities for progress in rural development, agricultural production, primary animal and human health care and regional and international trade, whereas the opposite is true for food that endangers human health. It is evident that the promotion of safe food of animal origin for all is an international priority. Thus health care professionals like veterinarians and other public health workers (medical officers/surgeons) are responsible for ensuring that food of animal origin poses no hazard to human health.

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Table no. 1: Heavy metal contaminants in food articles and its limit dose. (FSSAI manual, 2011)

Metal contaminant	**Article of food**	**Parts per million by weight**
Lead	Corned beef, luncheon meat, Cooked Ham, Chopped meat, Canned chicken, Canned mutton and Goat meat and other related meat products	2.5
	Canned fish, canned meats, edible gelatin, meat extracts and hydrolyzed protein, dried or dehydrated vegetables (other than onions)	5.0
	Infant Milk substitute and Infant foods	0.2
Copper	Edible gelatin	30.0
	Infant milk substitute and Infant foods	15.0 (But not less than 2.8)
Arsenic	Milk	0.1
	Preservatives, anti-oxidants, emulsifying and stabilising agents and on dry mattersynthetic food colours	3.0
Tin	Processed and canned products	250.0
	Corned beef, Luncheon meat, Cooked Ham, Chopped meat, Canned chicken, Canned mutton and Goat meat	250
Zinc	Infant milk substitute and Infant foods	50.0 (but) not less than 25.0)
Cadmium	Infant Milk substitute and Infant foods	0.1
Mercury	Fish	0.5
Methyl Mercury (Calculated as the element)	All foods	0.25
Nickel	All hydrogenated, patially hydrogenated, interesterified vegetable oils and fats such as vanaspati, table margarine, bakery and industrial margarine, bakery shortening, fat spread and partially hydrogenated soyabean oil	1.5
Chromium	Refined Sugar	20 ppb

Table no. 2: Crop contaminants in food article and its limit dose. (FSSAI manual, 2011)

Name of the Contaminants	**Article of Food**	**□imit □g/kg**
Aflatoxin	All articles of food	30
Aflatoxin M1	Milk	0.5
Patulin	Apple juice & Apple juice ingredients in other beverages	50
Ochratoxin A	Wheat, barley & rye	20

Table no. 3: Maximum limit level of naturally occurring toxic substances. (FSSAI manual, 2011)

Name of substance	**Maximum limit**
Agaric acid	100ppm
Hydrocyanic acid	5ppm
Hypericine	1ppm
Saffrole	10ppm

Table no. 4: Residues (insecticides) level in food article and their tolerance limit. (FSSAI manual, 2011)

Name of Insecticides	Food article	Tolerance limit mg/kg.ppm)
Aldrin, dieldrin (the limits apply to aldrin and dieldrin singly or in any combination and are expressed as dieldrin)	Food grains	0.01
	Milk and Milk products	0.15 (on a fat basis)
	Meat	0.2
	Eggs	0.1 (on a shell Free basis)
Carbaryl	Fish	0.2
Chlordane (residue to be measured as cis plus trans chlordane)	Milk and milk products	0.05 (on a fat basis)
D.D.T. (The limits apply to D.D.T., D.D.D. and D.D.E. singly or in any combination)	Milk and milk products	1.25 (on a fat basis)
Endosulfan (residues are measured and reported as total of endosulfan A and B and Cottonseed 0.5 endosulfan-sulphate)	Fish	0.20
Fenitrothion	Meat	0.03
Ethion (Residues to bedetermined as ethion and Its oxygen analogueand expressed as ethion)	Milk and Milk Products	0.5 (fat basis)
	Meat and Poultry	0.2 (carcass Fat basis)
Paraquat Dichloride (Determined as Paraquat cations)	Milk (whole)	0.01
Trichlorfon	Meat and Poultry	0.1
	Milk (whole)	0.05
Carbendazim	Eggs	0.10 (shell free basis)
	Meat & Poultry	0.10 (Carcass fat basis)
	Milk & Milk Products	0.10 (fat basis)
Captan	Fruit & Vegetables	15.00
Carbofuran (sum of carbofuran and 3-hydroxy carbofuran expressed as carbofuran)	Meat & Poultry	0.10 (carcass fat basis)
	Milk & Milk Products	0.05 (fat basis)
Cypermethrin (sum of isomers) (fat soluble residue)	Meat and Poultry	0.20 (carcass fat basis)
	Milk and Milk Products	0.01 (fat basis)
Pirimiphos-methyl	Eggs	0.05 (shell free basis)
	Meat & Poultry	0.05 (carcass fat basis)
	Milk & Milk Products	0.05 (fat basis)
Quinolphos	Fish	0.01
Phorate (sum of Phorate, its oxygen analogue and their sulphoxides and sulphones, expressed as phorate)	Eggs	0.05 (shell free basis)
	Meat & Poultry	0.05 (carcass fat basis)
	Milk & Milk Products	0.05 (fat basis)
Fenvalerate (fat soluble residue)	Meat and Poultry	1.00 (carcass fat basis)
	Milk and Milk Product	0.01 (fat basis)

Table no. 5: Tolerance limit of Antibiotic residues in fish and fish products. (FSSAI manual, 2011)

Name of Antibiotics	Tolerance limit mg/kg (ppm)
Tetracycline	0.1
Oxytetracycline	0.1
Trimethoprim	0.05
Oxolinic acid	0.3

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ETHNOPHARMACOLOGY: CHALLENGES AND OPPORTUNITIES

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"The time has come to replace the purely reductionist 'eyes-down' molecular perspective with a new and genuinely holistic, eyes-up, view of the living world, one whose primary focus is on evolution, emergence, and biology's innate complexity."Carl Woese (2004)

Abstract

India is one of the 12-mega biodiversity centers (10% of the world's biodiversity wealth), with 16 agro-climatic zones. Of 17,000 species of higher plants in India, 7500 have medicinal uses. This proportion of medicinal plants is the highest known against the existing flora of any country. Currently, 25% of drugs are derived from plants, and many others are synthetic analogues built on prototype compounds isolated from plant species. Ethnobotany and ethnopharmacology are rewarding areas of research, particularly in countries having a rich biodiversity of natural plant resources coupled with incidence of various infectious diseases of livestock/humans. The objective of a field-based ethnopharmacology, often is the selection of species for further pharmacological studies. However a pharmacologist should look for field studies and most importantly clinical assessment of functional herbal remedies in tune with the current concepts such as synergy, systems biology approach and reverse pharmacology using information flow analysis.

Introduction

In earlier times ethnopharmacology was an endeavour connected to folklore; now it is seriously pursued by not only main stream pharmacologists but also investigators trained in anthropology, botany, biochemists, and researchers in veterinary medicine, clinicians and others. To identify a drug that is safe, affordable and effective is a challenge to modern medicine today. Most chronic diseases are multigenic and thus multi-targeted approach, in drug development, is needed. As many as 500 gene products or proteins or kinases or signaling intermediates have been linked with any given chronic disease. Thus inhibition of a single kinase or a pathway is unlikely to treat the disease.

Microbiome - the all-encompassing web of nature at work

A multitude of microorganisms also associate with higher organisms and collectively function as a microbiome. It is now well established that every higher organism investigated, from plants, insects, fish, mice, apes, and humans, harbors a microbiome. These microbial communities do not simply inhabit our skin or intestine but also influence processes including behavior, appetite, and most importantly health. Increasing global Antimicrobial Resistance (AMR) is a major threat to human/animal health and undermines the safety of our food and environment. Antimicrobials play a critical role in the treatment of diseases of farm animals (aquatic and terrestrial) and plants. Their use is essential to food security, to our well-being, and to animal welfare. However, the misuse of these drugs, associated with the emergence and spread of antimicrobial-resistant micro-organisms, places everyone at great risk. (FAO, 2016). The aim of pharmaceutics is not waging a battle against microbes; rather an approach for peaceful co-existence of plant, animal and microbes is more

practical and safe.

Ethnoveterinary Medicine (EVM)

The medicinal use of plants for treating various disorders in humans and in their animals is a centuries-old tradition in many cultures. As for as the (traditional) ethno veterinary medicine system is concerned, the intensification of livestock production and the over-dependence on synthetics (antimicrobials) led to a decline in herbal knowledge base and less interest in providing scientific basis to herbal based medicine.

Even today a large majority of small and marginal rural livestock owners medicate their animals with traditional remedies largely based on plants. Documentation of EVM and validation of such plants has been initiated in the country, by ICAR, AYUSH, CSIR, and many NGOs, particularly the joint efforts by FRLHT and TANUVAS has been very effective from the point of view of clinical success in main stream veterinary medicine; but there is a pressing need to continue and complete this task before oral traditions of EVM are lost forever.

Ethno Pharmacology and Holistic drug development:

Ethnopharmacology focuses on the use of traditional medicine in local communities, including its commercial applications. It involves field studies, pharmacological and clinical studies of chemically profiled extracts, and studies on the quality and composition of naturally derived products. The perceived adverse effects of using antimicrobials and other synthetic compounds on human, animal and environmental health; livestock product quality and safety have rejuvenated interest in phytochemistry, phytopharmacology and phytotherapy; all these fields eventually require the consolidation of the discipline of ethnopharmacology.

How to design a drug that is safe, multi-targeted and yet affordable, we have turned to traditional medicine. Since reactive oxygen species produced (ROS) in the body are composed of many species, such as, oxygen ions, peroxides, hydroxyl radicals, etc.; one would require a combination of antioxidants to quench them altogether. Plant polyphenolics though are good source of antioxidants, but they differ in their abilities to quench difference species of ROS. Therefore, one may need to use a combination of phytochemicals.

Holistic treatment is the hallmark of treatment in ethnomedicine. This would probably the most ancient recommendation for a "Combinatorial and MutIti-targted Therapy". It is quite possible that a so called crude herbal formulation has a combination of compounds, where one compound either potentiates the effect other, or increases the bioavailibilty, or reduces the toxicity. A best example is the routine use of turmeric in combination with black pepper as a spice. It is now known that the bioavailibity of curcumin is increased by piperine by preventing the glucuronidation of the curcumin. Experimentation and documentation of more of such scientific information is highly desirable, and scientific researches to substantiate the use of mixtures of plants in ethnomedicine are a worthwhile venture.

Challenges in plant based drug development

Developing new drugs is very challenging. A cycle of biology (usually efficacy tests in animals) and chemistry (synthesis) has been used in the past. Unfortunately, many of the candidates developed in this manner had excellent properties in preclinical animal models but did not show clinical efficacy in target species. Two of the major problems have been bioavailability and toxicity, both of which can be related at least in part to metabolism. The issues are toxicology, pharmaceutics, and the process of absorption, distribution, metabolism, and excretion. The goal is to do a better job of selecting candidate drugs early in the development process (even as part of discovery) to avoid problems later in clinical trials, when more resources (including

time) have been invested, by restricting the development process to the compounds with the highest likelihood for success. It is generally assumed that traditional medicines are safe and efficacious, given that they have been used for centuries.

Nature is an attractive source of new therapeutic candidate compounds as a tremendous chemical diversity is found in millions of species of plants. For many living organisms, this chemical diversity reflects the impact of evolution in the selection and conservation of self-defense mechanisms that represent the strategies employed to repel or destroy predators. The development of novel agents from natural sources presents obstacles that are not usually met when one deals with synthetic compounds. For instance, there may be difficulties in accessing the source of the samples, obtaining appropriate amounts of the sample, identification and isolation of the active compound in the sample, and problems in synthesizing the necessary amounts of the compound of interest.

Traditional knowledge and evidence of activity

Curcumin (diferuloylmethane) is a yellow odorless pigment isolated from the rhizome of turmeric *Curcuma longa* L., Zingiberaceae. Curcumin, an anti-inflammatory agent used in traditional medicine, has been shown to suppress cellular transformation, proliferation, invasion, angiogenesis, and metastasis through a mechanism not fully understood. Curcumin inhibits mutagenicity of certain chemical carcinogens and also hampers their covalent DNA binding in vivo, as well as, in vitro. Curcumin also protected against chemically induced liver damage in experimental animals. Its antioxidant effects were confirmed in various systems. It is of interest to note that tetrahydrocurcumin, that is one of the plausible metabolites of curcumin, exhibits stronger antioxidative, and anti-inflammatory activities than does curcumin

Ginger *Zingiber officinale* Roscoe, Zingiber aceae is among the most frequently and heavily consumed dietary condiments throughout the world. Besides its extensive use as a spice, the rhizome of ginger has also been used in traditional oriental herbal medicine for the management of such symptoms as common cold, digestive disorders, rheumatism, neurologia, colic and motion-sickness. The ole oresin from rhizomes of ginger contains 6 gingerol - 1- 4 -hydroxy-3 -methoxyphenyl -5-hydroxy-3-de- canone and its homologs as pungent ingredients that have been found to possess many interesting pharmacological and physiological activities, such as anti-inflammatory, analgesic, antipyretic, antihepatotoxic, and cardiotonic effect. The chemical structure of 6 - gingerol is a pungent and pharmacologically active principle of ginger

Capsaicin *trans*-8-methyl-*N*-vanillyl-6-nonen amide; is a principal pungent ingredient present in hot red and chili peppers that belong to the plant genus *Capsicum* Solanaceae. The compound has been tested by many investigators for its effects on experimental carcinogenesis and mutagenesis. In several instances, it has been demonstrated that capsaicin modulates microsomal cytochrome *P*450-dependent monooxygenase activities, thereby affecting metabolism of carcinogens and other xenobiotics

Synergy: Phytochemicals

Generally, synergy is defined as the interaction of two or more agents to produce a combined effect greater than the sum of their individual effects. In medicinal research field, however, the understanding of synergy is complicated. The concept of synergy can be broadly divided into two main categories based on the mode of actionspharmacodynamic and pharmacokinetic synergy. The first type of synergy describes two or more agents that work on the same receptors or biological targets that result in enhanced therapeutic outcomes

through their positive interactions. The second type of synergy results from interactions between two or more agents during their pharmacokinetic processes (absorption, distribution, metabolism and elimination) leading to changes of the agents quantitatively in the body and hence their therapeutic effects .It is important not to confuse synergistic effect with additive effect. Synergy occurs when two or more drugs/compounds are combined to produce a total effect that is greater than the sum of the individual agents, while an additive effect is add up of individual effects where each individual agent is not affecting the other (no interactions).

Conclusion

To identify a drug that is safe, affordable and effective is a challenge to modern medicine today. Why modern drugs are unsafe, ineffective and costly ? Answering this requires serious "out of the box" thinking. For instance the realization that most chronic diseases are multigenic and thus multi-targeted approach, also called promiscuity in drug development, is needed. As many as 500 gene products or proteins or kinases or signaling intermediates have been linked with any given chronic disease. Thus inhibition of a single kinase or a pathway is unlikely to treat the disease.

Globally pharmacologists with other scientists are trying to elucidate the effects and mechanism of action of plant phytomedicines that have been discovered based on the knowledge of local and indigenous peoples of the world. Indigenous and local healers of the world continue to seek recognition, intellectual credit and sharing benefits to their communities for their contribution to modern medicine and phytotherapy. It is our foremost and bounden duty and responsibility to recognize, appreciate and thank the traditional healers of today and yesterday for their dynamic and ongoing contribution to the world's health care systems. Laboratory scientists continue to confirm now and then, the claims made by the traditional healers, which is testimony to the sophisticated knowledge of traditional healers around the world. Let us be open minded and seek truth in tune with nature and respect traditional knowledge on health which can help in the pursuit of one health agenda of the world.

NS-07

ROLE OF ETHNOBOTANICAL STUDIES IN ETHNOPHARMACOLOGICAL RESEARCH

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Ethnobotanical surveys are the forerunner or the greater applied science Ethnopharmacology. Though ethnobotany was established in India as early as (Janaki Ammal, Jain S.K.), the earlier ethnobotany was seen only as an added note on the use of plants by the local people in major taxonomic works and taxonomic theses. Almost all Indian Cybele as well as the floristic theses produced in Gujarat had such passing references. The exclusive ethnobotanical surveys started appearing in early seventies. The works had ethnomedicinal inclusions that lacked vital information on recipes, doses and diagnostic features.

Umadevi A.J. (1988) produced a most comprehensive document namely "Identification and status survey of medicinal plants of Gujarat". The work included information on about 750 taxa of Gujarat.

In the year 2004, Government of Gujarat launched a project through the nodal agency GEER (Gujarat Ecological Education and Research) Foundation, combining efforts of experts of seven universities of Gujarat to prepare the document on the ethnomedicinal heritage Gujarat. The document covers information on ca 1375 taxa. Anjaria et al. (2002) published a comprehensive work "Ethnovet Heritage" including information on about 450 taxa used in treating pet animals by the riebes of Gujarat.

The modern pharmaceutical research encompasses several approaches.

- Studying plants referred to in Ayurvedic classics.
- Studying plants referred to by ethnic source.
- Studying some known plants for some other activity hither to not mentioned in classics.
- Guided by the presence of certain phytochemical already known for its specific activity.
- Just picking up the plant, which is either easily available or grows plentiful in the surroundings.
- Standardizing plant based drug preparations through marker compounds.
- Isolating compounds to study their *in vitro* and *in vivo* activities.
- Major efforts are focused on the development of singly molecular drugs to treat ailments.

Pharmacy colleges of Gujarat are actively engaged in modern pharmacological research based on plants. The presentation shall include some plants having promising properties. A case study on the development and standardization of herbal antimalarial drug from *Calotropis procera* will be presented. Mention will be made on the different avenues of research and the author's views on the methodology of research will be discussed.

Authors strongly believe that the plant as a whole must be used. The focus on the search of single molecule drug is not supported as the single molecular drugs used against causal organisms fail after some time, when the causal organisms develop immunity against the molecule. The severe side effects of single molecule are proved beyond doubt against the whole raw drug.

ISVPT-2016

TECHNICAL SESSION - II

DR. JAYVIR ANJARIA AWARD

Chairperson : Dr. Dheer Singh

Co-chairperson : Dr. A. H. Ahmad

Rapporteur : Dr. R. K. Sharma

INDIAN SOCIETY OF VETERINARY PHARMACOLOGY & TOXICOLOGY • 2000 •

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NAVSARI AGRICULTURAL UNIVERSITY

JVAA-01

EVALUATION OF ANTIDIABETIC, ANTIHYPERLIPIDEMIC, ANTI-HYPERALGESIC, LOCOMOTOR ACTIVITY AND TOXICO-PATHOLOGICAL EVALUATION FOLLOWING ADMINISTRATION OF *OPUNTIA ELATIOR* AND QUERCETIN IN DIABETIC RATS

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The present study was carried out to evaluate antidiabetic, antihyperlipidemic, anti-hyperalgesic, locomotor activity and toxico-pathological evaluation following administration of *Opuntia elatior* (OE) and quercetin in diabetic rats. Rats of group C1, C2, and C3 were kept as normal, vehicle and diabetic control, respectively. Rats of group C4 were administrated with glibenclamide (5 mg/kg, P.O. for 28 days). Rats of group T1, T2 and T3 were treated with OE fruit juice (4 mL/kg, P.O.), quercetin (50 mg/kg, P.O.) and both, respectively for 28 days. Administration of OE fruit juice and quercetin to diabetic rats significantly ($P<0.05$) prevented a steep onset of hyperglycemia after streptozotocin administration compared to diabetic control rats. However, glibenclamide produced better glucose lowering effect than OE fruit juice and quercetin. Mean levels of total cholesterol and triglyceride were significantly ($P<0.05$) increased while levels of HDL-cholesterol and LDL-cholesterol were non-significantly higher in diabetic control group compared to other groups. The mean levels of total cholesterol, HDL-cholesterol and LDL-cholesterol in rats treated with quercetin along with OE were found comparable to normal control rats. Treatment with OE fruit juice and quercetin alone and in combination attenuated the hyperalgesic response as well significantly improved reduced locomotor activity due to diabetes in rats. The reduction in Hb (g/dL), packed cell volume (%) and total erythrocyte count (10^6/ml) and escalation in levels of ALT, AST, LDH and creatinine were normalized in diabetic rats when treated with above treatments. Pathological alterations in pancreas, liver and kidney due to diabetes were less severe in rats treated with OE fruit juice and quercetin. Thus, OE fruit juice along with quercetin may protect major organs from damage occurs in diabetes.

JVAA-02

STUDIES ON ANTIDIABETIC EFFECT OF *MORINGA OLEIFERA* IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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The present study was conducted on forty two (42) male Albino Wistar rats dividing them in various groups having six rats in each group. Group I served as vehicle control and received 0.5 % solution of sodium bicarbonate in normal saline orally once daily for 28 days. Group II served as diabetic control, group III served as standard treatment control and treatment groups IV, V, and VI received streptozotocin @ 60 mg/kg body weight, by dissolving it in 50 mM citric buffer (pH 4.5) solution as a single intraperitoneal injection for induction of diabetes. Group III received glibenclamide @ 5 mg/kg of body weight (p.o.) once daily after establishment of diabetes. Group IV, V and VI received alcoholic extracts of *M. oleifera* pods @ 100 and 200 and 400 mg/kg

respectively (p.o.) once daily respectively. Whereas group VII served as plant extract control were administered alcoholic extracts of *M. oleifera* pods @ 200 mg/kg orally once daily. On 29th day of study, animals were subjected to blood collection; blood and serum sample were analyzed for haematological and serum biochemical parameters respectively. Blood glucose was estimated by one touch select simple glucometer (Johnson & Johnson, India) on day 0 and weekly for 28 days. At the end of study period, animals were sacrificed and necropsy was performed; tissues (pancreas) were collected for histopathological studies. Upon acute oral toxicity testing, alcoholic extracts of *M. oleifera* pods were found safe. Phytochemical analysis by GC-MS revealed presence of many compounds in alcoholic extracts of pods. Administration of alcoholic extracts of *M. oleifera* pods @ 100, 200 and 400 mg/kg body weight and glibenclamide at 5 mg/kg body weight in diabetic rats for 28 days showed significant ($p<0.05$) reduction in the elevated level of blood glucose and TLC and significant ($p<0.05$) increase in the reduced level of Hb, RBCs, PCV, MCV, MCH and MCHC in dose- dependent manner as compared to rats of diabetic control group. Similarly produced significant ($p<0.05$) reduction in the elevated level of SGPT, SGOT, TC, LDH, CK and BUN and significant ($p<0.05$) increase in the reduced level of liver glycogen, albumin and total protein in dose- dependent manner compared to rats of diabetic control group. Microscopic examination of pancreas revealed destruction, decreased number, dearrangement, diminished size and shape of cells of islets of langerhans and damaged acinar cells, while histopathological examination of pancreas of glibenclamide and alcoholic extracts treated groups revealed restoration in damaged histoarchitecture structure. The hypoglycemic effect of glibenclamide, as a reference drug on reducing blood glucose was more potent and significant as compared to plant extracts treatment and brought all the hematological and biochemical parameters up to the normal level. Alcoholic extracts of the *M. oleifera* pods @ 400 mg/kg body weight showed better effect than dose rate of 100 and 200 mg/kg body weight. The antidiabetic activity of *M. oleifera* pods may be due to the presence of phytochemical constituents such as quercetin, flavonoids, phenol, glycoside and alkaloids. Further investigation to define its clinical efficacy in domestic animals would be highly desirable.

JVAA-03

EVALUATION OF TWO HERBAL FORMULATIONS FOR WOUND HEALING ACTIVITY ON PIG

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The present study was aimed to assess the wound healing activity of herbal formulations containing two potential plants *Pistacia lentiscus* and *Shorea robusta* in different proportions (ABHD1 and ABHD2) at second degree burn wound of pig. Twelve pigs of either sex were divided into four groups comprising of three animals in each group. After anaesthetizing the animals with xylazine @ 1-2 mg kg^{-1} and Ketamine @ 20 mg kg^{-1} IM, a brass rod of 8mm diameter heated in a boiling water bath was applied to the skin surface vertically for six seconds. Group I served as Untreated Control, Group II treated with Standard silver sulphadiazine (%) ointment, Group III and Group IV treated with herbal formulation ABHD1 and ABHD2 respectively applied topically once daily. The tissues were observed and collected on every 3rd, 5th, 7th and 14th day of post-wounding. Healing of wound was compared on the basis of physical, biochemical and histopathological studies. Wound

contraction size was decreased significantly (P<0.05) in Group III (ABHD1) compared to Group I (Untreated Control), II (Sulphadiazine) and IV (ABHD2) on day 5, 7 and on 14 of post wounding. Wound index score was significantly (P<0.05) decreased in Group III (ABHD1) as compared to Group I (untreated control) on days 7 and 14. DNA content was significantly (P<0.05) higher in Group III as compared to control group on days 3, 5 and 7, while it was higher on 14th day in all the treated groups compared to control group. RNA content was increased significantly (P<0.05) in all the treated groups (Group II, III and IV) on 7 and 14th day compared to control group (Group I). Total protein content was significantly (P<0.05) increased in test groups with higher in Group III followed by Group IV on 3rd, 5th and 7th day and on 14th day. Hydroxyproline content revealed that in group ABHD1 significantly (P<0.05) increased compared to Group II (standard) and Group IV (ABHD2). Haematoxilin and Eosin stained sections showed that ABHD1 treated wounds had marked proliferation of fibroblasts with collagen deposition, new and well-formed capillaries in granulating tissues covered by newly formed epithelial layer. From the study, it can be concluded that herbal formulation ABHD1 exhibited good wound healing potential with no scar formation in comparison to Silver sulphadiazine application. Further investigation in the form of quantification of proteins is envisaged.

JVAA-04

EVALUATION OF METHANOLIC LEAF EXTRACT OF *VOLKAMERIA INERMIS* L. FOR HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITY ON PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

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Paracetamol is a widely used analgesic and anti pyretic drug. In high dose, it leads to hepatotoxicity. Hepatotoxicity is a common cause of severe metabolic disorders and even death. The present study aimed to evaluate the phytochemical analysis, hepatoprotective and antioxidant activity of methanolic extract of *Volkameria inermis* in Wistar albino rats. Thirty rats were divided into five groups each group consisting of six animals. Group I (Control) and Group II (Positive Control) administered with vehicle carboxy methyl cellulose (CMC) for seven days orally. Group III was administered with silymarin @ 25 mg/kg for seven days orally. Group IV and V were administered with *Volkameria inermis* extract @ 200 and 400 mg/kg respectively for seven days orally. On seventh day, all the groups except Group I were administered with paracetamol @ 1g/kg orally. At the end of the experiment, serum levels of hepatic injury markers were assessed and liver specimens were processed for antioxidant profile, histopathological and immunohistochemical studies. Phytochemical analysis revealed the presence of alkaloids, flavonoids, anthracene derivatives and coumarins. Paracetamol treated group showed increase in the levels of serum AST, ALT, ALP, triglycerides and liver tissue malondialdehyde (MDA). Also depletion of glutathione (GSH) and superoxide dismutase (SOD) activity were

observed. Histopathological examination of liver sections of paracetamol treated group revealed degeneration and necrosis of hepatocytes. Central and portal veins were congested and invading infiltrative inflammatory cells appeared in association with marked in p53 cells. Both groups of *V. inermis* restored most of these morphological, immunohistochemical and biochemical alterations in dose dependent manner when compared with silymarin. *V. inermis* has protective effect similar to silymarin against liver damage induced by paracetamol in rats by reducing oxidative stress and apoptosis. Result confirmed that *V. inermis* could be used as source of drug for hepatoprotection which might be attributed to the phytochemical constituents present in the plant extract.

JVAA-05

ANTICANCER POTENTIAL OF HYDROETHANOLIC EXTRACT OF *TRIANTHEMA PORTULACASTRUM* LINN. IN 7, 12-DIMETHYLBENZ[A] ANTHRACENE INDUCED MAMMARY TUMOUR IN WISTAR RATS

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Trianthema portulacastrum Linn. has been known to possess various pharmacological properties and the plant has been traditionally used for treatment of cancer. The objective of the study was to investigate the curative anticancer potential of hydroethanolic extract of the *Trianthema portulacastrum* (TPHE) in 7,12-dimethylbenz[a]anthracene (DMBA) induced mammary tumour in Wistar rats. The tumours were induced in rats by administering the carcinogen, DMBA orally in two divided doses of 50 and 30 mg/kg at one week interval. The oral administration of DMBA induced mammary tumours in Wistar rats with around 76% incidence in approx. 5 months. The tumour induced animals were divided into various groups and given TPHE extract at two doses of 200 and 400 mg/kg for 30 days along with proper controls to evaluate the curative effect of these extracts in DMBA induced mammary tumour model. The tumour volume and the calculated tumour regression percentage indicated that TPHE at a dose rate of 400 mg/kg had comparatively better anti-tumour effects. TPHE 400 treated group showed a tumour regression of 48.23 ± 3.15% in 30 days. Since apoptosis is thought to be the major mechanism by which plants exert their anticancer activity, the expression of apoptotic genes like caspase-3, Bcl-2, TNF alpha and IL-10 were studied. The expression of pro-apoptotic genes, caspase-3 and TNF alpha were found to have increased while the expression of anti-apoptotic genes, Bcl-2 and IL-10 were found to be decreased in TPHE 400 treated group. Apoptotic cells in significantly high percentage were detected by flow cytometry in tumour tissues of TPHE treated groups as compared to cancer control establishes the fact that induction of apoptosis is the reason behind their anticancer activities. The mitochondrial transmembrane potential depolarization assessment also revealed significant percentage of

apoptotic changes in the tumour tissues of TPHE 400 treated animals. The decreased level of ROS detected in the treatment groups of TPHE may be due to the increased level of radical scavenging antioxidants present in their tumour tissues. Based on the above findings, it can be concluded that *Trianthema portulacastrum* produces anticancerous activity by induction of apoptotic mechanisms mediated via apoptotic genes and mitochondria-mediated pathways.

JVAA-06

APOPTOSIS MEDIATED ANTITUMOUR POTENTIAL OF FRACTION OF *ANNONA MURICATA* IN TRIPLE NEGATIVE MAMMARY TUMOURS

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Mammary tumours are the most common neoplasm observed in pet animals. The incidence of triple negative mammary tumours in dogs is reasonable. The pathobiological features of these tumours are in common to human triple negative breast cancers. The present study was undertaken to evaluate the antitumour potential of methanolic extract of seeds of *Annona muricata*, to derive the active fraction and to elucidate the probable mechanism through which the pharmacological action is accomplished, in triple negative mammary tumours. The methanolic extraction of seeds of *A. muricata* was performed using Soxhlet apparatus and fractionation of the methanolic extract of seeds of *A. muricata* was done. Crude extracts and the fractions of *A. muricata* were screened for *in vitro* cytotoxic potential in triple negative mammary tumour cell line, 4T1 mammary carcinoma cell line, using MTT assay. Cytotoxicity studies revealed that the methanolic extract of seeds of *A. muricata* and chloroform fraction of methanolic extract of seeds of *A. muricata* (CMAM) exhibited reduction in cell viability within the range of 62.43±1.23 to 78.47±1.21 and 22.53±0.72 to 46.9±0.55 per cent respectively. Chloroform fraction of methanolic extract of seeds of *A. muricata* was tested for its efficacy in 4T1 induced triple negative breast cancer model in Balb/c mice. The results revealed significant ($p<0.05$) reduction in tumour volume and serum tumour marker, CA 15/3 in CMAM treated groups. Histopathological examination of primary tumour tissue in CMAM treated groups showed reduction in cellularity, nuclear chromatin condensation and a few normal cells. Flow cytometric assay using FITC Annexin V/PI was performed *in vitro* to confirm the induction of apoptosis in 4T1 mammary tumour cells. Investigations were also made to determine whether the treatment induced a loss in mitochondria transmembrane potential using JC-1 staining method. Western blot analysis of CASPASE-3, PARP, BAX and BCl-2 proteins was conducted *in vitro* to confirm mitochondria mediated apoptosis. Liquid chromatography mass spectroscopic analysis of the plant fraction revealed the presence of annonaceous acetogenins. Thus the study concluded that CMAM exhibited its cytotoxicity through caspase activated mitochondria mediated apoptosis due to the presence of annonaceous acetogenins.

ISVPT-2016

TECHNICAL SESSION - III

DR. R. NATRAJAN AWARD

Chairperson : Dr. C. Nair

Co-chairperson : Dr. S. P. Singh

Rapporteur : Dr. Vinod Kumar

RNA-01

STUDIES ON HEPATOPROTECTIVE EFFECT OF BIHERBAL AQUEOUS EXTRACT OF *ANNONA SQUAMOSA* AND *MURRAYA KOENIGII* ON HEPATOTOXIC RAT MODEL

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The present study was conducted to evaluate hepatoprotective effects of biherbal aqueous extracts of leaves of *A. squamosa* and *M. koenigii* following repeated oral administration for 28 days in carbon tetrachloride induced hepatotoxic rats. The study was conducted on thity six (36) male albino Wistar rats dividing them in various groups having six rats in each group. Group I served as vehicle control and received the normal saline solution orally every day. Group II which was served as hepatotoxic control, group III served as standard treatment control and treatment groups IV, V and VI received 50 % carbon tetrachloride in olive oil @ 1 ml/kg body weight, intraperitoneally twice in a week throughout the study period for induction of hepatotoxicity. Group III received standard drug silymarin @ 50 mg/kg of body weight (p.o.) daily once for 28 days. Group IV, V and VI received biherbal aqueous extracts @ 100, 200 and 400 mg/kg (p.o.) respectively, daily once for 28 days. Upon acute oral toxicity testing, biherbal aqueous extracts of *A. squamosa* and *M. koenigii* were found safe. On 29th day of study, animals were subjected to blood collection; serum was separated and samples were analyzed for biochemical alterations. At the end of study period, animals were sacrificed and necropsy was performed; tissues (liver, kidney, spleen, heart and intestine) were collected for histopathological studies. The result showed significant increase in serum concentration of ALT, AST, GGT, ALP, creatinine kinase, bilirubin, creatinine and significant decrease in albumin, globulin and total protein in hepatotoxic control rats as compared to rats of vehicle control group. It suggests that carbon tetrachloride is useful substance for successful induction of hepatotoxicity in rats. Daily oral administration of standard reference compound silymarin significantly reduced serum ALT, AST, GGT, ALP, bilirubin, creatinine kinase and creatinine level and increased serum albumin, globulin and total protein level as compared to hepatotoxic control rats. Hepatotoxic rats receiving biherbal aqueous extracts showed similar changes in dose dependent manner as compared to rats of hepatotoxic control group. Gross pathological examination of liver from rats of hepatotoxic control group showed paleness and diffused necrotic foci and microscopically liver sections showed sinusoidal dilatation, cellular vacuolization, necrosis, distortion of the central venules and ballooning of hepatocytes, kidney sections showed congestion with degeneration, necrosis of renal tubular epithelium and cloudy swelling of tubular cells, spleen sections showed mild congestion and haemorrhage with multifocal area of necrosis and mild lymphoid depletion and heart sections reveled severe congestion. Treatment of hepatotoxic rats with biherbal aqueous extracts preserved normal histoarchitecture in dose dependent manner and standard treatment silymarin almost preserved normal histoarchitecture of all the organs as compared to rats of hepatotoxic control group. The findings of present study suggest that biherbal extracts of *A. squamosa* and *M. koenigii* has hepatoprotective effect at dose dependent manner.

RNA-02

EVALUATION OF IN VITRO ANTHELMINTHIC ACTIVITY OF *JASMINUM AURICULATUM* ROOT EXTRACT AGAINST *PHERETIMA POSTHUMA* AND *PARAMPHISTOMUM CERVI*

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The present investigation was executed to evaluate the anthelminthic potency of *J.auriculatum* root extract against Indian earth worm, *Pheretima posthuma* and parasitic rumen fluke, *Paramphistomum cervi.* The powdered root was subjected to extraction using soxhlet apparatus with solvents in increasing order of polarity. The extracts thus obtained were further subjected to preliminary phytochemical and TLC analysis. From the results obtained, the chloroform extract was selected for further experimentation. Initially the anthelmintic potency of extract was evaluated on *P. posthuma* and then based on the promising results obtained the research was further extended on intestinal parasitic worm *P. cervi* where the activity was performed on isolated parasites as well as parasites attached to goat flesh. All the helminthes were exposed to various concentrations of extract ranging from 10-50 mg/ml. The standard and control used were Albendazole (200 mg/5ml) and water, respectively. The parameters evaluated were time of paralysis and time of death, histopathological and SEM studies. Phytochemical screening and TLC analysis revealed the presence of flavonoids, tannins, steroids, alkaloids and phenolic compounds. The plant extract revealed a significant ($P<0.01$) dose dependent activity against both the helminthes when compared to standard Albendazole and control treated helminthes. In case of *P. posthuma* treated with *J. auriculatum*, mechanism of action explains that extract had acted upon typhlosole, seminal vesicle, cuticle, longitudinal & circular muscles, by which digestive system, reproductive system and locomotion got destructed leading to paralysis and death of the earthworm. In case of *P. cervi*, the extract had acted upon Mehli's gland, uterus, tegumental syncytium and caecum, by which reproductive system and digestive system got destructed leading to paralysis and death. The SEM studies revealed tegumental changes like appearance of wounds, grooves, ridges, constriction of papillae mainly near oral and ventral sucker. In conclusion, the chloroform extract of *J. auriculatum* root possess a potent vermicidal activity.

RNA-03

IN VITRO RELEASE AND PHARMACOKINETICS OF ENROFLOXACIN PHBV MICROSPHERE IN RATS

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Enrofloxacin loaded Poly 3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV) microspheres were synthesized by oil/water single emulsion technique. Encapsulation efficiency, *in vitro* release and pharmacokinetics of enrofloxacin loaded PHBV microspheres were evaluated in rats (5 mg/kg body weight). Optical microscopy demonstrated that the enrofloxacin loaded PHBV microspheres were regular and spherical in shape. The mean drug encapsulation efficiency of enrofloxacin loaded PHBV microspheres was 43.03 ± 2.36% and about 97.19 ± 0.35% enrofloxacin was released in vitro during first 24 hours due to burst release. While remaining amount of enrofloxacin was slowly release up to 13 days. After intramuscular administration of enrofloxacin and enrofloxacin loaded PHBV microsphere in rats (5 mg/kg body weight) the drug concentration of 0.02 ± 0.001 and 0.03 ± 0.001 µg/ml in plasma was detected up to 6 and 72 h respectively and beyond then the drug was not detected in plasma. Significant increase values of elimination half-life, apparent volume of distribution, area under curve, area under first moment curve, mean residence time and significant decrease in total body clearance were observed in rats given enrofloxacin loaded PHBV microspheres compare to conventional enrofloxacin. It is concluded that it is possible to prolong the release of enrofloxacin through its incorporation into PHBV microspheres and maintain the therapeutic concentration over extended time periods.

RNA-04

STRATEGIES FOR COMBATING CLINICAL RESISTANCE: CHITOSAN ENCAPSULATED MICROSPHERES CONTAINING SELECTED PHYTOCHEMICAL AND ENROFLOXACIN OR ALBENDAZOLE COMBINATION AS NOVEL ANTIBACTERIAL AND ANTHELMINTIC AGENTS

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Bacterial and anthelminthic resistance is growing worldwide due to inadvertent use of antibiotics and anthelmintics, necessitating the development of novel strategies to combat resistant organisms. In this study, the synthesis of chitosan encapsulated microspheres containing selected phytochemical and enrofloxacin or albendazole combination is presented along with the evaluation of their antibacterial and anthelmintic properties. Phytochemicals used in synthesis included piperine, curcumin, syringic acid, and alginic acid. Both

enrofloxacin (400 mg) and albendazole (400 mg) were individually combined with each phytochemical (20 mg) in sodium alginate (2.5%, 20 mL), and encapsulated with chitosan (0.4%) in calcium chloride (1.5%). The polysaccharide chitosan reacted with sodium alginate in the presence of calcium chloride forming microcapsules with a polyelectrolyte complex membrane through electrostatic interactions between the two oppositely charged polymers. These microcapsules were then studied for entrapment efficiency, and *in vitro* drug release kinetics using UV-Visible spectroscopy. The antibacterial activity of enrofloxacin + phytochemical microspheres was studied using microtitre broth dilution on MTCC cultures of Escherichia coli, Salmonella, Pseudomonas, Klebsiella, pGLO transformed *E.coli* and clinical isolates. The anthelmintic activity of albendazole + phytochemical microspheres was studied using egg hatching inhibition assay and larval development assay on gastrointestinal nematodes of ruminants. Both microspheres showed entrapment efficiency ranging from 20% to 80% depending on the type of drug and phytochemical used. These microspheres were free flowing, non-aggregated and spherical, between 600 and 800 nm in diameter. The *in vitro* drug release was found to be affected by the presence of phytochemicals. Significant antibacterial activity was observed with enrofloxacin+curcumin microspheres where as significant anthelmintic activity was observed with albendazole + piperine combination. In conclusion, combination of traditional antibiotics or anthelmintics with phytochemicals may result in synergistic activity with extended activity against resistant organisms. Alginate-chitosan encapsulation offers effective formulation for drug-phytochemical combinations.

RNA-05

EVALUATION OF THERAPEUTIC POTENTIAL OF URSOLIC ACID ON RENAL FIBROSIS IN ADENINE - INDUCED CHRONIC KIDNEY DISEASE MODEL IN RATS

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Renal fibrosis is the common final outcome of almost all progressive chronic kidney diseases (CKD) and is one of the critical concerns in small animal as well as in human medicine. Ursolic acid (UA) is a pentacyclic triterpenoid molecule possessing diverse pharmacological properties. The possible therapeutic potential of UA on renal fibrosis in CKD was investigated in this study. We established CKD in male Wistar rats by feeding adenine at 0.75% in feed. Adenine feeding increased the relative kidney weight of CKD control rats while UA mitigated this effect. CKD control rats showed elevated levels of serum blood urea nitrogen, creatinine and uric acid along with increased levels of kidney injury marker such as cystatin C reflecting the deteriorated kidney function. Further, evaluation of kidney samples from CKD control rats revealed increased levels of transforming growth factor-beta (TGF-beta), fibronectin, collagen type I and hydroxyproline indicating a pro-fibrotic response. These data were further fortified by Masson's Trichrome staining where kidney damage and

fibrosis were clearly evident with deposition of collagen fibers. However, the above mentioned findings in CKD rats were significantly reversed by UA-treatment revealing its nephroprotective potential especially anti-fibrotic activity. Thus, UA could be an adjunct agent for the CKD therapy.

RNA-06

FUNCTIONAL CHARACTERIZATION OF T-TYPE CALCIUM CHANNELS IN BUFFALO MYOMETRIUM

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Present study was undertaken to unravel the presence of T-type Ca^{2+} channels in buffalo myometrium and their functional role in mediating PGF-2-alpha induced contractions in myometrium of buffaloes. Uteri along with the ovaries were collected from nondescript adult buffaloes immediately after their slaughter from the local abattoir. The uteri were cut open from the mid-cornual region and myometrial strips were prepared and mounted in a thermostatically (37.0 ± 0.5°C) controlled organ bath containing RLS. Isometric tension in isolated myometrial strips were recorded using Labchart pro v 6.1.3 software under a resting tension of 2 gm. Inhibitory effect of cumulative concentrations of mibefradil (10^{-9} to 10^{-4}) evidently suggested the functional involvement of T-type Ca^{2+} channels in regulating myometrial tone in buffaloes. To further substantiate their involvement in regulating myometrial activity, effect of PGF_2alpha was studied in the presences of mibefradil (1 µm), nifedipine (1 µm) + mibefradil (1 µm) and NNC055-0396 (5 µm), nonspecific and selective blocker of T-type Ca^{2+} channel respectively. The DRCs of PGF-2-alpha (10^{-8} to 10^{-4} M) were significantly shifted to right in the presence of these antagonists in gravid myometrium and the E_{max} values of PGF-2-alpha were found to be 0.42±0.10 and 0.37±0.07 in the presences of mibefradil and NNC055-0396 compared to that of control (0.87±0.06). PGF-2-alpha produced concentration dependent-contraction and the DRCs of PGF-2-alpha were non-significantly shifted to right in the presence of mibefradil (1 µM) and NNC055-0396 (5 µM) in non-gravid uterus. Interestingly, PGF-2-alpha induced contraction in the presence of both nifedipine and mibefradil was further significantly reduced compared to either in presence of nifedipine or mibefradil alone in gravid uteri. However, in non-gravid myometrium, combination of nifedipine and mibefradil did not produce any additive inhibition compared to nifedipine alone. Thus implying that although T-type Ca^{2+} channels are present in non-gravid and gravid uteri of buffaloes but their functional role in mediating PGF-2-alpha induced contraction is more pronounced during gravid state.

RNA-07

PHARMACOLOGICAL AND MOLECULAR EVIDENCE OF HYDROGEN SULPHIDE MEDIATED UTERINE TONE IN WATER BUFFALOES *(BUBALUS BUBALIS)*

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Hydrogen sulphide (H_2S), a recent gasotransmitter is in limelight for its physiological action rather than as a toxicant. Present study was undertaken to investigate the effect of H_2S and its associated signaling pathways on myometrium of buffaloes using hydrogen sulphide donors- L-cysteine (10nM- 30mM) and sodium hydrogen sulphide (NaHS; 10nM 300µM). Both these produced contractile effect on non-pregnant myometrial uterine strips which was blocked by nifedipine (0.1µM). To further substantiate the involvement of extra-cellular Ca^{2+} in H_2S- induced uterotonic effect on buffalo myometrium, effect of L-cysteine was studied in the presence of Ca^{2+}- free RLS solution. L-cysteine failed to exhibit any contractile effect on myometrium in Ca^{2+}-free RLS solution. Aminooxyacetate (AOAA) (100µM) and D,L- proparylglycine (PAG) (100µM), the respective selective antagonists of CBS and CSE, significantly inhibited H_2S-induced myometrial contractions as the L-cysteine-induced contractions (E_{max}, 0.725±0.171; pD_2, 4.705±0.2821) were significantly attenuated ($P<0.05$) and DRCs were significantly shifted towards right both in the presence of AOAA (R_{max}, -1.538±0.258) and PAG (R_{max}, -0.674±0.114) with no significant changes in potency (pD_2). NaHS-induced contraction was also almost completely attenuated and the DRC of NaHS was significantly ($P<0.05$) shifted towards right but with non-significant ($P>0.05$) decrease in E_{max} value of 0.068±0.066g in case of AOAA and 0.134±0.047g in case of PAG and increase in R_{max} value to -1.433±0.383 g in the presence of PAG and -0.915±0.067g in the presence of AOAA. Using Western blot technique, existence of the biosynthesizing enzymes- CBS (~63kDa, 205bp) and CSE (~45kDa, 149bp) could be demonstrated. Hydrogen sulphide evidently seems to be an important endogenous physiological mediator of uterine tone in buffaloes.

ISVPT-2016

TECHNICAL SESSION - IV

ETHNOPHARMACOLOGY

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छात्रहिताय सर्वदा

NAVSARI AGRICULTURAL UNIVERSITY

LEAD-EP-01

INTEGRATED APPROACH TOWARDS PHYTO-PHARMACEUTICAL RESEARCH: AN OVERVIEW

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Abstract

The Government of India in the Ministry of Health and Family Welfare brought out the policy statement on phytopharmaceutical under Drugs and Cosmetics (Eighth Amendment) Rules, 2015 through vide notification number G.S.R. 702(E) in the Gazette of India on 30th November, 2015. In rule 2, "Phyto-pharmaceutical drug" includes purified and standardised fraction with defined minimum four bio-active or phyto-chemical compounds (qualitatively and quantitatively assessed) of an extract of a medicinal plant or its part, for internal or external use of human beings or animals for diagnosis, treatment, mitigation or prevention of any disease or disorder but does not include administration by parenteral route. The introduction of molecular biology, analytical chemistry, nanotechnology, in-silico biology into phyto-pharmaceutical research is essential to get more detailed information on phyto-pharmaceuticals. The elucidation of these phenomena can help to rationalize phytotherapy and to integrate it into an overall concept of modern medicine. In most countries, the herbal drugs are poorly regulated and are often neither registered nor controlled by the health authorities. The safety of phytomedicine remains a major concern. The new phyto-pharmaceuticals regulation in India permits the development of the plant-derived drugs using advanced techniques of solvent extraction, fractionation, potentiating steps, modern formulation development. The new regulation is expected to promote innovations and development of new drugs from botanicals in a scientific way and would help in the acceptance of the use of phyto-phamaceutcals by modern medical profession as an important alternative to modern allopathic medicine.

Preamble

An interest in medicinal herbs is increasing as a precursor of pharmacological actives because of its traditional knowledge as well as some experimental evidences published in peered reviewed journals. Worldwide, phytomedine has been considered an important alternative to modern allopathic medicine. Self medication and greater orientation towards preventive health care, the growing desire of the aging population to stay young and healthy, and the increasing healthcare costs of therapy provided by modern medicine have led to more usage of traditional remedies [1] or phytomedicine. The Government of India in the Ministry of Health and Family Welfare brought out the policy statement on phytopharmaceutical under the Drugs and Cosmetics (Eighth Amendment) Rules, 2015 through vide notification number G.S.R. 702(E) in the Gazette of India on 30th November, 2015. "Phyto-pharmaceutical drug" includes purified and standardised fraction with defined minimum four bio-active or phyto-chemical compounds (qualitatively and quantitatively assessed) of an extract of a medicinal plant or its part, for internal or external use of human beings or animals for diagnosis, treatment, mitigation or prevention of any disease or disorder but does not include administration by parenteral route[2]. The introduction of molecular biology, analytical chemistry, nanotechnology, *in-silico* biology into phyto-pharmaceutical research is essential to get more detailed information on phyto-

pharmaceuticals. The safety of herbal medicines remains a major concern. Herbal medicine products include herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants, other plant materials, or combinations thereof as active ingredients.[3] Herbs include crude plant material, for example, leaves, flowers, fruit, seed, and stems. Herbal materials include, in addition to herbs, fresh juices, gums, fixed oils, essential oils, resins, and dry powders of herbs. Herbal preparations are the basis for finished herbal products and may include comminuted or powdered herbal materials, or extracts, tinctures, and fatty oils of herbal materials. Finished herbal products consist of herbal preparations made from one or more herbs.

The regulatory scenario regarding herbal preparations varies from country to country. In most countries, the herbal drugs are poorly regulated and are often neither registered nor controlled by the health authorities [4]. Phyto-Pharmaceutical drug development process should have same vigor of that synthetic drugs/New Chemical entity undergo to establish their safety and efficacy. The challenges in phyto-pharmaceutical drug development are due to the complex combinations leading to difficulty in assessment of their activity and risk/benefit ratio. Major issues in Herbal drug development are as follow; (i) very few pharmaceutical companies are involved in herbal drug research this may be due to the high cost involved in isolation and identification of pure compounds, difficulty in collection, the complex nature of plants, and absence of clear-cut regulatory guidelines for natural products (ii) In the conventional drug discovery process, a single pure active constituent is isolated, purified, and standardized. Multi-constituent herbal formulations can be standardized with newer techniques such as DNA fingerprinting, high-pressure thin layer chromatography (HPTLC), and liquid chromatographymass spectroscopy (LCMS) and the major challenges in Herbal Drug Development are (i) the reproducibility of biological activity of herbal extracts (ii) its toxicity and adverse effects (iii) its adulteration and contamination (iv) herbal drug interactions issues (v) standardization issues. To ensure the reproducibility and quality of R& D work related to herbal product development, the R& D work should implement the standard operating procedures (SOP) as Good Agricultural Practice (GAP), Good Supply Practice (GSP), Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP), Good Clinical Practice(GCP).

In India, Phyo-pharmaceutical drug development guidelines recommends that Investigational New Drug (IND) must contain sufficient information to demonstrate that the drug is safe for testing in humans and that the clinical protocol is properly designed for its intended objectives. Clinical trials for phyto-pharmaceutical drugs to be conducted as per applicable rules and guidelines for new drugs/new chemical entity, as per the Schedule Y. In addition to general regulatory requirements for new drug application (NDA), nonclinical pharmacology/toxicology studies, clinical evidence of efficacy and safety for botanical drugs there are special requirements to ensure safety and quality of botanicals [2].

In India, regulatory requirements for phyto-pharmaceuticals are under the purview of the Central Drugs Standards Control Organization (CDSCO) and regulatory submission requirements for scientific data on quality, safety, and efficacy to evaluate and permit marketing for an herbal drug on similar lines to synthetic, chemical moieties. The new phyto-pharmaceuticals regulation in India permits the development of the plant-derived drugs using advanced techniques of solvent extraction, fractionation, potentiating steps, modern formulation development. The new regulation is expected to promote innovations and development of new drugs from botanicals in a scientific way and would help in the acceptance of the use of phyto-phamaceutcals by modern

medical profession as an important alternative to modern allopathic medicine.

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LEAD-EP-02

FLAVONOIDS: A POTENT SOURCE FOR ANTI CANCER ACTIVITY

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Cancer is the second leading cause of mortality in human diseases worldwide. According to a national statistic report on the incidence and mortality in the USA, there were a total of 1,529,560 new cancer cases and 569,490 deaths from cancer occurring in 2010 [1]. Although modern cancer therapies have been significantly increased patient survival rate in human medicine, the cancer patients are now facing new challenges resulting from severe chronic tumor induced bone loss and pain. The cancer induced bone loss and pain is due to release of local and systemic inflammatory factors such as endothelin, TNF-alpha, prostaglandins or RANKL etc or due to tumor related *i.e* is by invasion of cancer cells to bone tissue and nerve tissue or due to chronic chemotherapy treatment [2-3]. Cancer induced pain and bone loss may affect the social, behavioral aspects of humans and hence considered as a severe consequence of cancer [4]. Among the cancer associated painful symptoms, neuropathic pain is found to occur in 40-80 % of patients [5]. Significant efforts have been made in the last two decades to develop novel therapeutic strategies against cancer and its complications. However, the available pharmacological options couldn't increase the patient's quality of life in most of the case. Further, chemotherapy- induced peripheral neuropathy and bone loss suffer from disadvantages such as adverse effects and patient tolerability [6]. Therefore, there is a need to develop more effective therapeutic strategies to combat cancer, cancer induced bone loss and pain. In this context, it is noteworthy that some plants and plant based products seem to be effective against cancer, cancer-induced bone loss and neuropathy pain.

Although there is no 'magic bullet' to overcome cancer, but the risk could be abridged by eliminating the tobacco or at least minimizing exposure to carcinogen containing foods. But, without complete identification of the corresponding risk factors, such primary prevention might be difficult to apply. Furthermore, the avoidance of some risk factors could involve large lifestyle changes, which are not so easy to implement. Moreover, conventional cancer therapies such as surgery, radiation and chemotherapy evoke severe side effects and in many cases, patients die before they could recover due to organ failure and immunosupression.

In view of these limitations, alternate preventive approaches which could require minimal or no life style changes and fewer side effects are urgently needed. One such strategy with promising clinical implications include the development of more effective and less toxic cancer preventive agents, i.e. chemoprevention.

Chemoprevention is regarded as one of the best way of inhibiting, delaying or reversing carcinogenesis by using natural, dietary or synthetic agents. A number of effective chemopreventive measures have been introduced substantially to reduce both the incidence and mortality due to lung cancer; among them use of dietary agents have shown to be promising and realistic approach owing to their ubiquitous nature, inexpensiveness and broad safety window. Chemoprevention through dietary means and/or use of pharmacological and natural agents with efficacy and acceptable toxicity are considered to be the winning strategy in reducing the morbidity and mortality of cancer. A large number of epidemiological and experimental studies, preclinical and clinical observations have suggested that diet plays a beneficial role in the chemoprevention of cancer. It is estimated that there are greater than 5000 individual phytochemicals in plant-based foods. Their identification and mechanism of action evaluation need to be resolved before we can fully understand the health benefits in humans. The phytochemicals demonstrated antiproliferative, anti-inflammatory, antiangiogenic, and proapoptotic effects, or the ability to reduce oxidative stress, and thus they are of high interest to scientists around the world and the general public. *In vitro* studies on different cancer cell lines also proved the role of polyphenols as anti-cancer agents. Among dietary factors, certain flavonoid phytochemicals particularly those in the diet have marked lung cancer chemopreventive properties.

Flavonoids, the naturally occurring polyphenolic compounds, represent one of the major class of compounds consumed in human diet such as vegetables, fruits, nuts and beverages like tea, coffee and red wine, have demonstrated their chemoprevention and chemotherapeutic properties in certain cancers. They exert wide variety of pharmacological activities, including antioxidant, anti-inflammatory, anti-diabetic and anticancer.

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EP-01

IN-VITRO ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF HYDRO-ALCOHOLIC EXTRACT OF *OPUNTIA ELATIOR* (OE) FRUIT AS WELL AS QUERCETIN

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The present study was carried out to evaluate *in-vitro* antioxidant and antidiabetic activity of hydro-alcoholic extract of *Opuntia elatior* (OE) fruit as well as quercetin. Antioxidant activity was carried out by using DPPH free radical scavenging assay and antidiabetic activity was carried by using alpha-amylase assay. Hydro-alcoholic extract of OE fruit and quercetin showed 38.14 ± 1.07 and 37.74 ± 1.06% inhibition of DPPH free radical scavenging activity, respectively at 200 µg/mL concentration. Alpha amylase inhibition by hydro-alcoholic extract of OE fruit and quercetin were 54.68 ± 0.11 and 54.64 ± 0.20%, respectively at 500 µg/mL concentration. Phytochemical screening of OE fruit revealed the presence of various phytochemicals i.e. carbohydrate, flavonoids, anthocyanin, phenols and protein. The extract of OE fruit as well as quercetin showed significant antioxidant and antidiabetic activities compared to the standard antioxidant ascorbic acid and antidiabetic agent acarbose in a dose dependent manner respectively.

EP-02

EVALUATION OF *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY OF *GLYCYRRHIZA GLABRA* AND *TINOSPORA CORDIFOLIA*

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The present study was carried out to evaluate *in-vitro* anti-inflammatory properties of extracts of *Tinospora cordifolia* stem and *Glycyrrhiza glabra* root in combinations, by bovine serum albumin denaturation method. Chloroform and methanol extracts of both plants were prepared by soxhlet extraction. The anti-inflammatory activity of different concentrations of extracts of both nutraceutical plants in combinations 2:1 and 1:2 were evaluated. Aspirin was used as standard anti-inflammatory drug. The percentage inhibition of combination of *G. glabra* and *T. cordifolia* chloroform extracts in ratios of 2:1 and 1: 2 were found to have significant (p<0.05) inhibition up to 79.45 ± 0.337 and 64.36 ± 0.27% at 25 µg/ml, respectively. Whereas, the percentage inhibition values of methanolic extract of both plants in ratio of 1:2 and 2:1 were found significantly (p<0.05) higher up to 83.21 ± 0.024 and 71.28 ± 0.51% at 25 µg/ml, respectively. It was concluded that compounds responsible for anti-inflammatory activity in above both nutraceutical plants may be better extracted with methanol, further isolation and identification of active principles are needed to validate the ethno-medical use of these plants.

EP-03

SURVEY ON ETHNOVETERINARY USE OF MEDICINAL PLANTS IN JUNAGADH REGION OF GUJARAT, INDIA

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The present study was conducted to document ethnoveterinary knowledge livestock farmers of Junagadh region of Gujarat, India. Three tehsils and 4 villages from each tehsil of Junagadh region were selected as study area. A total 121 informants were contacted personally in the survey with a semi-structured questionnaire. Meetings were either unstructured or grouped during the study. Some of the gaushala (cow sheds) owners were also contacted. The data obtained from the survey were quantitatively analysed by Informant Consensus Factor (ICF), Use Value (UV) and Frequency of Citation (FC).Out of 121 male informants, 82 were included in this study which have fulfilled the selection criteria. Informants were 40 to 70 years of age having an education up to secondary school level. Most of the plants informed by the informants were either medicinal or folklore plants from this region. Sixty-seven (67) medicinal plants from 40 different families have been reported to be used in 13 different ailments. Highest ICF value was found in intestinal and worm related disease, wound healing and skin related problems. Three plants shown highest UVs viz. *Annona squamosa*, *Ennicostema littiolare* and *Aloe barbadensis*. Various plant parts like leaf (27.59%), fruit (18.97%), bark (15.52%), seed (10.34%), root (9.48%), stem (6.03%), whole plant (4.31%), flowers (3.45%) and others (4.31%) were used in ethnoveterinary practices. Data obtained in the present study is valuable as till date no systemic documentation of ethnoveterinary practices was carried out in Junagadh region of Gujarat, India. *In-vitro* and *in-vivo* pharmacological evaluation is required to validate the efficacy of commonly used medicianl plants.

EP-04

NEPHROPROTECTIVE EFFECT OF *AEGLE MARMELOS CORREA* ON GENTAMICIN INDUCED NEPHROTOXICITY IN WISTAR RATS

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Gentamicin (GM) is an aminoglycoside antibiotic commonly used in treating life-threatening gram-negative bacterial infections (Ali, 1995). However, about 30% of the patients treated with GM for more than 7 days show some signs of nephrotoxicity; and serious complications resulting from GM-induced nephrotoxicity were a limiting factor for its clinical usage (Mathew, 1992). In rural and backward area of India, several plants are commonly used as herbal medicine for the treatment of kidney diseases. *Aegle marmelos* correa more commonly found plant was screened for potential Nephroprotective effect. The study was conducted at KNP College of veterinary science; Shirval Maharashtra to evaluate Nephroprotective effect of ethanolic leaf

extract of *Aegle Marmelos* Correa on Gentamicin induced nephrotoxicity in wistar rats. It was therefore, planned to study the effect of *A. marmelos* Corr. leaf extracts on experimentally induced Gentamicin nephrotoxicity in Wistar rats. Preliminary qualitative phytochemical investigation of *A. marmelos* ethanolic leaf extract revealed presence of alkaloids, carbohydrate, Saponin, phenolic compound tannin, fixed oils and fats and absence of glycosides. The Gentamicin (40mg/kg body weight) treated group show significant reduction in body weight, Hb, TEC, and TLC while, they had shown significant increase in relative kidney weight, BUN serum creatinine, ALT and AST levels. Rats treated with preventive regimen dose of *Aegle marmelos* leaf extract (250 mg/kg body weight) showed 0.55% percent decrease in body weight as compared to Taurine (1000mg/kg) and curative regimen dose of *Aegle marmelos* leaf extract However rats treated with curative regimen of *A. marmelos* Corr. leaf extract (500mg/kg) revealed significant increase in body weight compared to control and Taurine treated group. The rats treated with preventive and curative regimen dose of *A. marmelos* leaf extract revealed significant increase in Hb, TEC, and TLC and significant decrease in relative kidney weight, BUN serum creatinine, ALT and AST levels. Histopathological observation of liver showed derangement of hepatic cords with granular changes, cellular swelling of hepatocytes, congestion of central and portal blood vessels with sinusoidal congestion, focal and necrotic degenerative changes in few sections in Gentamicin treated animals at both the intervals studied. The treatment with *A. marmelos* Corr. leaf extract and Taurine partially reversed histopathological changes in liver. The mechanism involved in protective effects might be due to antioxidant activity of phytoconstituents of *A. marmelos* Corr. leaf extract which might act as free radical scavengers that restored Gentamicin induced oxidative stress in animals. However further studies are needed for better understanding of Nephroprotective effect of *Aegle marmelos.*

EP-05

EFFECT OF AQUEOUS EXTRACTS OF *ANNONA SQUAMOSA* ON HEMATO-BIOCHEMICAL PARAMETERS IN HEPATOTOXIC RAT MODEL

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The present study was conducted to evaluate hemato-biochemical alterations following repeated oral administration of aqueous extracts of *Annona squamosa* leaves in carbon tetrachloride induced hepatotoxic rats. The study was conducted on thirty six (36) male albino Wistar rats dividing them in various groups having six rats in each group. Group I served as vehicle control and received the normal saline solution. Group II was served as hepatotoxic control, group III served as standard treatment control and rats of treatment group IV, V and VI received 50 % carbon tetrachloride in olive oil @ 1 ml/kg body weight, intraperitoneally twice in a week throughout the study period for induction of hepatotoxicity. Group III received standard drug silymarin @ 50 mg/kg of body weight (p.o.) and group IV, V and VI received aqueous extracts of *A. squamosa* @ 100, 200 and 400 mg/kg (p.o.) respectively, daily once for 28 days. Upon acute oral toxicity testing, aqueous extracts of *A.*

Squamosa were found safe. Phytochemical analysis by GC-MS revealed presence of many compounds in *A. Squamosa* aqueous extracts. On 29th day of study, rats were subjected to blood collection; blood and serum sample were analyzed for haematological and serum biochemical parameters respectively. The result showed significant decrease in hematological parameters like Hb, TEC, PCV, MCV, MCH, MCHC, WBCs/TLC and platelets, significant increase in serum concentration of ALT, AST, GGT, ALP, creatinine kinase, bilirubin, serum creatinine and significant decrease in albumin, globulin and total protein in hepatotoxic control rats as compared to rats of vehicle control group. It suggests that carbon tetrachloride is useful substance for successful induction of hepatotoxicity in rats. Daily oral administration of silymarin significantly reduced serum ALT, AST, GGT, ALP, bilirubin, creatinine kinase and serum creatinine and increase albumin, globulin and total protein level as compared to hepatotoxic control rats. Hepatotoxic rats receiving aqueous extracts of *A. squamosa* @ 100, 200 and 400 mg/kg body weight also showed the same changes as compared to rats of hepatotoxic control group in dose dependent manner except *A. squamosa* (100 mg/kg). However, above parameters level did not reach to the normal. The findings of present study suggest that aqueous extracts of *A. squamosa* has significantly altered hemato-biochemical parameters in carbon tetrachloride induced hepatotoxic rat model.

EP-06

EVALUATION OF KAEMPFEROL PRETREATMENT ON HEMODYNAMIC FUNCTIONS IN ISOPRENALINE-INDUCED MYOCARDIAL INJURY IN RATS

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The Present study was undertaken to evaluate the effect of kaempferol in isoprenaline-induced myocardial injury in the rats. Pre-treatment of different doses of kaempferol was done for seven days before induction of myocardial injury in the rats. Isoprenaline was injected subcutaneously for two consecutive days to induce myocardial injury in the rats. Various hemodynamic functions such as systolic, diastolic, mean arterial blood pressure and heart rate were assessed in the isoprenaline-induced myocardial injury in the rats. Isoprenaline administration for two consecutive days significantly decreased the mean arterial blood pressure and diastolic blood pressure. However, heart rate was significantly enhanced in the cardiac injury group. Further, Pretreatment with kaempferol for seven days did not improve the mean arterial blood pressure and diastolic blood pressure in the cardiac injured rats in comparison with isoprenaline alone administered rats. However, kaempferol pretreatment for seven days in rats significantly ($p<0.05$-0.001) improved the heart rate in comparison with myocardial injured rats. No improvement was observed in the systolic blood pressure of the rats. The present study suggests that kaempferol pre-treatment for seven days partially improved the hemodynamic functions in the isoprenaline-induced myocardial injury.

EP-07

TOTAL THIOLS AND OXIDATIVE STRESS INDEX IN BLOOD AND HEPATIC TISSUE OF EXPERIMENTALLY INDUCED HEPATOTOXIC RATS: ATTENUATING POTENTIAL OF *CALENDULA OFFICINALIS* EXTRACTS

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Calendula officinalis have high medicinal potential and approved by food and drug administration for safe use in food industry. Study was aimed to determine the levels of total thiols and oxidative stress index in blood and hepatic tissue of experimentally induced hepatotoxic rats and its attenuation by administration of floral extracts of *C. officinalis*. Forty two rats were randomly divided into 7 groups with 6 rats in each received different treatments for 7 days. Significantly ($P<0.05$) increased plasma activities of phosphatases, transferases, reduced levels of total proteins and conjugated bilirubin following single oral administration of acetaminophen (APAP) indicated acute hepatotoxicity. Hepatotoxic rats also exhibited significant reduced levels of total thiols (TTH), total antioxidant status (TAS) and antioxidant enzymes, and increased oxidative stress index (OSI), total oxidant status (TOS) and malondialdehyde (MDA) levels in blood and hepatic tissue. Treatment with either silymarin or ethanolic floral extract of *C. officinalis* restored hepatic blood biomarkers, increased ($P<0.05$) the levels of TTH, TAS and antioxidant enzymes, and reduced the levels of MDA, TOS and OSI in blood and hepatic tissue of hepatotoxic rats. These findings were further confirmed in histopathological alterations in hepatic tissue of APAP administered rats. Correlation analysis of oxidant and antioxidant parameters revealed the negative ($p<0.05$) correlation between MDA levels and TAS levels in blood. Study suggested that ethanolic floral extract of *C. officinalis* increased the levels of TTH which may be responsible for the hepato-protection during APAP induced oxidative damage.

EP-08

ANTIDIABETIC, WOUND HEALING AND ANTIOXIDANT POTENTIAL OF QUERCETIN IN STREPTOZOTOCIN INDUCED DIABETIC WISTAR RATS

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Quercetin, important flavonoid widely distributed in fruits and vegetable and has recognized for having interesting clinical applications. The present study was aimed to evaluate the hypoglycemic, wound healing and antioxidant potential of quercetin in diabetic rats. Diabetes was induced with streptozotocin at the dose of 55 mg/kg intra-peritoneally. Thirty six wistar rats (180-200 gm BW) were divided into six groups of six animals each and were subjected to various daily oral treatment regimes for 21 days to determine the effect of quercetin on diabetic wound healing. Diabetes was induced in all Groups (Group-II to Group-VI) except Group-

I, that served as a vehicle control group receiving carboxy-methyl-cellulose (CMC) @1ml/100gm body weight/day orally and petroleum jelly topically for 21 days. For the creation of wound ($2x2cm^2$), the wistar rats of Group I-VI dorsal surface a full thickness excision wound was created along the markings. The wound was then monitored and the area of wound was measured at day 4^{th}, 8^{th}, 12^{th}, 16^{th} and 21^{st} day. Oral administration of quercetin @100mg/kg B.W for 21 days in diabetic rats normalized the altered blood glucose, TC, HDL, triglycerides, LDL, protein profile, plasma urea nitrogen, creatinine and antioxidant biomarkers. Topical application of quercetin ointment (1%) on the excised wound in diabetic wistar rats was sufficient enough to heal the wound area. Furthermore, the lipid profile was improved along with other biochemical (BUN, creatinine, SGOT, SGPT) and oxidative stress parameters. Therefore, it is concluded that quercetin @ 100mg/kg BW has antidiabetic potential. Wound healing property has been excellently produced by 1% topical application alone and 1% topical application + 100mg/kg B.W orally.

EP-09

IN VITRO ANTIBACTERIAL EVALUATION OF ACETONE EXTRACTS OF *ANDROGRAPHIS PANICULATA*, *OROXYLUM INDICUM*, *TERMINALIA BELLIRICA*, *BIXA ORELLANA* AND *DRYPETES ROXBURGHII* LEAVES

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The present study was planned to explore *in-vitro* antibacterial activity of acetone extracts of *Andrographis paniculata*, *Oroxylum indicum*, *Terminalia bellirica*, *Bixa orellana* and *Drypetes roxburghii* leaves. Plant leaves were collected, shade dried, powdered and further serial cold extractions were carried out using various solvents like hexane, chloroform, acetone, ethanol and water based on their increasing polarity. Solvents from acetone extracts were evaporated using rotatory vacuum evaporator. Reduced extracts were weighed and serial dilutions were made in 10% DMSO to evaluate their antibacterial activities using micro-broth dilution technique in which tetrazolium chloride dye was used to check viability of bacteria in microtiter plate. All the dilutions were made in triplicate. Gentamicin and enrofloxacin were used as positive control. MIC values of *Andrographis paniculata* against *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes* and *Proteus mirabilis* were found 2.56 mg/ml each. MIC of *Oroxylum indicum* against gram positive bacteria *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes* were observed 0.64 mg/ml, 1.28 mg/ml and 1.28 mg/ml, respectively and against gram negative bacteria *Salmonella typhimurium* and *Proteus mirabilis* were found 5.12 mg/ml and 1.28 mg/ml, respectively. MIC value of *Terminalia bellirica* against *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes* were found 0.32 mg/ml, 0.64 mg/ml and 1.28 mg/ml, respectively and against *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Proteus mirabilis* were observed 5.12 mg/ml, 5.12 mg/ml, 2.56 mg/ml and 1.28 mg/ml, respectively. MIC of *Bixa*

orellana leaves acetone extract against *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes* and *Proteus mirabilis* were observed 1.28 mg/ml, 2.56 mg/ml, 1.28 mg/ml and 2.56 mg/ml, respectively. MIC value of *Drypetes roxburghii* against gram positive organisms *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes* were observed 1.28 mg/ml, 5.12 mg/ml and 2.56 mg/ml, respectively. In conclusion, acetone extracts of *Andrographis paniculata*, *Oroxylum indicum*, *Terminalia bellirica*, *Bixa orellana* and *Drypetes roxburghii* leaves found to have antibacterial efficacy against various micro-organisms.

EP-10

IN VITRO ANTIOXIDANT SCREENING OF ACETONE EXTRACTS OF *BIXA ORELLANA* AND *DRYPETES ROXBURGHII* LEAVES AND BARK OF *FICUS RACEMOSA*

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Present research was conducted to evaluate *in-vitro* free radical scavenging and antioxidant properties of acetone extracts of *Bixa orellana* and *Drypetes roxburghii* leaves as well as *Ficus racemosa* bark. Leaves and barks of plant were collected, shed dried and powdered to evaluate its antioxidant activity using 2,2-diphenyl 1-picrylhydrazyl (DPPH) and 2,2-azinobis (3-ethylbenzothiazoline 6-sulfonic acid) (ABTS) free radical scavenging assays. Serial extraction was done using various solvents like hexane, chloroform, acetone, ethanol and water based on their increasing polarity to prepare extracts of plant material. Acetone from the crude extracts was evaporated and measured to make serial dilutions in 10% DMSO. Percentage inhibition and half maximal inhibitory concentration (IC_{50}) were calculated for each extracts. Trolox was taken as standard antioxidant and free radical scavenging agent. Each extracts were tested in triplicate. IC_{50} of trolox in DPPH assay was observed 0.074 mg/ml and in ABTS assay 0.022 mg/ml. Compared to standard drug, acetone extracts of *Bixa orellana* leaves, *Drypetes roxburghii* leaves and *Ficus racemosa* bark showed IC_{50} values for DPPH assay were 0.12 mg/ml, 0.11 mg/ml and 0.06 mg/ml, respectively. Whereas, IC_{50} values of acetone extracts of *Bixa orellana* leaves, *Drypetes roxburghii* leaves and *Ficus racemosa* bark were 0.09 mg/ml, 0.04 mg/ml and 0.09 mg/ml, respectively. Based on observation it can be concluded that acetone extracts of *Bixa orellana* and *Drypetes roxburghii* leaves as well as *Ficus racemosa* bark were found to have *in-vitro* antioxidant and free radical scavenging activity.

EP-11

EVALUATION OF IMMUNOMODULATORY AND ANTIOXIDANT ACTIVITY OF *TINOSPORA CORDIFOLIA*, *AZADIRACHTA INDICA* AND *ANDROGRAPHIS PANICULATA* EXTRACTS IN BROILER CHICKENS

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An experimental study was aimed to evaluate the individual effects of *Tinospora cordifolia* (stem), *Azadirachta indica* (leaves) and *Andrographis paniculata* (aerial parts) as alternatives to antibiotic growth promoter (BMD) in broiler chicks. Extracts of plants were obtained by Soxhlet's extraction in the mixture of 50% methanol and 50% water. In this study, total 210 day old Ven Cobb broiler chicks were used for stress and anti-oxidant parameters, one hundred fifty chicks were randomly assigned to 5 groups and each group of 3 replicates and 10 chicks in each replicate. The group T1 (Control diet), T2 (Standard growth promoter; BMD @ 0.05% in feed), T3 (TCE @ 0.4g/L), T4 (AIE; 0.4 g/L)) and T5 (APE @ 0.4g/L) were treated in drinking water daily for 42 days. The birds of Group T3 and T5 had significantly lower level of MDA and significantly higher levels GSH and GPx in RBC haemolysate as compared to control. The result indicated that both APE and TCE minimized oxidative stress and improved antioxidant status. The birds of group T4 did not show any significant difference in the levels of MDA and GSH but showed significantly lower level of GPx as compared to control. In cell mediated immunity, Groups T5 and T3 showed significantly higher skin thickness in DNFB skin sensitization test both at 24 hours and 48 hours after sensitization. The humoral immunity were assessed by micro HA test against sheep red blood cell (SRBC). Group T5 (APE) and T3 (TCE) showed significantly higher HA titer value as compared to the T1 and T2. But no significant difference was found among groups T1, T2 and T4. APE and TCE caused stimulation of both CMI and humoral immune responses in broiler chicks. AIE showed only stimulation of CMI at 48 hours after sensitization but did not show any significant effect on humoral immune response in broiler birds.

EP-12

STUDY OF INHIBITORY POTENTIAL AND PERCENT INHIBITION OF OIL OF *SYZIGIUM AROMATICUM* AND LEAVES OF *OCIMUM SANCTUM* ON EXTENDED SPECTRUM BETA LACTAMASE ENZYME FROM *E.COLI* OF BROILERS IN JABALPUR

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In the present study 400 caecal swabs were taken from the freshly slaughtered healthy broilers of various poultry sale outlets of Jabalpur and screened for the presence of ESBL producing *E.coli* using standard methods. ESBL enzyme was obtained by the standard method from the samples found to be positive for the resistant isolates. Enzymes were further studied for the inhibitory potential and per cent inhibition of oil of *Syzigium aromaticum* and fresh leaves juice of *Ocimum sanctum* using colorimetric method based on

microtitre plate assay method using CENTA and NITROCEFIN as the chromogenic substrate at the wave length of 405 and 486nm respectively. The inhibitory potential and percent inhibition was obtained on the basis of absorption value. *Syzigium aromaticum* exhibited mean value of 0.4±0.02 with 28 per cent of the average per cent inhibition of six samples with CENTA and mean absorbance value of 0.41±0.03 and 27 per cent of inhibition with Nitrocefin, in case of *Ocimum sanctum* mean absorbance value and per cent inhibition with CENTA and Nitrocefin was 2.03±0.02 and 10.0 and 1.97±0.06 and 10.0 respectively with ($p>0.05$) no significant difference was seen in the activity of both the chromogenic substrate Tazobactum (100µM) was taken as the standard control and it exhibited 0.12±0.01 and 0.13±0.01 (Mean±S.E.) of inhibitory potential, per cent inhibition observed was 99.88 percent and 98 percent against CENTA and NITROCEFIN respectively.

EP-13

HYPOLIPIDEMIC EFFECT OF *CALOCYBE INDICA* (MILKY MUSHROOM) IN HYPERCHOLESTEROLEMIC RATS

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Hypercholesterolemia and reduced HDL-C levels occur as a consequence of several interrelated factors that affects the concentration of various plasma lipoprotein. Herbal remedies are increasingly being employed in an attempt to manage hyperlipidemia. Previous literature reported that the edible mushroom *Calocybe indica* possesses many medicinal properties. Hence this study has been undertaken to evaluate hypolipidemic effect of *Calocybe indica* in high fat diet induced hypercholesterolemic model in rats. Forty-eight Wistar rats procured from Small Animal Breeding station were divided randomly into six groups of eight animals each. Group I-Normal control, Group II-Hyperlipidemic control, Group III,IV,V-Hyperlipidemic rats fed with *Calocybe indica* at dose rates of 250, 500, and 750 mg/kg and Group VI- Hyperlipidemic rats administered with reference drug, rosuvastatin at a rate of 10mg/kg orally. All the animals except normal control were fed with high fat diet for 45 days. The high fat diet was prepared by mixing 77% standard diet, 20% coconut oil, 2% cholesterol and 1 ml coconut oil supplemented with egg. The extract and reference drug were given from 16th day of high fat diet feeding. The blood was collected from all animals on day 0, 15, 30 and 45 and serum was separated for biochemical analysis. Administration of *Calocybe indica* ethanolic extract at all dose levels studied revealed hypolipidemic property by significant reduction in serum lipid profile in a dose dependent manner. *Calocybe indica* extract at 750 mg/kg lowered serum triglyceride and VLDL in a manner similar to rosuvastatin treated group. The protective effect of *Calocybe indica* may be attributed to the combined effect of many factors like presence of mushroom secondary metabolites. Thus the mushroom, *Calocybe indica* is very effective in lowering hyperlipidemia induced by high fat diet.

EP-14

ISOLATION, MORPHOLOGICAL IDENTIFICATION AND ANTIBACTERIAL ACTIVITY OF ENDOPHYTIC BACTERIA ISOLATED FROM ALOE VERA LEAVES

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The word endophyte literally means 'in the plant' (Gr. endon = within; phyton = plant). The term is used in a broad sense as per its definition to include bacteria, fungi, actinomycetes residing inside the plant tissues without causing any apparent disease symptoms in the plants. The present study was done to isolate endophytic bacteria from aloe vera (*Aloe barbadensis*) leaves, observe there *in vitro* antibacterial activity on some gram positive and gram negative bacteria. Five isolates of aloe vera were taken and divided into five isolate (25 isolate). The leaves were sterilized and incubated into kings B agar medium and then again sub cultured into 5% sheep blood agar and then transferred into BHI broth. The growth characteristics of endophytic bacteria isolated from aloe vera were studied. Endophytic bacteria isolated from aloe vera leaves were irregular in shape, had flat elevation on petri plate, margin of the colonies were entire, the surface of the growth was smooth the growth was opaque and green in colour. The antibacterial activity was observed against *Bacillus cereus, Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae and Salmonella* Typhimurium. The endophytic bacteria isolated from aloe vera had shown antibacterial activity as follows: 84% of isolate inhibited the growth of *Staphylococcus aureus,* 16% of isolate inhibited the growth of *Streptococcus pyogenes,* 16% isolate inhibited the growth of *Bacillus cereus,* 84% of isolate inhibited the growth of *Escherichia coli,* 12% of isolate inhibited the growth of *Salmonella* Typhimurium, 8% isolate inhibited the growth of *Klebsiella pneumoniae.*

EP-15

ASSESSMENT OF XANTHINE OXIDASE INHIBITION ACTIVITY OF *A. CEBA*, *A. INDICA* AND *P. BETLE* ALONE AND IN COMBINATION USING SPECTROPHOTOMETER

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Gout is a common metabolic disorder that results in abnormal accumulation of urates in domestic birds. Gout affected broiler birds were shown mortality rate of above 15% and feed conversion ratio were drastically increased (>2), which in turn results in heavy economic loss to farmers. Xanthine oxidase enzyme majorly involved in the development of gout in birds. The inhibition of Xanthine oxidase has been recognized as one of the promising targets on the treatment of poultry gout. Xanthine oxidase inhibitors like Allopurinol are used in the treatment of gout and it may cause the adverse effects like superoxide generation, hepatitis. Hence the

present study has focused on the natural herbs which are devoid of such disadvantages. Herbs like A. ceba, A. indica and P. betle were selected for this study based on the available literatures and their *in-vitro* Xanthine Oxidase inhibitory activity alone and in combinations were measured in UV/Visible Spectrophotometer. The extracts of all three herbs were prepared and the qualitative phytochemical analysis was carried out. The Xanthine Oxidase inhibitory activity of fresh extracts of all three herbs were measured at 100µg/ml level and in combination each at 50 µg/ml level in Spectrophotometer in comparison with Allopurinol. The results revealed that the *P. betle* extract treated at 100 µg/ml level were produced potential xanthine oxidase inhibitory activity (72.39 ± 0.60 per cent) in comparison with Allopurinol (89.30 ± 0.77). Combination of *A.ceba*, *P. betle* and *A. ceba*, *A. indica* and *P. betle* extracts showed moderate inhibition of enzyme. Therefore the outcome of this study indicated that *P. betle* could be used as a potential antigout agent instead of Allopurinol. Further detailed in-vivo trial of these plants on the gout affected live birds may give detailed insight about their use as possible antigout agents in poultry.

EP-16

DEVELOPMENT OF POLYHERBAL FORMULATION FOR CALF DIARRHEA AND SCREENING FOR ANTIBACTERIAL ACTIVITY ON ISOLATED BACTERIAL PATHOGENS - EXPERIMENTAL AND COMPUTATIONAL STUDIES

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The present study was conducted to explore the antidiarrheal potential of herbal formulation containing *Zingiber officinale, Piper nigrum, Capsicum fructescens, Annona squamosa* leaf and *Psidium guajava* leaves. Entero-pathogenic organisms (*E.Coli, Salmonella* spp, *Staphylococcus aureus* and *Vibrio cholera*) were isolated from the calf having acute diarrhea and cultured in selective media. Methanolic extract of Polyherbs was tested for antibacterial activity in different concentrations against the isolated pathogens, MIC and MBC values were recorded. Antidiarrheal activity of herbal formulation was evaluated using castor oil induced diarrhea model in albino Wistar rats. A total of 24 animals divided in to four groups each containing 6 animals. Loperamide (4 mg/kg, po) was used as standard drug, Methanolic herbal formulation @ 200 and 400 mg/kg (Per oral) was tested for activity. The severity of diarrhea was assessed by loose stool incidence rate (LSIR), average loose stools grade (ALSG) and Diarrhea index (DI). Computational molecular docking was carried out to identify the interaction between the bioactive compounds of polyherbs and their molecular targets. The herbal formulation was proved to have potent antibacterial activity with zone of inhibition ranging from 6-25mm. Upon 12 hour of observation, the complete cessation of wet motion was noticed in animals treated with 400 mg/kg body wt by 6 h. LSIR of control rats were higher ($P<0.05$) than that of plants treated groups, while LSIR of positive control group was lower ($P<0.05$). There was no difference in ALSG between different

groups. Model control group has higher DI than herbal group, while positive control group had the least DI. Docking studies revealed multifarious degree of affinity between protein and ligand groups, with highest binding energy of -10.24 kcal/mol. In conclusion, the herbal formulation shows potent antidiarrheal activity which proves the traditional use of the preparation for diarrhea in calves.

EP-17

EVALUATION OF ANTIDIARRHOEAL, ANTIBACTERIAL AND ANTI-INFLAMMATORY ACTIVITIES OF ETHANOLIC LEAF EXTRACT OF *DALBERGIA SISSOO*

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Among the various diseases confronted by animals diarrhoea is major cause of concern of morbidity and mortality. Diarrhoea is a commonly observed health disorder in goats. Many formulations of plant origin had been used since long time with no notable adverse effects unlike synthetic drugs. And herbs are better assimilated in animal body and well tolerated by animals. In order search cheaper, ecofriendly and potent antidiarrhoeal the present study was undertaken to evaluation antidiarrhoeal, antibacterial and anti-inflammatory activities of *Dalbergia sissoo.* The *Dalbergia sissoo* ethanolic leaf extract (DSELE) prepared by cold hydroethanolic extraction. In phytochemical qualitative analysis conducted by standard procedure, *D. sissoo* found to possess flavonoids, anthraquinones, amino acid, protein, saponins, tannins, sterols, glycosides and phenols. In acute oral toxicity studies, mice were administered with *DSELE* up to 2000 mg/kg P.O. did not produce any toxicity or mortality in mice. In evaluation of antidiarrhoeal activity using castor oil induced diarrhoea in mice, *DSELE* showed significant (p<0.01) percent inhibition of frequency of defecation at 400 and 800 mg/kg compared to control. In charcoal meal test per cent inhibition of charcoal meal transit by *DSELE* at 400 and 800 mg/kg doses was found to be 42.99 and 53.20. Thus, *DSELE* showed marked dose dependant inhibition of gastric intestinal motility induced by castor oil. In clinical cases of diarrhoeal goats presented to TVCC, PGIVAS, Akola, the animals treated with *D. sissoo* decoction showed restoration of feaces to normalcy from 2nd day of treatment and goats recovered completely on 4th day. For evaluation of antibacterial activity five different concentrations of *DSELE* were used against four different pathogenic bacteria by disc diffusion method. *DSELE* at 100 mg/disc concentration showed significantly highest zone (14.49±0.72) of inhibition against all test bacteria. Amongst the test bacteria *DSELE* showed highest zone of inhibition against *S. aureus* (15.66±0.47mm) followed by *E. coli* (14.33±0.47), *S. typhimurium* (14.33±0.94mm) and *B. cereus* (13.66±0.94mm). For evaluation of in-vitro anti-inflammatory activity *DSELE* was subjected to hypotonic solution induced HRBC membrane stabilization. The *DSELE* showed dose dependant HRBC membrane stabilization with maximum per cent stabilization was 73.09±5.63 at 1000 µg/ml concentration. Estimation of anti-arthritic activity was evaluated by inhibition of protein denaturation method. The maximum per cent inhibition of protein denaturation shown by *DSELE* was 78.39±3.39 at 1000 µg/ml indicating anti-arthritic potential in *DSELE*. In conclusion, the present study demonstrates that *D. sissoo* has potent antidiarrhoeal

activity with mild to moderate antibacterial and anti-inflammatory actions with considerable margin of safety. Thus *D. sissoo* could be a potential compound in the clinical application of diarrhea in animals.

EP-18

SCREENING OF *CLERODENDRUM INERME* (L). FOR PHARMACOLOGICAL ACTIVITY ON CENTRAL NERVOUS SYSTEM IN MICE

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The *Clerodendrum inerme* L. belongs to family *Verbenaceae* and is popular among the traditional practitioners for the treatment of pain, inflammation, skin diseases, topical burns, fever in human beings and it was also used by tribals as an antidote of poisoning from fish, crabs and toads. However, scientific studies to ascertain pharmacological activities are limited. The present study was undertaken to determine the phytochemistry and to screen the hydro-alcoholic extract of *Clerodendrum inerme.* L (HACI) for central nervous system activities, viz; to spontaneous motor activity (SMA), forced locomotor activity (FLA) and analgesic activity in mice. Phytochemical analysis of HACI revealed essentially the presence of alkaloids, triterpenoids, tannins, flavanoids and absence of glycosides, steroids and saponins. HACI at a dose rate of 400, 800 mg/kg significantly (p<0.05) reduced the SMA in mice and results were comparable to diazepam (2 $mg.kg^{-1}$ i.p.). HACI showed significant influence on voluntary locomotor activity in all the tested doses as assessed by the rota rod. Further HACI (400 and 800 $mg.kg^{-1}$) significantly (p<0.05) had showed analgesic activity (Eddys hot plate) after single per oral administration and the effects were comparable to acetyl salicylic acid. Thus, there exists a vast scope to subject the HACI for further investigations as it showed potent significant activity on central nervous system activity in mice and correlate with the folkloric claim for its use in the pain and inflammation.

EP-19

EVALUATION OF OXIDATIVE AND IMMUNOLOGICAL EFFECTS OF ARSENIC AND THEIR AMELIORATION BY *ECLIPTA ALBA* IN POULTRY

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This study was undertaken to assess the ameliorating potential of dried powder of *Ecliptaalba* plant (DPEA) on oxidative and immunological parameters following prolonged administration of arsenic in diet for 90 days in white leghorn cockerels. The 50% hydroethanolic extract of the plant was also screened for qualitative phytochemical analysis. The dried powder of *Eclipta alba* (DPEA) and hydroethanolic extract (HEEA) were prepared from the whole plant. Arsenic was given in the feed @100ppm and *DPEA* was given in two doses @

1000 ppm and 2000 ppm in feed for 90 days to evaluate its protective efficacy by determining antioxidant and immunological parameters. Phytochemical analysis revealed the presence of alkaloids, flavonoids, glycosides, resins, sterols, saponins, tannins, terpenes and absence of anthraquinones, proteins and amino acids. Thirty five cockerels were divided randomly and equally into five groups viz. Groups I as control, II for arsenic only, III for arsenic+ silymarin only, IV for arsenic+ DPEA @1000ppm and V, arsenic+ DPEA @2000ppm. Immunological parameters showed prominent alterations indicating immunosuppressive effect of arsenic; however DPEA treated group showed immunomodulation in comparison to arsenic treated group. Arsenic enhanced lipid per oxidation and reduced GSH, SOD levels in groups II and IV. $DPEA_{2000}$ was more effective in reversing these parameters significantly ($p<0.05$) as compared to silymarin. In tissues, DPEA significantly ($p<0.05$) restored the values of LPO, GSH and SOD altered by arsenic intoxication. DPEA also showed protective effect on LPO, GSH and SOD in tissues. $DPEA_{2000}$ showed better protective efficacy than $DPEA_{1000.}$ It is concluded from this study that *Eclipta alba* produced protective efficacy against arsenic induced oxidative stress and immunosuppression in cockerels.

EP-20

A STUDY ON APHRODISIAC EFFECT OF *CANNABIS INDICA* (LEAVES) & *MADHUCA LONGIFOLIA* (FLOWERS) ON MALE POULTRY

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Aphrodisiac is the word derived from Aphrodite, the Greek goddess of sexual, love & beauty. An aphrodisiac is defined as an agent (food or drug) that arouses sexual desire. But present time regular use of pesticides in our country decrease the fertility day by day. India uses approximately 85,000 tons of pesticides annually and an increase of 8% is expected every year .The residue of such environmental pollutant remain in soil, water, air, feed & fodder items for a longer period, to contaminate them. Chicken are especially vulnerable to pesticides toxicity because poultry houses are dusted with pesticides that decrease the all the parameter of semen those related the aphrodisiac potential. The present study was conducted to investigate the aphrodisiac effect of ethanolic extract of *Cannabis indica* leaves($1/10^{th}$ & $1/5^{th}$ of LD_{50}) and *Madhuca longifolia* flowers ($1/10^{th}$ & $1/5^{th}$ of LD_{50}) on poultry birds doses used for evaluation of all semen parameter (conc. of spermatozoa, live, general motility, progressive motility & volume). There was a significant ($p<0.05$) decrease in the values of conc. of spermatozoa, live, general motility, progressive motility & volume after 7, 14 & 28 days after given the *Cannabis indica* leaves ($1/10^{th}$ & $1/5^{th}$ of LD_{50}) as compare to control group. Ethanolic extract of *Madhuca longifolia* flowers also produced significantly ($p<0.05$) increase in level of above semen parameter as compare to control group after 7, 14 & 28 days treatment. Both doses of *cannabis* i.e.$1/10^{th}$ & $1/5^{th}$ of LD_{50} significantly reduced all the parameter indicating adverse effect on libido. Both doses of Mahua i.e.$1/10^{th}$ &$1/5^{th}$ of LD_{50} there was a significant increase in all the parameters reflecting positive aphrodisiac effect. Comparing $1/10^{th}$ & $1/5^{th}$

of LD_{50} of Mahua, 1/5 showed better result i.e. better aphrodisiac effect. These finding suggests that Mahua has positive aphrodisiac effect where *cannabis* does not. Similar findings were observed at 14 and 28 days but there was a continuous improvement recorded in all the parameters. From the present study, it may be concluded that Mahua has positive aphrodisiac effect whereas *Cannabis indica* has negative effect. There is a scope of further investigation regarding Mahua flower extract.

EP-21

EXPLORATION OF IMMUNOMODULATORY AND GROWTH PROMOTING POTENTIALS OF *KEDROSTIS FOETIDISSIMA* (JACQ.) COGN HERB IN IMMUNOSUPPRESSED BROILERS

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Herbal medicines are used throughout the world for their promising therapeutic potentials in human and animals. In poultry farming, the usage of herbs is increasing in recent years as growth promoters, immunomodulators, antivirals and antimicrobials. This study was taken to assess the immunomodulatory and growth promoting effects of *Kedrostis foetidissima* (Jacq.) Cogn herb in immunosuppressed broilers. A total number of 96 broiler birds used in this study, comprised of six birds in eight groups with two replicates. Groups 1 - 3 were fixed as normal, positive (Levamisole-30 mg/kg BW) and negative (Cyclophosphamide) controls respectively and groups 4 - 8 were fixed as *Kedrostis foetidissima* (whole plant crude powder) treatment groups with the inclusion levels of 0.1%, 0.2%, 0.5%, 1.0% and 2% respectively with an immunosuppression drug (Cyclophosphamide at 50 mg/kg, BW) on 21st day. Growth performance was assessed by evaluating weekly weight gain, feed intake and feed conversion efficiency (FCR) and body immunity was assessed by weekly Hemagglutination Inhibition (HI) titre against NDV LaSota antigen. Out of five inclusion levels, 1% (T7) and 2% (T8) received groups showed significant difference ($P< 0.05$) in producing body weight gain, feed intake and good FCR with T1, T2, T3, T4, T5 and T6 groups. But there is no significant noticed between T7 and T8 groups. On immunity, there is no significant difference noticed between T2, T7 and T8 groups, but there is a significant difference ($P< 0.05$) noticed with T3, T1, T4, T5 and T6 groups. This study revealed the growth promoting and immunostimulant potentials of Kedrostis foetidissima was proved in immuno-suppressed broilers. Since this herb was proved for having potentials, and is locally available, they may become an alternative source and further studies on this herb may lead towards discovery of novel immunomodulatory compounds for poultry in future.

EP-22

DOCUMENTATION OF ETHNOVETERINARY PRACTICES IN NAMAKKAL DISTRICT OF TAMILNADU

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Ethnoveterinary practices are still important in treating livestock diseases. But the knowledge of ethnoveterinary practices is declining due to improper documentation and oral passage of herbal heritage verbally. Documenting the indigenous knowledge is important for conservation and utilization of biological resources. Hence the ethnoveterinary practices followed in Namakkal district was documented from the traditional ethnoveterinary practitioners through a questionnaire. Existing scientific information about the ingredients used in the ethnoveterinary practices was also recorded. Ethnoveterinary practitioners are using *Lippia nodiflora* leaves, *Commiphora caudate* bark and *Terminalia arjuna* bark, that are scientifically proved to have antidiarrhoeal action for treating enteritis. *Andrographis paniculata* leaves, *Corallocarpus epigaeus* root and *Piper nigrum* seeds are being used to treat envenomation. The above mentioned two ingredients are having anti-venom action. Ethnoveterinary practitioners are using *Blepharus madraspatensis* and turmeric powder for wound healing, *Crotalaria verrucosa* leaves for treating contusion, *Dodonaea viscose* leaf decoction for treating yoke gall, *Acalypha indica* leaf, *Piper nigrum* seeds and *Cuminum cyminum* for treating skin lesions and *Clerodendrum phlomidis* leaf, *Cardiospermum halicacabum* leaves, *Piper nigrum* seeds and dried ginger for treating inflammatory conditions of muscle and joints. *Blepharus madraspatensis* was proved to have wound healing, anti-inflammatory, analgesic, antimicrobial and antioxidant properties *Crotalaria verrucosa* was proved to have clot lysis and wound healing property. *Dodonaea viscose, Acalypha indica* and *Piper nigrum* was proved to have anti-inflammatory and antimicrobial properties. *Cuminum cyminum* is rich in Vitamin E that helps in treating the skin lesions. *Clerodendrum phlomidis and Cardiospermum halicacabum* was proved to have anti-inflammatory, analgesic, anti-arthritic, antimicrobial and antioxidant properties. Thus the existing scientific information supports the use of these herbs for treating the above mentioned conditions.

EP-23

EVALUATION OF *IN-VITRO* ANTI-DIABETIC ACTIVITY OF *GLYCYRRHIZA GLABRA* AND *TINOSPORA CORDIFOLIA*

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Glycyrrhiza glabra and *Tinospora cordifolia* are traditional medicinal plants which possess anti-spasmodic, anti-inflammatory, anticancerous and antioxidant properties. The present study was carried out to evaluate an *in-vitro* anti-diabetic activity of different extracts of *G. glabra* root and *T. cordifolia* stem in proportions of 1:2 and 2:1 by alpha-amylase inhibition method. Chloroform, methanol and water extracts of both plants were prepared by soxhlet apparatus. Various concentrations from 10 to 100 µg/mL were used to determine the activity. Acarbose was used as standard drug. Water extracts in 1:2 and 2:1 ratio inhibited alpha-amylase significantly (p<0.05) with 53.69±2.14 and 52.89±1.40 percent, respectively. In case of methanolic extract 2:1 ratio of both plants produced significant inhibition 53.95±0.66 percent (p<0.05), where as 1:2 ratio of same extract inhibited alpha-amylase by 48.12±1.40 percent at 100 µg/mL concentration. Combinations of chloroform extract did not show any inhibition against alpha-amylase. Presence of triterpenoid in the methanol and water extracts of both plants might be responsible for antidiabetic activity.

EP-24

PHARMACOLOGICAL EVALUATION AND ELECTRONMICROSCOPY STUDY OF QUERCETIN AND IBUPROFEN IN COMPLETE FREUND'S ADJUVANT INDUCED RHEUMATOID ARTHRITIS IN RATS

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Arthritis is joint inflammation and refers to a group of more than hundred rheumatic diseases and other conditions that cause pain, stiffness and swelling particularly in the joints. Thirty male *Wistar* rats aged about 60 days were randomly assigned into five groups comprising of six rats in each and treated as follows: Group 1 was kept as normal control throughout the experimental period. Remaining 4 groups were induced rheumatoid arthritis (RA) by sub plantar injection of CFA. After 72 h, all those induced rats were diagnosed for RA, and were included in the study. Treatment protocols were initiated from day 0 post-confirmation of RA and continued for 21 days. Group 1: Non-RA control; group 2: CFA (0.1 mL @ 10% single dose, sub plantar route) - induced RA control; group 3: Quercetin (160 mg/kg b.wt mixed with tween 80 given orally by gavage on alternate days from 0th day of induction); group 4: Ibuprofen (53 mg/kg b.wt *P.O* from 0th day of induction) treatment in RA rats; group 5: Ibuprofen and quercetin in RA rats. In the present study, significant alterations in

body weights, relative organ weights arthritic index, paw volume and thickness and biochemical parameters in RA control group 2 as compared to normal control group 1. All the treated groups (3, 4 and 5) revealed significant improvement in all the parameters as compared to group 2. TEM of synoviocytes from RA control group showed increased nucleus to cytoplasm ratio, stained granules in nucleus, ill-defined nuclear membrane, altered cellular architecture and loss of intercellular matrix.TEM of quercetin-treated group showed plenty of collagen fibers. Ibuprofen-treated group showed modified cristae, rough endoplasmic reticulum and many free ribosomes. TEM of synovial membrane of quercetin + ibuprofen treated group showed retained cellular architecture i.e. compactness in the arrangement of synoviocytes having distinct cell walls with plenty of matrix in between. From this study, it is concluded that CFA-induced RA and its effects can be reverted by using quercetin and ibuprofen, combination treatment with both the drugs in study was found superior when compared to individual treatments.

EP-25

NEPHROPROTECTIVE POTENTIAL OF *TINOSPORA CORDFOLIA* ON GENTAMICIN INDUCED NEPHROTOXICITY IN RATS

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Gentamicin, an aminoglycoside antibiotic, accumulates in the biological membranes causing necrotic and degenerative changes in renal tubules resulting in acute nephrotoxicity. Many phytochemicals possess the potential of protecting and curing nephrotoxicity. The present study was conducted to evaluate the nephroprotective potential of *Tinospora cordifolia* methanolic stem and leaf extract against gentamicin induced nephrotoxicity in rats. Twenty four male rats were divided into four groups of six animals each. Animals of Group I served as normal control. Nephrotoxicty was induced in all other groups by intraperitoneal administration of Gentamicin at the dose rate of 100mg/kg daily for 8 consecutive days. Gentamicin treated rats of Group III and Group IV were co-administered with methanolic extract of *Tinospora cordifolia* stem and leaf respectively, at the dose rate of 300mg/kg orally. The plasma urea, blood urea nitrogen, albumin and total protein was evaluated along with histopathological investigation of the kidney. *Tinospora cordifolia* extract (both stem and leaf) at 300 mg/kg treatment recorded a significant reduction in the elevated plasma urea, blood urea nitrogen, albumin and total protein at the end of the treatment. Histopathology revealed significant reduction in renal tubular degeneration among both Group III and Group IV which correlated with the biochemical observations. From the findings it can be concluded that the stem and leaf of *Tinospora cordifolia* has nephroprotective action on Gentamicin induced nephrotoxicity and the leaf extract was more potent than the stem extract.

EP-26

EVALUATION OF *EUCALYPTUS CITRIODORA* LEAVES HOT METHANOLIC EXTRACT AGAINST EXPERIMENTALLY-INDUCED ENDOMETRITIS IN WISTAR RATS

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Present study was undertaken to investigate the modulatory effect of *Eucalyptus citriodora* hot methanolic leaves extract @ 25 mg/kg body wt. and cefixime @ 15 mg/kg body wt. on inflammatory biomarkers in experimentally induced endometritis in female Wistar rats. Rats were divided into five groups (Control, sham operated, endometritis control, endometritis treated with *E. citriodora* and endometritis treated with cefixime) of eight animals in each. Rats endometritic model was developed by inoculating the mixture of *E. coli* ($1x\ 10^6$) and *Staphloccocus aureus* ($1x\ 10^8$) in to uterine horns during diestrus stage followed by cervical ligation and the model was confirmed based on presence of visible pus in the uterus, edematous uterine horn, thinning of endometrial lining and presence of large number of polymorphonuclear cells and bacterial load in uterine flushing. After 7 days of induction of inflammatory condition, endometritic rats were treated with Eucalyptus leaves extract and cefixime once daily for next five days. After twelve days of the experimental period blood was collected from retro orbital plexus of each rat, and serum was harvested for further analysis. Tumor necrosis factor alpha (TNF alpha), pro- and anti-inflammatory cytokines like interleukin 1 beta (IL1 Beta) and interleukin10 (IL-10), serum amyloid A (SAA) and intercellular adhesion molecule 1 (ICAM-1), myleoperoxidase (MPO), toll like receptor 4 and 9 (TLR-4, TLR9), cyclooxygenase 1 and 2 (COX-1, COX-2), inducible nitric oxide synthase (iNOS) and nitric oxide (NO) were found to be significantly reduced after treatment with Eucalyptus leaves extract. Histopathological changes in uterus also showed efficient induction of endometritis by presence of inflammatory cells which are lessened effectively after treatment with Eucalyptus leaves extract. Results of the present study revealed that *Eucalyptus citriodora* produced curative and protective effect against endometritis and was more efficacious than cefixime. Based on the present study, it may be inferred that *Eucalyptus citriodora* leaves extract possesses promising efficacy against experimental bacterial endometritis and, therefore, can be exploited in drug development program for treatment of endometritis in animals.

EP-27

ANTI INFLAMMATORY EFFECT OF SILVER NANO EUGENOL IN CARAGEENAN INDUCED PAW OEDEMA IN WISTAR ALBINO RATS

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Eugenol (4-allyl 2-methoxyphenol) a naturally occurring phenolic compound is a major component of basil oil and exists to a lesser extent in oil of several other plants. Nanotechnology has emerged as an exciting approach in the drug development process and among the various nanoparticles; silver nanoparticles have been explored for its variety of medical applications. In the present study the anti-inflammatory activity of silver nano-eugenol particle was assessed using carageenan induced rat paw model. Acute toxicity study of silver nano particle was conducted in wistar albino rats as per OECD guideline 425. Silver nano particle was made with silver nitrate and its properties were characterised using zeta potential, dynamic light scattering and size was measured using scanning electron microscope and transmission electron microscope. Three groups of animals (n= 6) namely control (Corn oil), standard (diclofenac 30mg/kg oral) and nano eugenol (80 mg/kg orally) were given for each group prior to administration of 0.1ml of 1% carageenan by sub-plantar injection. Paw volume was measured using digital plythesmometer with data acquisition system for every one hour up to 4 hours. The silver nano particle was having a zeta potential of -5.7mV and were conforming to the size of nanoparticle. In paw oedema model it was shown to possess a comparable effect as that of diclofenac as indicated by an increase of 16.61±6.116% in nano eugenol compared to 26.56±6.288 % in diclofenac and 41.68±4.01% for vehicle at one hour, which persisted up to 4 hours. Hence it can be concluded that silver nano particle of eugenol is having acute anti inflammatory activity.

EP-28

EFFECT OF EUGENOL ON AMELIORATING THE HYPER RESPONSIVENESS OF AORTA TO PHENYLEPHRINE AND 5-HYDROXYTRYPTAMINE IN DIABETIC RATS

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Diabetes mellitus is a major contributing factor for mortality associated with cardiovascular diseases. Hyper responsiveness to vasoconstrictors and diminished response to vasorelaxants are the reasons for vascular complications in diabetes. Eugenol is a phenylpropene phytochemical present in clove oil, nutmeg, cinnamon basil and bay leaf. The present study was carried out to know the effect of eugenol on vascular hyper responsiveness of aorta associated with diabetes to vasoconstrictors like phenylephrine (PE) and 5-hydroxy tryptamine (5-HT). Wistar albino rats were randomized into control, eugenol treated control, diabetic and eugenol treated diabetic (n=6) groups. The rats were rendered diabetic by giving 10% fructose in water for initial 4 weeks followed by single dose of streptozotocin at the rate of 40 mg/kg by i/p administration.

Development of diabetes was assessed by blood glucose levels. Eugenol was given for 8 weeks in eugenol treated control group and eugenol treated diabetic group at a daily dose rate of 80 mg/kg orally. 4 weeks after streptozotocin administration, rats were sacrificed and thoracic aorta was isolated for functional studies using polygraph with digital data acquisition system. Hyper-responsiveness to PE was observed in diabetic animal as evidenced by EC50 value of $8.51X10^{-8}$ M compared to the value of control (1.99×10^{-7} M) whereas in eugenol treated diabetic rat it was $1.45 X 10^{-7}$ M which was comparable to the normal group. EC50 value of 5-HT was moderately enhanced in diabetic group ($3.56 X 10^{-6}$ M) when compared to the control ($1.70 X 10^{-6}$ M) and eugenol treated diabetic group ($3.24 X 10^{-6}$ M) which did not differ from each other. Eugenol treated control animals showed no difference from control animals for PE ($1.91 X 10^{-7}$ M) and 5-HT ($1.62 X 10^{-6}$ M). Hence it can be concluded that eugenol treatment can ameliorate the aortic hyper-responsiveness to PE in diabetic animals.

ISVPT-2016

TECHNICAL SESSION - V

ANTIMICROBIALS AND ANTIMICROBIAL RESISTANCE

Chairperson : Dr. C. Varshneya

Co-chairperson : Dr. M. M. Gatne

Rapporteur : Dr. Binita Angom

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World Health Organization's 2014 report on global surveillance of antimicrobial resistance reveals that antibiotic resistance is no longer a prediction for the future; it is happening right now, across the world, and is putting at risk the ability to treat common infections in the community and hospitals. It is an increasingly serious threat to global public health that requires action across all government sectors and society. There are high proportions of antimicrobial resistance (AMR) amongst bacteria that cause common infections (e.g. urinary tract infections, pneumonia, bloodstream infections) throughout the world. Resistant microorganisms (including bacteria, fungi, viruses and parasites) are able to survive attack by antimicrobial drugs, such as antibacterial drugs (e.g., antibiotics), antifungals, antivirals, and antimalarials, so that standard treatments become ineffective and infections persist, increasing the risk of spread to others. The evolution of resistant strains is a natural phenomenon the use and misuse of antimicrobial drugs accelerates the emergence of drug-resistant strains. The evolving public health threat of AMR is driven by both appropriate and inappropriate use of antimicrobial agents. Overuse plays an important role in the emergence of AMR. Paradoxically, underuse through inappropriate choice, inadequate dosing, poor adherence to treatment, and substandard antimicrobials, also plays an important role in the emergence and spread of AMR. Hence, there is need to monitor the use of antimicrobials at all levels of health care, study the antimicrobial use practices in various infections and behavior of stakeholders for antibiotic use and resistance.

Antibiotic use in food animals began almost as early as it did in people and has grown steadily, with little oversight. Today, far more antibiotics are consumed by animals than by people, the vast majority for growth promotion and disease prevention, as a substitute for hygiene and nutrition. The growing demand for meat and other animal products over the next few decades presages a potentially massive increase in antibiotic use, even greater than the increase in demand as intensive large-scale production replaces small-scale operations in LMICs. Now is the time to make sure that conditions are established to safely eliminate most animal use of antibiotics. This may entail an economic cost but should not harm animal health and is likely to decrease the burden of antibiotic resistance in the human population. Some of the antibiotics used by people and animals end up in ground, surface water and soil. The consequences of this antibiotic load in the environment are just beginning to be studied. Early research suggests that it adds to the total burden of antibiotic resistance in the world, although effects on humans cannot yet be measured.

Currently, few laws in India govern antibiotic use in food animals, and most pertain only to animal products for export. General Statutory Rule (GSR) 28(E) mandates a withdrawal period for use of antibiotics in food producing animals from the time of administration until the production of foodstuffs. GSR 588 (E) specifies that all drugs in the H1 category, including many antibiotics, require a prescription, and requires separate pharmacy documentation of those prescriptions that are subject to review. Statutory Order (SO) 722(E) restricts some antibiotic use in aquatic animals for export, and the Export Inspection Council monitors for

antibiotic residues in eggs, honey, milk and poultry for export. In the European Union (EU), the use of antibiotics for growth promotion has been banned since 2006, resulting in some decreases in antibiotic use and resistant bacteria.

In addition to veterinary-specific regulations on antibiotic use, the Second Amendment of the Drugs and Cosmetics Rules (2006) contains a list of 536 drugs that fall under Schedule H. These drugs, which include antibiotics, require by law a prescription for their use (Ministry of Health and Family Welfare, Department of Health 2006). In 2013, a new category of H1 drugs was added in a Fourth Amendment to the Drugs and Cosmetics Rules (GSR 588 (E)).

Laws aim to limit the amount of antibiotic residue ingested by consumers and to reduce antibiotic use with the aim of slowing the evolution and spread of antibiotic-resistant bacteria in animals and humans. In the EU, a critical step in this process was the banning of antibiotic use for growth promotion. India has no such ban, and at least eight antibiotics deemed 'highly' or 'critically' important for human health that are banned for growth promotion purposes in the EU are used for such purposes in India (Center for Science and Environment 2014).

There has been opposition to banning the use of antibiotics as growth promoters in many countries due to the potential negative economic impact. A recent assessment (Laxminarayan *et al*. 2015) estimates that the impact would be marginal in countries where farm production systems are already optimized, and more significant in countries with non-optimized systems.

In India, projected production losses were estimated at about 1 to 3 percent of annual meat production, or $1,110 to 2,599 million USD. Commercial poultry farmers account for one half to three quarters of total production in India, and would face the greatest impact (Center for Science and Environment 2014) . In addition to laws, the Codex Alimentarius, developed by FAO and the WHO, specifies a series of recommendations to 'ensure safety and quality in international food trade'. The Maximum Residue Limits for Veterinary Drugs in Foods, updated in July 2015, recommends maximum residue limits (MRLs) for commonly used veterinary drugs, including antibiotics (Codex Alimentarius Commission 2015). It includes detailed recommendations for MRLs in specific types of animal tissue. These are designed to assist countries as they consider adopting national MRLs.

Strategies contribute to successful national policies for antibiotic resistance and access

1. Reduce the need for antibiotics through improved water, sanitation, and immunization. The most attractive strategy is to reduce the need for antibiotics by reducing the burden of infectious diseases requiring antibiotics. This can be achieved by improving vaccination coverage (Okeke *et al*. 1999; Zhou *et al*. 2008), improving access to clean water and sewerage systems (Cairncross *et al*. 2010), and ensuring a safe and healthful food supply (Katona and Katona-Apte 2008). Some bacteria, such as Clostridium dif?cile, a diarrhea-causing pathogen spread through the fecal-oral route, are especially likely to spread through ?ngers, devices, and surfaces. The long-term use of antibiotics can destroy normal gut ?ora and increase susceptibility to *C. difficile* infection (Owens *et al*. 2008).

2.Reduce and eventually phase out sub therapeutic antibiotic use in agriculture. In many parts of the world, food animals consume more antibiotics than humans do, and with even less oversight. The few available studies on antibiotic resistance in livestock show that farm animals carry a large load of resistant organisms. In most low to mid income countries, little is known about antibiotic use in agriculture or antibiotic-resistant

organisms in animals. Documenting levels and patterns of antibiotic use in agriculture will provide a sound basis for reviewing and strengthening laws and regulations. Incentivizing the rational use of antibiotics is important in the veterinary field as well. *Helping farmers optimize production as they transition to large scale farming, for example, could avoid reliance on antibiotics in place of improved water, sanitation, and immunization (Laxminarayan et al.* 2015).

3. Educate and inform health professionals, policymakers, and the public on sustainable antibiotic use. *Though international attention to the issue is growing, antibiotic resistance is still not widely recognized or understood as a serious threat to human health.* Awareness campaigns have decreased antibiotic use, with some indications of corresponding decreases in resistance (Huttner *et al*. 2010). In France, which had among the highest rates of antibiotic consumption in Europe, an awareness campaign with the slogan "Antibiotics are not automatic" resulted in an average 27 percent decrease in rates of antibiotic prescriptions between 2000 and 2007 across all 22 regions of France. The decrease was greatest 36 percentin children aged 6 to 15 years (Sabuncu *et al*. 2009). The educational component of ASPs is often conducted at the hospital level, but guidance on antibiotic prescribing, antibiotic stewardship, and infection control can be incorporated into both undergraduate and postgraduate medical programs to instill appropriate prescriber practices early on. Medical students in Europe, the United States, and some LMICs reported interest in additional education on antibiotic prescribing (Dyar *et al*. 2014; Abbo *et al*. 2013; Thriemer *et al*. 2013).

4. Ensure political commitment to address antibiotic resistance. Generating local interest and pressure by healthcare professionals and the public and undertaking a thorough situation analysis are necessary to build political commitment and cooperation for combating antibiotic resistance. Thereafter, politicians need to allocate time, money, and resources to designing and implementing strategies to promote the rational use of antibiotics. In addition, government can convene academics and stakeholders from other government sectors health, social development, environmental health, agriculture and food production, education, science and technology to create locally relevant, evidence-based policies. Examples of such political efforts include the Jaipur Declaration on Antimicrobial Resistance, in which WHO Southeast Asia member states committed to developing multisectoral national alliances to develop national antibiotic policies (WHO 2011c). WHO called for the creation of national-level strategies on antibiotic resistance in each member state as a part of its 2015 Global Action Plan (WHO 2015).

Guidelines for Optimizing Use of Key Antimicrobials

A. Antimicrobial Prescribing: Good Practice

1. Send for the appropriate investigations in all suspected infections as recommended. These are the minimum required for diagnosis, prognosis and follow up of these infections.
2. All attempts shall be made to send microbiological samples prior to initiating antimicrobial therapy. Rapid tests, such as Gram stain, can help determine therapeutic choices when decision on empiric therapy is required.
3. Differentiation between contamination, colonization and infection is important to prevent overuse of antimicrobials. Use hospital guidelines based on local antibiograms when choosing antimicrobial therapy whenever possible. If alternatives to those recommended as used, reasons in the case records should be documented.

4. Prescribing antibiotics just in case an infection is present is rarely justified. Where patients are in hospital close observation is usually better options till the diagnosis is made.
5. Choice of antibiotics: This depends on antibiotic susceptibility of the causative organism. There are some infections, which can be treated by one of several drugs. The choice can be based on Toxicity, Efficacy, Rapidity of action, Pharmacokinetics and Cost. Use the most effective, least toxic and least expensive antibiotic for the precise duration of time needed to cure or prevent infection. Pathogens specific guidance in hospital policy is encouraged. Before prescribing consider the following:
   a. Which organism is likely to cause the disease?
   b. What is the clinical diagnosis and what other steps should be taken to reach diagnostic precision
   c. Which antimicrobial agents are available and active against the presumed cause of the illness? Is their range of antimicrobial activity appropriate and what information is available about the likelihood of drug resistance?
   d. Check for factors, which will affect choice of drug and dose, e.g., renal function, interactions, allergy, pregnancy and lactation.
   e. Check that the appropriate dose is prescribed. If uncertain, contact registered veterinarian or clinical microbiologist. Alternatively, check in the formulary.
   f. What is the duration of treatment?
   g. Is treatment working?
6. Clinical Diagnosis: The antibiotic treatment chosen must be based on presumptive diagnosis made on some assumption regarding the nature of disease. The treating doctor may not have difficulty in choosing the appropriate antibiotic to treat a disease caused by a single microorganisms e.g scarlet fever, typhoid, anthrax, as microbiological diagnosis is implicit in clinical diagnosis. However, diseases such as pneumonia, meningitis and urinary tract infection can be caused by spectrum of bacterial species and doctor may be wrong if he has to guess which antimicrobial agent to use. In such instances one should seek a bacteriological diagnosis.
7. Empiric Therapy If the causative agent is not known and where delay in initiating therapy would be life threatening or risk serious morbidity, antimicrobial therapy based on a clinically defined infection is justified and the following points should be taken into consideration:
   a. Do not rush to treat.
   b. Collect the necessary specimens before commencing therapy.
   c. Cover all possible microbial causes.
   d. Try to attain synergy.
   e. Consider possible interaction with other drugs.
   F. Accuracy of diagnosis should be reviewed regularly and treatment altered/stopped when microbiological results become available.
   g. Use less costly drugs where possible.
8. The need for antimicrobial therapy should be reviewed on a daily basis. For most infections 5 - 7 days of antimicrobial therapy is sufficient (simple UTI can be adequately treated with 3 days of antibiotic).
9. All IV antibiotics may only be given for 48 - 72 hours without review and consideration of oral

alternatives.

10. Once culture reports are available, the registered veterinarian should step down to the narrowest spectrum, most efficacious and most cost effective option. If there is no step down availed, the reason shall be documented and is subjected to clinical audit.
11. Treatment with antibiotic combinations: In order to avoid antagonism between drugs and undesirable side effects of several antibiotics it is advisable to use a single drug where ever possible. There are situations however, when the use of antibiotic combination is desirable. The situations are:
    a. A temporary expedient during the investigation of an obscure illness.
    b. To prevent the development of bacterial resistance in long term therapy e.g treatment of tuberculosis.
    c. To achieve synergistic effect, e.g. in treating infective endocarditis.
    d. Mixed infection, when one drug is not effective against the pathogen.
    e. To permit a reduction of the dose of potentially toxic drug.

    Antimicrobial drug therapy cannot be considered in isolation and other aspects of therapy must be taken into account in judging the effect of treatment. Even an appropriate antibiotic may be ineffective unless pus is drained, septic shock treated and hypoxia and anemia corrected. There are several conditions in which chemotherapy alone cannot eliminate an infection. Obstructive lesions can cause infection to recur, unless they can be dealt with surgically. Also, chemotherapy cannot obviate the necessity for draining an abscess or removing sequestra or calculi. Failure of treatment can also be due to a super-added infection, e.g. phlebitis, development of resistance during therapy or poor tissue penetration.
12. Laboratory control of the effects of treatment: Whether treatment has been successful or not is best judged by clinical criteria, but it is useful to know whether the infecting organism has been eliminated. Repeated cultures are, therefore sometimes indicated.
13. Reserve Antimicrobials: These reserve antimicrobials will be made available following a recommendation from the Microbiology Department as per culture report or if included in an antimicrobial policy for a clinical specialty that has been agreed with antibiotic management team. They are held in reserve to maintain their effectiveness in treating certain difficult infections by reducing the spread of microbial resistance and to encourage cost effective prescribing. Before a reserve antibiotic is issued to the ward, the pharmacist is instructed to ascertain the indication and if this falls outside the approved policy, to request that the prescriber consult the registered veterinarian/clinical microbiologist.
14. Alert Antimicrobials: To Prevent and Control the Emergence and Spread of Antimicrobial-Resistant Micro-organisms in Hospitals" one major strategic goal is to "define guidelines for use of key antibiotics", ("Alert" antibiotics) targeted in these guidelines are ciprofloxacin, ceftazidime, cefotaxime, ceftriaxone, vancomycin (or teicoplanin), imipenem, levofloxacin, meropenem, moxifloxacin,piperacillin-tazobactam, linezolid (oral/IV), voriconazole, caspofungin, valganciclovir, ertapenem and newer preparations of amphotericin. Collectively, these are among the drugs most frequently prescribed irrationally which is largely responsible for the current escalation of antibiotic

costs. They also account for a significant proportion of serious antibiotic toxicity including *Clostridium difficile* diarrhoea and CNS toxicity/seizures as well as the emergence of major antimicrobial resistance.

15. Educate farmers, veterinarians, and consumers on the dangers of antibiotic resistance Veterinarians, farmers, and consumers should be educated on appropriate use of antibiotics and the benefits of antibiotic-free meat.
16. Change incentives to discourage unnecessary antibiotic use in animals Subsidies and alternatives to antibiotics are necessary to offer incentives for farmers to decrease antibiotic use without causing economic harm.
17. Track rates of veterinary antibiotic use, resistance, and residues through a nationwide surveillance and monitoring system. Too little is known about antibiotic use and resistance patterns in India; the establishment of a nationwide surveillance system is required to inform policymaking.

CONCLUSIONS

Every country has a responsibility for maintaining antibiotic effectiveness. Successful efforts have direct benefits to local communities in the form of lower rates of antibiotic resistance, as well as to the global community and to future generations. New tools may make the job easier, but changing norms for antibiotic use and infection control are effective means of reducing unnecessary and inappropriate use. Local expertise and resolve are essential in every country. To date, it is mostly high-income countries that have established effective antibiotic use policies, but developing countries are also represented among the success stories. With global support, success should be achievable everywhere.

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ANTIBACTERIAL RESISTANCE

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Introduction

Antimicrobial resistance is the broader term for resistance in different types of microorganisms and encompasses resistance to antibacterial, antiviral, antiparasitic and antifungal drugs. Antimicrobial resistance is not new, but the number of resistant organisms, the geographic locations affected by drug resistance, and the breadth of resistance in single organisms are unprecedented and mounting (Meka and Gold, 2004). Diseases and disease agents that were once thought to be controlled by antibiotics are returning in new leagues resistant to these therapies. It should be stressed, however, that antimicrobial resistance is also visible in other microorganismsnamely, parasites, fungi and viruses (Ash, 1994). When the microorganisms become resistant to most antimicrobials they are often referred to as "superbugs". This is a major concern because a resistant infection may kill, can spread to others, and imposes huge costs to individuals and society.

AMR results in reduced efficacy of antibacterial, antiparasitic, antiviral and antifungal drugs, making the treatment of patients difficult, costly, or even impossible. The impact on particularly vulnerable patients is most obvious, resulting in prolonged illness and increased mortality. The magnitude of the problem worldwide and the impact of AMR on human and animal health, and on costs for the health-care sector and the wider societal impact, are still largely unknown.

Some estimates of the economic effects of AMR have been attempted, and the findings are disturbing. For example, the yearly cost to the US health system alone has been estimated at US $21 to $34 billion dollars, accompanied by more than 8 million additional days in hospital. Because AMR has effects far beyond the health sector, it was projected, nearly 10 years ago, to cause a fall in real gross domestic product (GDP) of 0.4% to 1.6%, which translates into many billions of today's dollars globally.

AMR is a complex global public health challenge, and no single or simple strategy will suffice to fully contain the emergence and spread of infectious organisms that become resistant to the available antimicrobial drugs. The development of AMR is a natural phenomenon in microorganisms, and is accelerated by the selective pressure exerted by use and misuse of antimicrobial agents in humans and animals. The current lack of new antimicrobials on the horizon to replace those that become ineffective brings added urgency to the need to protect the efficacy of existing drugs. The development and implementation of effective strategies to curtail the emergence and spread of AMR, and to evaluate the effect of interventions to do so, depend on the collection of accurate representative information on the extent of the problem and its impact (WHO, 2014).

Causes of drug resistance

Antimicrobial resistance occurs naturally over time, usually through genetic changes. However, the misuse and overuse of antimicrobials is accelerating this process. As underlined by the European Centre for Disease Prevention and Control (ECDC) they are three main types of misuse:

1. The unnecessary prescription of antibiotics for viral infections, against which they have no effect;
2. Too frequent prescription of "broad-spectrum antibiotics", in place of a better targeted antibiotic, through

more precise diagnosis;

3. The inadequate use by the patient, not respecting either dosage or duration of the treatment, which means that some of the bacteria may survive and become resistant

Some other cause, that increases the incidence of resistance in micro-organisms are excessive antibiotic use in animal husbandry is also creating some drug resistant bacteria, which can be transmitted to humans, Increased globalization along with increasing movement of man, animal and animal food also increases the spread of resistant organisms. Very Close association of animal and man also increases the chance of exchange of resistant infectious agents between them. Poor infection control, inadequate sanitary conditions and inappropriate food-handling encourage the spread of antimicrobial resistance. Millions of kilograms of antimicrobials are used each year in the prophylaxis and treatment of people, animals and agriculture globally (Mellon *et al.*,, 2001), driving the resistance problem by killing susceptible strains and selecting those that are resistant.

Further antibiotics are also used in animals as growth promoters and an alternative to hygiene and cleanliness in animal farms, this further ad to the development of resistance. In developing countries like India, antibiotics control is very lax, and almost all antibiotics are available as OTC drugs, further promotes the indiscriminate and careless use of antibiotics by animal owners. In this globalised word, were movement of animals and animal products are at its peak, the spread of resistant organisms is difficult to check.

How do bacteria acquire resistance?

Microorganisms were increasingly becoming resistant to ensure their survival against the arsenal of antimicrobial agents to which they were being bombarded. They achieved this through different means but primarily based on the chemical structure of the antimicrobial agent and the mechanisms through which the agents acted. The resistance mechanisms therefore depend on which specific pathways are inhibited by the drugs and the alternative ways available for those pathways that the organisms can modify to get a way around in order to survive. According to Fluit *et al*. (2001) following can be the mechanism of acquired resistance.

Mechanisms for acquired resistance

1. The presence of an enzyme that inactivates the antimicrobial agent
2. The presence of an alternative enzyme for the enzyme that is inhibited by the antimicrobial agent
3. A mutation in the antimicrobial agent's target, which reduces the binding of the antimicrobial agent
4. Post-transcriptional or post-translational modification of the antimicrobial agent's target, which reduces binding of the antimicrobial agent
5. Reduced uptake of the antimicrobial agent
6. Active efflux of the antimicrobial agent
7. Overproduction of the target of the antimicrobial agent

Changes in bacterial genome through mutation or horizontal gene acquisition, lead to a change in the nature of proteins expressed by the organism. Such change may lead to an alteration in the structural and functional features of the bacteria involved, which may result in changes leading to resistance against a particular antibiotic. This is referred to as acquired resistance, which is limited to selected isolates of that particular species or group of microorganisms. For example, we know that methicillin resistance of *Staphylococcus aureus* is primarily due to changes that occur in the penicillin binding protein (PBP), which is the protein which

beta-lactam antibiotics bind and inactivate to consequently inhibit cell wall synthesis. This change is actually rendered by the expression of a certain mecA gene in some strains of these bacteria, which is said to have been induced by the excessive use of penicillin. Expression of this mecA gene results in an alternative PBP (PBP2a) that has a low affinity for most ß-lactam antibiotics, thereby allowing these strains to replicate in the presence of methicillin and related antibiotics (Enright,2003).

Some antimicrobial resistance is brought about by multiple changes in the bacterial genome. For example, Isoniazid resistance of Mycobacterium tuberculosis results from changes in the following genes: katG gene which encodes a catalase; inhA gene which is the target for isoniazid; the oxyR gene and neighboring aphC gene and their intergenic region.

In the absence of plasmids and transposons (which generally mediate high-level resistance), a step-wise progression from low-level to high-level resistance occurs in bacteria through sequential mutations in chromosomes (Wang *et al*., 2001,). This process was responsible for the initial emergence of penicillin and tetracycline resistance in *N. gonorrhoeae*. The organism later acquired transposons bearing genes with high-level resistance to these drugs. Strains of *E. coli* and other Enterobacteriaceae have evolved increasing resistance to fluoroquinolones, the result of mutations in the target enzymes (topoisomerases) and an increase in the expression of membrane proteins that pump the drugs out of the cell (Schneiders *et al*., 2003).

Resistance to ß -Lactam group of antibiotics

Resistance to ß -lactams in many bacteria is usually due to the hydrolysis of the antibiotic by a beta-lactamase or the modification of PBPs or cellular permeability. b-Lactamases constitute a heterogenous group of enzymes which are classified according to different ways including their hydrolytic spectrum susceptibility to inhibitors, genetic localization (plasmidic or chromosomal), and gene or amino acid protein sequence (Bush *et al*.,1995)

Resistance to Tetracycline group of antibiotics

Tetracyclines are another of the very commonly used antimicrobial agents in both human and veterinary medicine in developing countries because of their availability and low cost as well as low toxicity and broad spectrum of activity. The tetracyclines were discovered in the 1940s. They inhibit protein synthesis by preventing the attachment of aminoacyl-tRNA to the ribosomal acceptor (A) site. They are broad-spectrum agents, exhibiting activity against a wide range of gram-positive and gram-negative bacteria, atypical organisms such as chlamydiae, mycoplasmas, and rickettsiae, and protozoan parasites. Examples of these include drugs such as tetracycline, doxycycline, minocycline, and oxtetracycline. Resistance to these agents occurs mainly through three mechanisms (Roberts, 1996), namely

1. Efflux of the antibiotics,
2. Ribosome protection, and
3. Modification of the antibiotic

Efflux of the drug occurs through an export protein from the major facilitator superfamily (MFS). These export proteins are membrane-associated proteins which are coded for by tet efflux genes and export tetracycline from the cell. Export of tetracycline reduces the intracellular drug concentration and thus protects the ribosomes within the cell.

Ribosome protection occurs through ribosome protection proteins that protect the ribosomes from the action of tetracyclines (Taylor and Chau, 1996). Ribosome protection proteins are cytoplasmic proteins that bind to

the ribosome and cause an alteration in ribosomal conformation which prevents tetracycline from binding to the ribosome, without altering or stopping protein synthesis. Modification of the antibiotic on the other hand occurs through enzymatic alteration of the drugs. Some of these genes are coded for by tet (X) genes.

Resistance to Chloramphenicol antibiotics

Resistance to chloramphenicol is generally due to inactivation of the antibiotic by a chloramphenicol acetyltransferase (Traced *et al*., 1993). Various enzymes have been described and are coded for by the cat genes found in gramnegative and gram-positive bacteria and usually show little homology (Kehrenberg *et al*., 2001). Sometimes decreased outer membrane permeability or active efflux is responsible for the resistance in gram-negative bacteria (Butaye *et al*., 2003).

Resistance to aminoglycoside group of antibiotics

Resistance to aminoglycosides such as gentamicin, tobramycin, amikacin,and streptomycin is widespread, with more than 50 aminoglycoside-modifying enzymes described (Schmitz and Fluit, 1999). Most of these genes are associated with gram-negative bacteria. Depending on their type of modification, these enzymes are classified as aminoglycoside acetyltransferases (AAC), aminoglycoside adenyltransferases (also named aminoglycoside nucleotidyltransferases [ANT]), and aminoglycoside phosphotransferases (APH) (Shaw *et al*., 1993). Aminoglycosides modified at amino groups by AAC enzymes or at hydroxyl groups by ANT or APH enzymes lose their ribosome-binding ability and thus no longer inhibit protein synthesis. Besides aminoglycoside-modifying enzymes, efflux systems and rRNA mutations have been described.

Common antibiotic resistant bacteria

Antibiotic-resistant bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase producers, and carbapenem-resistant Enterobacteriaceae, are increasing in prevalence worldwide, resulting in infections that are difficult and expensive to treat.

Methicillin-resistant *Staphylococcus aureus*:

MRSA is a common pathogen responsible for skin and soft tissue infections, severe bloodstream infections, and pneumonia. MRSA was once a predominantly hospital acquired infection but in recent years has been increasingly found in community-onset infections. The proportion of *S. aureus* that is resistant to methicillin has declined in Europe and the United States over the past eight years, from 22 to 18 percent and from 53 to 44 percent, respectively. However, in India, a steep increase in MRSA, from 29 percent of *S. aureus* isolates in 2009 to 47 percent in 2014 (CDDEP 2015).

Extended-spectrum beta-lactamase producers

Extended-spectrum beta-lactamases (ESBLs) are a family of enzymes, produced by Gram-negative bacteria, that confer resistance to some of the world's most widely prescribed antibiotics (Reuland *et al*. 2014). ESBLs can inactivate all penicillins and cephalosporins, including third generation cephalosporins (e.g., ceftriaxone, cefotaxime, and ceftazidime) and monobactams (aztreonam). In Europe, 17 of 22 countries reported that 85 to 100 percent of *E. coli* isolates were ESBL positive. In United States, ESBL-producing Enterobacteriaceae made up 14 percent of *E. coli* isolates and 23 percent of *K. pneumoniae* isolates. In New Zealand, ESBL-producing Enterobacteriaceae incidence increased from 10 people per 100,000 population in 2000 to 213 per 100,000 in 2013 (Heffernan and Woodhouse 2013).

New Delhi metallo-beta-lactamase 1

New Delhi metallo-beta-lactamase 1 (NDM-1) is a genetic element with multiple resistance genes that can be

harbored by and transmitted between Gram-negative bacteria, originally identified in a Swedish patient returning from New Delhi, India, in 2008. NDM-1 is highly resistant to most antibiotics except polymyxins Moellering 2010). E. coli and Klebsiella spp. carrying NDM-1 now account for the majority of carbapenem esistance in some countries (Pillai *et al*. 2011). From their original detection in 2008, NDM-1 carrying Enterobacteriaceae have been identified in more than 70 countries in all regions. NDM-1 has also been identified in environmental samples from water sources in India indicating that the gene is present in both community and hospital settings (Johnson and Woodford, 2013).

Clostridium difficile

Antibiotic treatment destabilizes the balance of intestinal microflora by killing off large numbers of bacteria, allowing *C. difficile*, which is naturally resistant to most antibiotics, to proliferate. *C. difficile* can be thought of as a serious adverse event related to antibiotic use, whether appropriate or inappropriate (CDC 2013; McDonald et al. 2012). The infection can be lethal, especially to elderly people and those with impaired immune systems or other serious comorbidities (Fridkin *et al*. 2014), and is responsible for more than 14,000 deaths and 250,000 infections per year in the United States (CDC 2013). Although hospitals are the source of most *C. difficile* infections, Antibiotic use increases the risk of *C. difficile* infections by seven- to 10-fold for up to one month after discontinuation (Brown *et al*. 2015; Hensgens *et al*. 2012). *C. difficile* can be treated with antibiotics and is not significantly resistant to the available drugs.

Managing resistance in man and animals

1. Develop awareness and understanding of antimicrobial resistance, its implications and actions to combat it in all stakeholders.
2. Implement rational use of antimicrobials in animal and human health practices. Ensure appropriate and judicious prescribing dispensing and administration of antibiotics
3. Develop national database for the use of antibiotics and resistance development.
4. At hospital and town level, frequent surveillance should be carried out keep an eye on antimicrobial resistance.
5. Improve infection prevention and control measures across human health and animal care setting to help prevent infections and the spread of antimicrobial resistance.
6. At national and international level discovery and development of new antibiotics should be taken at highest priority.
7. At human hospital and veterinary care centers utmost care should be taken for prevention of any possible spread of infection to susceptible individuals.
8. With proper hygiene and cleanliness the need for antibiotics and occurrence of infectious disease can be significantly reduces so extra care should be taken to maintain cleanliness surround the human and animal habitat.
9. Antibiotic resistance is a global problem and mutual partnership among the countries is essential to control this menace, so effort should be made to build the global partnership in this regard.
10. Stringent control should be impose on movement of sick human and animals from areas of high incidence of antibiotic resistance to prevent spared of same to other areas.

Conclusion

The global community has an ongoing and worsening crisis of antibiotic-resistant infections in man and animal. We cannot count on new antibiotics to save us from this crisis as development pipeline is almost empty. Since a long time, no new antibiotic has developed and in future, there is little possibility of any new breakthrough. We need to preserve the effectiveness of existing antibiotics and prolong their life as much as possible. We must therefore use antibiotics judiciously in human and veterinary practice. Along with judicious use as far as possible we need to adopt greater prophylactic and management practices to minimize the use of antibiotics. If timely care is not taken these resistant organisms could create havoc like pre antibiotic era.

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LEAD-AMR-03

SMART VETERINARY PRESCRIPTIONS ADDRESSING ANTIMICROBIAL DRUG RESISTANCE

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Antimicrobials are widely used for the treatment and control of infectious diseases as well as for growth promotion in animals. For the first 40 years of the antibiotic era, the fall of one antibiotic was followed by the discovery and rise of another. The last entirely new class of antibiotics to reach the market was discovered nearly 30 years ago. The excessive use of antibiotics over the last century has provided an evolutionary drive for bacteria to develop resistance to antibiotics and become fitter and potentially more deadly in some cases. Inappropriate or over use of antimicrobial drugs, its use in animals for production performance as well its entry in human or animal body by residue sources are some of the reasons responsible for pathogens resistant to antimicrobial drugs. The consequences of growing antimicrobial resistance increasingly making difficult to control infection in human and animals. In veterinary medicine, it will be more difficult to maintain animal health and protect animal welfare, potentially impacting food supply. An integrated approach is required to tackle antibiotic resistance as part of the 'One-Health' approach at national and international levels. This new 'One health' concept aims at strengthening the links between human and animal health and management of the environment in order to protect public health through the control of pathogens in animals at the interface of men/animals/the environment.It is high time to improve the knowledge and understanding of antimicrobial resistance. Prescribing practices need to be optimized through enhanced dissemination and implementation of best use of laboratory data and existing and new rapid diagnostics, with PK/PD data usage, with use of combination of antimicrobials, with the use of herbal therapeutics as growth promoters, with new antimicrobials developed from different sources and lastly with possible antimicrobial alternatives. This will definitively be solution initiative to control the danger of superbug.Prescription of antimicrobial drugs should be with the knowledge of relationship between pharmacokinetics and pharmacodynamics of the drugs (concentration independent versus concentration dependent) that determines the success of clinical cure of disease. Pharmacokinetics mathematically describes the relationship of antimicrobial drug concentration to time whereas pharmacodynamics describes the relationship of antimicrobial concentration to pharmacological effect or microorganism death. Important PK/PD parameters of a drug like peak to minimal inhibitory concentration (peak/MIC), the AUC to MIC ratio (AUC/MIC) and the time the drug concentration remains above the MIC(T>MIC) are suggestive while selecting drug for the treatment. Examples of concentration independent antimicrobials include: beta-lactams, macrolides, carbapenems, tetracyclines whereas the examples of concentration dependent antimicrobials include: fluoroquinolones, aminoglycosides and amphotericin B.

Enhancing use of laboratory data like analysis of antimicrobial sensitivity and MIC tests for guiding therapy, assisting infection control and characterizing resistant epidemiology are useful for controlling antimicrobial resistant bacteria. Treatment of repeated cases in challenging infection, prescriptions of antimicrobial drugs

should always be based on laboratory analysis of bacteria.

The use of double coverage (use of two antimicrobials) is based upon the following assumptions: the combination provides a broad spectrum of coverage for empiric treatment before the knowledge of identification and susceptibility of the causative pathogen. Such combination may provide additive or synergistic effects against the pathogens and also the combination of antimicrobials may decrease or prevent the emergence of resistant bacteria. Double beta-lactam combination should be avoided and also double anaerobic coverage is not necessary and put patient at risk for additional toxicities.

Animal agriculture system depends on thousands of tons of antimicrobial products per year to treat and prevent infections among livestock including cattle, pig and poultry. Furthermore, small doses of certain antimicrobials, used over long period of time increases the reasonable body mass of animals giving antimicrobials a new off-label use referred to as "increasing growth production and production efficiency" in the industry. This revolutionary discovery rapidly pushed the industry towards antimicrobial-centric production system. Almost all antimicrobials used in animal agriculture are identical to, or analogue of, human and veterinary antimicrobials. Because of this, it is likely that antimicrobials-pressured bacteria will become resistant to human and animal therapeutics. Prescription of herbal therapeutics for increasing growth production and production efficiency should be practiced.

The discovery and development of new drugs takes time (about 10 to 15 years) and the efforts to develop new antibiotics are not keeping pace with the growth in microbial resistance. Another barrier to developing new antibiotics is their relatively low commercial return on investment, relative to investments in other therapeutic areas. The good news is that with the availability of multiple types of antibiotics, we may have an opportunity to selectively withdraw certain drugs or at least to reduce their use by managing the simultaneous use of multiple types of drugs. The more types of drugs that are available, the more easily this can be done. Recently new antimicrobials invented from synthetic peptides or mines from human guts (Humymycin A and Humymycin B) and marine sponge (Darvinolide) have shown potential sensitivity towards certain deadly pathogens.

Antimicrobial alternatives have pivotal role in controlling resistance towards pathogens. In future prescriptions of such antimicrobial alternatives would be used in clinical practice. Engineered particles called "phagemids" , bacteriophages that attack bacteria but leave human and animal cells unscathed, engineering the gut bacterium *Escherichia coli* to produce peptides that kill *Pseudomonas aeruginosa*, a microbe that causes pneumonia, peptides with antibacterial activity isolated from frogs, alligators and cobras, PPMO(peptide-conjugated phosphorodiamidate morpholino oligomer) that disrupt the bacteria' genes, artificial bait for bacterial toxins (engineered artificial nanoparticles made of lipids, "liposomes"), Interleukin 10 (IL-10) , bacterial gene-editing enzymes, metals such as copper and silver, zinc chelator that upsets bacterial balance and vaccines are few examples of antimicrobial alternatives promising to control and spread of drug-resistant strains of pathogens.

AMR-01

ANTIBIOGRAM OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM BOVINE CLINICAL MASTITIS CASES IN NORTH GUJARAT

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Bovine mastitis is disastrous economic threat to dairy industry affecting livestock farmers across the globe. Appraising the emerging antimicrobial resistant strains of pathogens is most serious issue in mastitis therapy. *Staphylococcus aureus* is one of major bacterial pathogen responsible for the bovine mastitis. The present investigation was carried out to assess *in vitro* antimicrobial sensitivity of field isolates of *S. aureus* derived from clinical bovine mastitis cases in North Gujarat region, against eight commonly used antimicrobials in same region for the treatment of clinical mastitis. A total of 68 milk samples of clinical mastitis cases were collected from TVCC, Deesa, Veterinary College, Sardarkrushinagar, and Animal health division of Dairy Co-operatives and screened for the presence of *S. aureus* by cultural, colonial, microscopic and biochemical characteristics. HiStaph™ Identification Kit, a biochemical test kit, was used to differentiate species of genus *Staphylococcus*. Out of 68 clinical mastitis samples, 16 samples (23.53 %) were confirmed for presence of *S. aureus*. Antibiogram was determined by E-test (agar diffusion with epsilometer test) using HiComb MIC™ test or Ezy MIC™ strips, useful for quantitative determination of susceptibility of bacteria to antibacterial agents. Amoxycillin-clavulanate (co-amoxiclav) and ceftizoxime were least resistant antimicrobials with susceptibility of 81.25 and 68.75%, respectively. Order of susceptibility for other antimicrobials were as ampicillin-sulbactam (62.50%) > gentamicin (62.50%) > enrofloxacin (56.25%) > ceftriaxone (56.25%) > ciprofloxacin (43.75) > Tetracycine (18.75%).

AMR-02

TREATMENT EFFICACY AGAINST GASTROINTESTINAL PARASITES IN CAPTIVE WILD ANIMALS

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The emphasis on the study of parasitic disease manifestations in captive wild animals is equally important as domestic animals. The collected fresh faecal samples of herbivores, omnivores and carnivores inhabitant of Surat Municipal Corporation Zoo, Gujarat, were stored in the containers containing appropriate amount of 10% formalin and brought to the laboratory for the assessment of gastrointestinal (GI) parasitic status. Out of 69 faecal samples, 6 (8.69%) captive lives were found positive for different GI parasitosis. The faeces/

droppings of, sloth bear and a bird recorded coccidian oocyst, the starred tortoise, saras and peacock recorded nematode egg while monkey recorded protozoan cyst. After treatment with sulphadimidine for 5 days, the sloth bear become free from the condition GI coccidiosis while treatment failure was observed in the bird. Saras and Peacock successfully cleared the infection of GI nematode upon dosing with ivermectin while no effect was recorded in the Starred tortoise. The infected monkey constantly shed the protozoan cyst even after simultaneous treatment with ivermectin and metronidazole. The frequent observation of treatment regimen failure in the selected wild lives might be due to development of resistance against the drugs.

AMR-03

AN ANTIBIOGRAM PATTERN OF BACTERIAL ISOLATES OBTAINED FROM SUBCLINICAL MASTITIS (SCM) IN ORGANIZED DAIRY CATTLE FARM, ANAND

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A present study was aimed to advocate remedial measures of subclinical mastitis (SCM) by determining *in vitro* antibiotic sensitivity pattern of the isolated bacteria. SCM was determined among 86 dairy cattle (49 Holstein Friesian and 37 Jersey) in organized Jersey Bull Mother Farm (Indian Dairy Corporation Project) of Gujarat Agricultural University (now, it is Anand Agricultural University) in Anand, by using various direct (cultural isolation) and indirect tests. A total of 232 bacterial isolates were tested for their antibiotic sensitivity pattern against ten commonly used antibiotics. The number and per cent isolates sensitive to various antibiotics in descending order of frequency were as follow, pefloxacin 229 (98.70%), ciprofloxacin 227 (97.84%), chloramphenicol 224 (96.55%), gentamicin 222 (95.68%),erythromycin 210 (90.51%), streptomycin 194 (83.62%), oxytetracycline 182 (78.44%), cloxacillin 181 (78.01%), penicillin 160 (68.96%) and ampicillin 152 (65.51%). On the basis of drug sensitivity test, the highest numbers of isolates (more than 90%) was sensitive to pefloxacin, ciprofloxacin, chloramphenicol, gentamicin and erythromycin while more than 75 per cent of the isolates were moderate sensitive to streptomycin, oxytetracycline and cloxacillin whereas least sensitive to penicillin and ampicillin. Therefore, pefloxacin, ciprofloxacin, chloramphenicol, gentamicin and erythromycin must be given preferential consideration for mastitis therapy as has been indicated from the results of the present study.

AMR-04

INCIDENCE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN THE MILK OF SURTI GOAT

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Staphylococcus aureus is pathogenic to man and animals and it has been implicated in food borne outbreaks. Present investigation was carried out to judge incidence of *Staphylococcus aureus* in the milk of Surti goats reared at Livestock Research Station, NAU, Navsari as well as from two rural goat rearing pockets i.e. Amalsad and Dharampur. The pathogenic nature of the microorganism was delineated on the basis of identifying toxigenic gene, *mecA* sequence by performing PCR. Systematic bacteriological examination of total 179 Surti goat milk samples yielded 37 (20.67%) *S. aureus* isolates. All the 37 isolates *S. aureus* isolates were subjected to antibiotic susceptibility testing against 9 selected antibiotics by agar disc diffusion method. The results showed cent percent sensitivity to Amikacin, Ciprofloxacin, Enrofloxacin, Gentamicin and Streptomycin and lower levels of susceptibility of Kanamycin (78.38 %), Ampicillin (72.97%), Methicillin (48.65%) and Cephalexin (29.73%). However, *S. aureus* isolates were resistant to Cephalexin (62.16%), Methicillin (40.54%), Ampicillin (18.92%) and Kanamycin (2.70 %). All MRSA strains identified by antibiotic sensitivity test were subjected to PCR to detect methicillin resistant gene *mecA.* Agarose Gel electrophoresis revealed single compact band of 533 bp, indicative of successful amplification of target *mecA* gene from the genomic DNA of *S. aureus.* Out of 37 *S. aureus*, 15 isolates amplified 533 bp of *mecA* gene, result show that 40.54 per cent *S. aureus* isolates carried *mecA* gene.

AMR-05

IN VITRO ANTIBACTERIAL ACTVITY OF ETHANOL EXTRACTS OF LEAVES OF *ANDROGRAPHIS PANICULATA*, *OROXYLUM INDICUM*, *TERMINALIA BELLIRICA*, *HEMIDESMUS INDICUS, BIXA ORELLANA* AND BARK OF *CAREYA ARBOREA & FICUS RACEMOSA*

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The present study was planned to validate folklores claimed by tribal healers for antibacterial properties of various medicinal plants. Leaves and barks of plants were collected, shed dried and made ready to evaluate its antibacterial activity. Extracts were made using various solvents like hexane, chloroform, acetone, ethanol and water based on their increasing polarity. Ethanol from the crude extracts were evaporated and measured to make serial dilutions in 10% DMSO. Antibacterial efficacy was evaluated of these extracts using micro-broth dilution technique and viability of organism was checked by tetrazolium chloride dye keeping gentamicin and enrofloxacin as positive control. All the dilutions were made in triplicate. MIC values of *Andrographis paniculata* leaves against *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes and Proteus*

mirabilis were observed 2.56 mg/ml, 5.12 mg/ml, 5.12 mg/ml and 5.12 mg/ml, respectively. Ethanol extract of *Oroxylum indicum* leaves showed antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes* and *Proteus mirabilis* with MIC values 0.64 mg/ml, 1.28 mg/ml, 2.56 mg/ml and 2.56 mg/ml, respectively. MICs of *Terminalia bellirica* leaves against gram positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes* were observed 1.28 mg/ml, 1.28 mg/ml and 0.64 mg/ml, respectively and against gram negative bacteria *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Proteus mirabilis* were observed 2.56 mg/ml, 2.56 mg/ml, 2.56 mg/ml and 1.28 mg/ml, respectively. MIC values of *Hemidesmus indicus* leaves against *Bacillus subtilis*, *Streptococcus pyogenes* and *Proteus mirabilis* were detected 1.28 mg/ml, 2.56 mg/ml and 2.56 mg/ml, respectively. MICs of *Bixa orellana* leaves against *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Proteus mirabilis* were observed 0.32 mg/kg, 2.56 mg/kg, 0.08 mg/kg, 2.56 mg/kg, 0.32 mg/kg and 0.64 mg/kg, respectively. Ethanol extract of *Careya arborea* bark also showed antibacterial properties against *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes* and *Proteus mirabilis* with MIC values 1.28 mg/kg, 2.56 mg/kg, 2.56 mg/kg and 5.12 mg/kg, respectively. MICs of *Ficus racemose* bark observed against *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pyogenes* and *Proteus mirabilis* were 2.56 mg/kg, 5.12 mg/kg, 1.28 mg/kg and 2.56 mg/kg, respectively. In conclusion, this study validates the folklore claims of tribal healers regarding antibacterial properties of medicinal plants.

AMR-06

STUDY OF ANTIMICROBIAL RESISTANCE DUE TO ESBL PRODUCING *E.COLI* IN BROILERS

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Antimicrobial resistance, within a large range of infectious agents is a rising health risk of broad concern to countries and multiple sectors. In intensively reared poultry, antibiotics are administered to whole flocks rather than individual animals. In addition to this, poultry farmer also use low doses of antibiotics as growth-promoting substances, which result in the high antibiotic selection pressure for resistance with relatively high proportion of resistant bacteria in poultry faecal flora. ESBLs are a group of enzymes that break down beta lactam antibiotics like penicillin and cephalosporin groups and render them ineffective. ESBL has generally been defined as transmissible beta-lactamases that can be inhibited by clavulanic acid, tazobactam or sulbactam. In the present investigation prevelance of ESBL producing E.coli in broilers was studied in 400 samples collected randomly from various poultry sale outlets of Jabalpur. Prevalence rate was found to be 33.5 percent. Phenotypic and genotypic characterization of extended spectrum beta lactamase producing *E.coli* was also under taken in the positive samples by CDDT method, DDST method and Enzyme MIC strip method. 135 samples were positive for the same. In genotypic characterization and 76 samples were positive for bla TEM and bla SHV and bla CTX genes.

AMR-07

PREVALENCE OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING *ESCHERICHIA COLI* IN CHICKEN MEAT

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Extended-spectrum beta-lactamases (ESBLs) are variants of beta-lactamases that confer resistance to the cephalosporin antibiotics such as cefotaxime, ceftazidime, ceftriaxone and monobactams such as aztreonam. Production of ESBL is the most common mechanism of resistance among Enterobacteriaceae especially *Klebsiella pneumoniae* and *Escherichia coli.* ESBL producing *E.* coli are now being found in increasing numbers in animals and they might be an infection source or reservoir for spread of this organism. Currently, there is paucity of information regarding ESBL producing E. coli in food animals. Present experiment was performed to study the prevalence of genes coding for Extended Spectrum Beta lactamase gene in E coli isolates collected from chicken meat. The objective of the present study was to investigate the prevalence and genetic characterization of ESBL-producing *E. coli* in chicken meat. One hundred and five meat samples were collected from retail broiler shops. After enrichment and selective inoculation, E. coli were isolated and identified. E. coli isolates were screened for the production of ESBL by double disc diffusion method as per CLSI guidelines. Molecular detection of ESBL genes, bla TEM, bla SHV and bla CTX-M was carried out by PCR method. From the 105 chicken meat samples, 31 samples were found to be positive for *E. coli* and the prevalence rate of *E. Coli* in chicken meat was found to be 29.52%. Out of which, 16 isolates were found to be positive for ESBL production in the phenotypic screening. CTX, TEM and SHV genes were detected in 15 isolates (93.75%), 11 isolates (68.75 %) and 14 isolates (87.5 %), respectively. 62.5% isolates (10 isolates) showed the presence of all the three genes. This study showed the prevalence of ESBL producing E. coli in chicken meat and could be a potential source for the transfer of antibiotic resistant bacteria to human. Hence, surveillance programme are required to monitor ESBL producing organism and the spread of beta-lactamase resistance.

ISVPT-2016

TECHNICAL SESSION - VI

FOOD SAFETY / XENOBIOTIC RESIDUE

Chairperson : Dr. J. S. Sanganal

Co-chairperson : Dr. U. D. Patel

Rapporteur : Dr. P. K. Verma

NAVSARI AGRICULTURAL UNIVERSITY

LEAD-FS-01

ANTIBIOTIC RESIDUES: A GLOBAL HEALTH HAZARD

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The introduction of antibiotics to the Veterinary field started soon after the use of antibiotics for the treatment of bacterial diseases in humans. The main use of antibiotics in animal rearing was for the treatment and prevention of diseases. Indeed, antibiotics have been used for the treatment of mastitis, arthritis, respiratory diseases, gastrointestinal infections and other infectious bacterial diseases (Draisci *et al.* 2001). More recently antibiotics have been used for improved growth, especially in broilers and fatteners. Antibiotics also favor growth by decreasing the activity of the immune system, reducing the waste of nutrients, and reducing toxin formation. In most cases, however, only young growing animals and poultry are responsive to antibiotic-mediated health maintenance (Nisha, 2008).This approach actually is problematic as these feed additives are usually used without prescription and for very long periods, in both large and small doses, which leads to drug residues entering animal-derived food.

Food borne diseases caused by microbial agents, biotoxins and chemical pollutants constitute a serious public health problem (FAO/WHO, 2004). Among the chemical pollutants found in food, antibiotics residues occupy a prominent place (Votimir *et al.*, 2011). Serious food borne infections of epidemiological proportions have been reported globally in the past decades, showing their importance both for public health and social plan. Worldwide, consumers are increasingly concerned about these epidemics such as bovine spongiform encephalopathy, avian influenza etc. (Lantier *et al.*, 2004). The anarchic (lawless) use of antibiotics for therapeutic purposes or as a growth promoter in lives in livestock is causing serious problems associated with the presence of these residues in food animals such as milk, meat, eggs and fish (Catery *et al.*, 2003; Ben-Madhi and Ouslimani, 2009).

The frequent use of antibiotics in clinical practice causes the occurrence of antibiotic residues in various food products of animal origin including milk, egg and meat. Presence of drugs or antibiotics residues in food above the maximum acceptable level has been recognized worldwide by various public authorities (Kempe and Verachtert, 2000). The antibiotic contamination of milk was reported to be due to intramammary infusions of antibiotics for treating mastitis (92%), injections (6%) and other causes 2% (Booth, 1998). In case of eggs, the pattern of appearance of drug residues will be influenced by the formation of yolk and white. Presence of antibiotic residues in milk produces difficulties in validation of certain quality control tests (Mishra *et al*, 2011). For human concern, antibiotic residues in food of animal origin produces potential threat to direct toxicity in human (cancers, allergic reactions, etc.) and low levels of antibiotic exposure results in alteration of microflora, and the possible development of resistance (Ahaduzzaman, 2014) which cause failure of antibiotic therapy in clinical situations.

DRUG RESIDUES IN FOOD

Antibiotics are commonly used for therapeautic purpose then how can be these antibiotics are dangerous. The

answer is complex, as is the subject and reflects a number of factors. The first is that toxicity can be the result of a single, large, acute exposure or can result from long-term exposure to much lower concentrations. Whether an acute or chronic toxicity will be observed reflects the exposure dose, the nature of the toxicity, pharmacokinetics, and the exposed population. While exceptions abound, in general long-term exposure can result in toxicity following a lower concentration in the diet than would be seen from a single acute exposure. Regulatory agencies typically anticipate that Veterinary drug toxicity, including antibiotics, will generally result from long-term, low-level exposure in the diet. As will be discussed later, the bulk of the approach to establishing the safety of any given concentration of antibiotic residue for the human diet is based on chronic exposure. Adverse impacts on the human consumer resulting from years, or even decades, of exposure to residues of a veterinary antibiotic in the food would be very difficult to trace back to the source of the problem. On the other hand, there are a few examples where Veterinary drug residues can cause an acute toxicity, sometimes from a single meal.

ANTIMICROBIAL RESIDUES IN MILK AND MEAT

Antibiotic residues occur in milk supplies throughout the world. In the US, public health is protected by regulations that prohibit the presence of antibiotics in milk intended for human consumption UCM (2011) and the dairy industry bears the primary responsibility for ensuring the safety of milk and milk products. The FDA is responsible for verifying that the industry is complying with regulations and initiates regulatory action when necessary. Individual bulk milk samples from every farm are tested once monthly, 4 times in every 6 month period. Additional random testing for other drug classes is also performed and individual state regulatory agencies or individual milk processors may test more frequently. Results of official drug testing are compiled annually in the National Milk Drug Residue Database The prevalence of positive antibiotic test results for bulk milk tankers has been steadily declining and about 70% less milk was discarded because of the presence of antibiotic residues in fiscal year 2012.

PUBLIC HEALTH IMPORTANCE OF ANTIBIOTIC RESIDUES IN FOODS

Antibiotic residues in milk that is used to produce fermented products can interfere with the fermentation process by affecting desired lactic acid bacteria. Normally this is just a technical problem resulting in financial loss, but, when it occurs, pathogens present in the milk may grow and pose a health hazard later. For these reasons many countries have regulations prohibiting the sale of milk from cows being treated for mastitis and milk is routinely tested for the presence of antibiotic residues (Nisha, 2008). Disruption of normal human flora in the intestine is another harmful effect of drug residues in human food. The bacteria that usually live in the intestine act as a barrier to prevent incoming pathogenic bacteria from becoming established and causing disease. Antibiotics might reduce total numbers of these benign bacteria or selectively kill some important species (Myllyniemi *et al*, 2000).

ENVIRONMENTAL ISSUES RELATED WITH ANTIBIOTIC RESIDUES

Subject to the type of animal production system being considered, antimicrobial agents used in the livestock industries may enter the environment (Boxall, 2010). In the case of manure or slurry, which is typically stored before being applied to land, anaerobic degradation of antimicrobials occurs to differing degrees during storage. For example, â-lactam antibiotics rapidly dissipate in a range of manure types whereas tetracyclines are likely to persist for months. Compared to the situation in manure or slurry, the degradation of

antimicrobials in soil is more likely to involve aerobic organisms. In fish production systems, medicated food pellets are added directly to pens or cages to treat bacterial infections in fish. This practice results in the sediment under cages becoming contaminated with antimicrobials (Bjorklund, 1991).

More recently, the literature has described tetracycline (Boxall *et al*; 2006) and chloramphenicol (Berendsen *et al*; 2010) produced by soil organisms being taken up by plants. This raises the possibility that food-producing species may consume naturally derived antimicrobials when grazing herbs and grasses.

Risks of antibiotics in the environment to human health

Measured concentrations of pharmaceuticals in water and crops in the studies described above, typically result in exposures that are well below human therapeutic dose levels and acceptable daily intakes (ADIs) (Boxall *et. al*, 2006). However, there is concern among the scientific and regulatory communities and the general public that exposure to pharmaceuticals, including antibiotics, in the environment may affect human health. These concerns arise from the following facts:

- Individual antibiotics do not occur in the environment on their own but occur as a mixture, which introduces the possibility of synergistic or additive interactions or environmental contraindications between an environmental residue and a medicine taken by a patient for an existing condition.
- Humans will be exposed to antibiotics via a number of routes, whereas most risk assessment studies have considered only one route of exposure.
- Degradation processes, particularly in drinking water treatment processes, may result in transformation products that may be of greater health concern than the parent compound. For example, some pharmaceuticals with amine functionality are possible precursors for nitrosamineswhich can be mutagenic and carcinogenic (Krasner, 2009).
- Indirect effects of residues in the environment, such as the selection of antibiotic-resistant microorganisms, cannot currently be ruled out (Byrne-Bailey, 2009).

DETECTION AND DETERMINATION OF ANTIBIOTIC RESIDUES

Maximum residue limits (MRLs) for residues in food are recommended to the FAO/WHO food-standards-setting body, the Codex Alimentarius Commission (CAC). Established in 1963, the CAC sets non-binding food standards with the goal of protecting consumer health while ensuring fair international trade. The CAC, on reaching international consensus among its attending members, sets international standards for the maximum residue of the Veterinary drug (or pesticide) that may be contained in food (the MRL). While these standards are recommendations, they are also the principal food standards recognized by the 1995 World Trade Organization Agreement on Sanitary Phytosanitary Measures.

Setting Residue Concentrations Not Allowed in Food

National and regional authorities responsible for the protection of public health must consider the concentration of residues of Veterinary drugs, pesticides, and other chemicals that may be in food regardless of whether the substance is allowed for that use. In many regions, in the absence of an approval for the substance, the concentration of residues allowed in food is considered to be zero. In practical terms, this is frequently defined by the technical capability of the analytical method. Attempts to improve on "zero" include the ALARA (as low as reasonably achievable) approach, which recognizes that absolute zero is unattainable and describes an approach that considers what is technically achievable, the resources needed to achieve that

technical goal, and the benefit gained.

Setting Residue Concentrations Allowed in Food

Residues are evaluated to determine the extent of uptake of the Veterinary drug, its distribution throughout the body, and its elimination. Normally, contemporary residue depletion studies establish tissue concentrations in a radio labeled drug study, in which total residues and parent compound are determined at several pre-determined times between zero time and a time beyond the proposed withdrawal time. As well as total residues, which include free and bound components, the study quantifies major metabolites. These are compounds contributing 10% or more of total radioactivity or that are present at a concentration of =0.10 mg/kg. Biotransformation studies enable identification of the marker residue and target tissue. The marker residue must give assurance that, when its concentration is at or below the MRL, total residues satisfy acceptable daily intake (ADI) requirements.

Both toxicological ADI and MRL/tolerance are linked through biotransformation studies in the laboratory animal species used to determine the toxicological NOEL, as well as each food-producing animal species. A qualitatively similar metabolite profile between the laboratory animal species and the target species ensures the validity of the toxicology studies, by demonstration of broadly similar exposure to the same range of compounds. Comparative biotransformation studies involve analysis of metabolites in blood and its fractions, excreta, kidney, fat, liver, and bile in both the target and laboratory animal test species.

As a consequence, both MRLs and tolerances may be considered as derived food safety standards. However, the exact way in which this connection back to the ADI is made differs for MRLs and tolerances. Table-1 represents the Maximum Residue Limit (MRL) for some Veterinary residues.

Table-1: Maximum Residues Limit (MRL) for veterinary residues.

ANTIBIOTIC	MRL(µg/kg)
Benzyl penicillin	4
Ampicillin	4
Amoxycillin	4
Oxacillin	30
Cloxacillin	30
Dicloxacillin	30
Tetracycline	100
Oxytetracycline	100
Chlortetracycline	100
Streptomycin	200
Dihydrostreptomycine	200
Gentamicin	200
Neomycin	100
Sulphonamides	100
Trimethoprim	50
Spiramycin	200

ANTIBIOTIC	MRL(µg/kg)
Tylosin	50
Erythromycin	40
Quinolones	75
Polymyxin	50
Ceftiofur	100
Cefquinome	20
Nitrofurans	0
Nitromidazoles	0
Other chemotherapeutics (Chloramphenicol, Novobiocine)	0
Benzyl penicillin	4
Ampicillin	4
Amoxycillin	4
Oxacillin	30
Cloxacillin	30
Dicloxacillin	30
Tetracycline	100

Method of detection and determination of Antibiotic residues

The widespread use of antimicrobial compounds in animal husbandry and the stringent food safety legislation demand the availability of rapid and sensitive screening techniques for residue detection. For these reasons there is an increased need for rapid, easy-to-use, reliable, cost effective and broad-spectrum screening methods, which can be readily implemented in survey, surveillance and compliance monitoring schemes. Another important aspect to consider when choosing to introduce a screening method is the extent of assay compliance with internationally recognized validation criteria. When choosing to implement detection or screening assays for antibiotic residue, the laboratory must ensure that the performance criterion is fit for the intended purpose and in line with the appropriate legislative requirements within that country or the country for which the testing is undertaken.

Because of the diverse physicochemical properties of antimicrobial compounds, a variety of analytical techniques are commonly employed to screen for their residues in food of animal origin.

Detection and/or determination of antibiotic residues in food fall into two categories:

(1) Screening methods such as microbial inhibition tests and rapid test kits

(2) Quantitative and/or confirmatory methods, including gas chromatography with electron capture, flame ionization, or mass spectrometry detection, as well as liquid chromatography (LC) with ultraviolet (UV), fluorometric or electrochemical detection or mass spectrometry (MS).

Microbial inhibition assays (MIAs) are routinely used screening techniques offering the advantage of detecting the total biological activity associated with unknown residues (non-targeted analysis). Microbial inhibition assays are the methods of choice when an estimate of antimicrobial potency is required (Stead D.A., 2000).The MIAs are sensitive to compounds that inhibit or disturb the growth of a test microorganism. For these reasons microbial inhibition assays are a widely employed screening method for determination of the presence or absence of antimicrobial residues in milk, animal tissues and food products. A negative secondary screening result indicates the presence of one (or more) classes of antimicrobial compound in the sample. Positive samples resulting from the secondary screening assays may be rapidly directed to the appropriate quantitative/ confirmatory chemical analysis, such as liquid chromatography coupled to a tandem mass spectrometer (LC-MS/MS).

Enzyme-Linked Immunosorbent Assay (ELISA) is the commonest type of immunoassay. The ELISA technique was developed through the pioneering work of Engvall and Perlmann (1971) in the 1970s. By immobilizing the reagents to a surface, the facile separation of bound and unbound material is achieved, making ELISA a powerful tool for the measurement of analytes in crude sample preparations. ELISA has become the basic immunoassay on which many of the modern assay formats are based. In its simplest form, ELISA is the direct competitive assay in which the antigen is bound to the solid surface. ELISA offer numerous advantages over other immunoassay techniques because the end signal is amplified by the formation of a large number of product molecules. Some laboratory offers the most complete portfolio of sensitive and easy to use veterinary drugs residue test kits for the detection of drug residues in various samples) e.g., tissue, muscle, urine, milk, milk, egg, honey and feed samples. The analyte specific test kits use ELISA technology and have low detection limits.

Surface Plasmon Resonance (SPR) Biosensor Technology is also a technique for the detection of antibiotic

residues in foods of animal origin with the help of biosensors. The term biosensor describes a device that responds to analyte(s), and can interpret concentration as an electrical signal via a combination of a biological recognition element (BRE) and an electrochemical transducer. The earliest biosensors were catalytic systems that integrated bioreceptors, that is, enzymes, cellular organelles, or microorganisms with transducers to convert the biological response into digital electronic signals (Turner A.P.F., 2000). When biological interactions take place, changes in other physiochemical parameters, including enthalpy, ionic conductance, and mass, also occur. Such effects can be exploited by coupling the biocatalytic reaction with a transducer (Higgins and Lowe, 1987). Optical, electrochemical, thermometric, piezoelectric, and magnetic transducers are all commonly used transduction mechanisms. The application of optical, surface plasmon resonance (SPR) biosensors for the detection of antibiotic residues in foods of animal origin. SPR offers the advantage of detecting the specific binding event between a target and a recognition element without the use of the enzyme labels or fluorescent tags required by the majority of immunochemical techniques (Karlsson, 2004). SPR can provide information about the concentration, binding specificity, binding affinity, kinetics and cooperativity of a target molecule.

Liquid chromatography and mass spectrometry (LC-MS) are methods for detection of pharmaceutically active compounds. It was first developed in the mid-1990s by Ternes group at the Institute for Water Research and Water Technology, Wiesbaden, Germany (Ternes *et al*., 1998). Several antibiotics, beta-blockers, X-ray, contrast media and neutral drugs in different water matrices were identified by using GCMS and LCMSMS.

Solid-phase extraction method (SPEM) is the most common method for sampling prior to LCMSMS analysis. Stoob *et al*, 2005 described the use of an on-line SPELCMSMS system which includes three-directional autosampler, three LC pumps with large volume dispenser, two six-port valves (Lotto and Purves 1999). An important aspect of method validation and procuring consistent result, precision and detection limits are determined by batch to batch performance of methods (Langeveld *et al*., 2008).

With the advent of *Mass spectrometry* based method for identification and quantification of organic compounds, detection of antibiotics in environment had become much easier and cost effective. The method is an inevitable tool for detection of trace amount of organic moieties and providing unambiguous identification by library search as well as quantification with reference to standard. The combination of liquid chromatography and mass spectrometry in late nineteen-eighties with highly selective and sensitive tandem mass spectrometry (MSMS) has made LCMS and/or LCMSMS as the best tool for identification and quantification of antibiotics. The greatest achievement with mass spectrometry over the other spectrometry such as NMR lies with its requirement of minute quantity of sample and the requirement of derivatization step for the analysis made the GCMS method more time consuming and labour intensive.

GUIDELINES FOR RESIDUES BY REGULATORY AUTHORITIES

All advanced and several emerging economies have well established, legally binding procedures for evaluating applications for marketing authorizations (MAs) for VeterinZary medicinal products (VMPs). In the case of the EU of 27 member states, as well as the supranational authority, there are also national authorities; MAs can be obtained through four possible channels: centralized, decentralized, mutual recognition, and a solely national channel. For products containing anti-microbial drugs, all authorities require the submission of data packages that establish their quality, safety, and efficacy (QSE). Considerable progress has been made in harmonizing

QSE registration requirements in the form of guidelines, at international level under the auspices of VICH.

INTERNATIONAL HARMONIZATION

There have been considerable international efforts to harmonize evaluation of the safety of veterinary drug residues. The International Cooperation on Harmonization of Technical Requirements for Veterinary Products (VICH) was established in 1996 under the auspices of the World Organization for Animal Health, formally known as Office International des Epizooties, which has retained its historical acronym (OIE). VICH incorporates representatives of both government and industry of VICH member states (EU, Japan, and USA) and observers (Australia, Canada, and New Zealand).

The program addresses a broad scope of requirements for the approval of veterinary drug products by the national authorities, including what would be needed to show safety for residues of the veterinary drug in food. Agreement has been reached for a number of requirements for the toxicology needed to establish the safety of veterinary drug products for the human consumer. The Codex Alimentarius, created by the United Nations Food and Agriculture Organization (FAO) and World Health Organization (WHO), establishes maximum residue limits (MRLs) for the residues of veterinary drugs in food; the MRLs serve as international standards for safety setting an upper concentration for residues of veterinary drugs in foods in international trade (Codex Alimentarius Commission, 2010) .

The Joint FAO/WHO Expert Committee on Residues for Veterinary Drugs in Food (JECFA) performs independent expert toxicological evaluations used to establish an acceptable daily intake (ADI) of residues of the veterinary drug for the human consumer and residue evaluations that result in MRLs recommended to the CAC for their consideration. The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) performs similar evaluations for pesticides, which may include antimicrobial compounds used in fruit and vegetable production and also recommends MRLs to the CAC. In the United States, the USDA Foreign Agriculture Service offers a consolidated database of international MRLs for pesticides and veterinary drugs (USDA, 2010).

RESIDUE VIOLATIONS: THEIR SIGNIFICANCE AND PREVENTION

Residue violations may occur as a consequence of the use of drugs and pesticides or from environmental contaminants and naturally occurring toxicants in foods. Drugs (including pesticides registered for veterinary use) are the most commonly detected chemicals in animal-derived foods and, of these, a large majority of positive findings are AMDs. Dowling (2006) has outlined the roles of the US Department of Agriculture's Food Safety and Inspection Service (FSIS) and the Canadian Food Inspection Agency (CFIA) in monitoring meat, poultry, eggs and honey for residues of chemicals, including AMDs. FSIS monitors tissues through its National Residue Program (NRP). Both agencies utilize hazard analysis and critical control point (HACCP)-based systems as the basis for conducting risk analyses.

Annually, FSIS and CFIA analyze approximately 300,000 and 200,000 samples, respectively, from all market classes of food-producing animals. When a noncompliant residue is detected in a slaughter animal or food animal product, it is condemned. FSIS informs USFDA of residues violations and seeks to obtain the names of producers of products and/or to identify other parties offering animals or products for sale. Appropriate action by the federal agency may include follow-up inspections, seizure and recall of products, and, on the basis of a surveillance plan, further sampling. The action taken depends on the magnitude of the health risk and emphasis is placed on avoidance of any repeat occurrence and/or further distribution of products.

Croubels *et al.* (2004) list problem compounds as sulfonamides, tetracyclines, nitroimidazoles, nitrofurans, nicarbazin and ionophore coccidiostats. In some jurisdictions, products can be used in a non-approved species, under the responsibility of the prescribing veterinarian and based on estimation of a suitable withholding time (WhT). Such recommendations may be based on estimations lacking sufficient accuracy. Gehring *et al.* (2006) have discussed the application of risk management principles to the extra-label (off-label) use of drugs.

RECOMMENDATIONS FOR THE REDUCTION OF ANTIBIOTIC CONTAMINATION IN FOOD

There is no doubt that neither humans nor animals can live without antibiotics as they are some of the most effective antimicrobial treatments. However, at the same time, the misuse of antibiotics may result in the various health hazards. Thus, the reduction of antibiotic use constitutes a challenge for the world. In order to achieve such a reduction, the following steps should possibly be considered with regard to all antibiotics:

- The effective prevention of infectious diseases and the adoption of strict hygiene standards and rearing skills may reduce our need for antibiotics, particularly in the veterinary field.
- The use of alternatives to antibiotics, such as plant-derived antimicrobial substances and probiotics, may represent a promising option; vaccination against some bacterial diseases may be of great value in the near future.
- The reduction of unnecessary antibiotic use in animals in captivity should be pursued, as should antibiotic use for the treatment of viral disease in animals; the reduction of prophylactic antibiotic use should also be considered.
- Strict national legislation must be passed around the world to avoid the unnecessary use of antibiotics.
- National monitoring of antibiotic residues in foods and updating of the maximum permissible limits of these residues for each country should be undertaken.
- Antibiotics use in feed additives should be ceased.
- Avoid using antibiotics in the veterinary field without a veterinarian's prescription.
- Strict observation of antibiotic cessation times should be made; the avoidance of antibiotics lacking clearly documented pharmacokinetic and pharmacodynamic properties must be considered.
- The heat treatment of meat, milk, and eggs may inactivate antibiotic contaminants in feedstuffs.
- The freezing of animal-derived foods may also contribute to the reduction of some antibiotic contamination.

CONCLUSION

Antimicrobial drug use in food animal production is fundamental to animal health and well-being and to the economics of the livestock industry. Therefore the prudent use of antimicrobials is critically important because few new drugs are entering the market, and existing uses need to be preserved for as long as is practicable. Prudent use will minimize the development of antimicrobial resistance and maximize therapeutic effect. When introducing new products onto the market, pharmaceutical companies need to rule out the presence of cross-resistance to old products in the same class, some of which may no longer be used in animals. From a food safety perspective, responsible use of antimicrobials in food-producing species as reflected by the results of residue-monitoring programs is of paramount importance to reassure the community that the food supply is safe.

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METABOLOMICS: A NEW FRONTIER IN FOOD SAFETY AND QUALITY

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Introduction

Metabolomics, the study of ''as-many-small-metabolites-as-possible'' in a system, has become an important tool in many research areas. Metabolomics is a science of interest in food analysis to describe and predict properties of food products and processes (Cevallos-Cevallos *et al*., 2009).

It includes the development of analytical methods with the ultimate goal being the identification of so-called 'quality markers' (*i.e.* sets of metabolites that correlate with, for example, quality, safety, taste, or fragrance of foodstuffs). In turn, these metabolites are influenced by factors such as genetic differences of the raw food ingredients (such as animal breed or crop species differences), growth conditions (such as climate, irrigation strategy, or feeding) or production conditions (such as temperature, acidity, or pressure). In cases where the routine-based measurement of a food property faces some limitations such as the lack of knowledge regarding the target compounds to monitor, monitoring based on a limited set of crucial biomarkers is a good alternative, which is of great interest for food safety purposes regarding growth promoting practices (Dervilly Pinel *et al*., 2012).

Two important aspects of metabolomics include targeted and non-targeted categorisation of metabolome. The targeted metabolites need accurate identification and quantification (Ramautar *et al.*, 2009). The identification of targeted metabolites / analyses is important for assessing the behaviour of a specific group of compounds in the sample under determined conditions. Untargeted metabolomics do not need to be accurate

identification and quantification. Their presence in samples with distinct patterns provides avenues for chemical finger printing of samples (Monton and Soga, 2007).

The Metabolomics studies applied for food quality and standard are broadly classified as discriminative, informative, and/or predictive. Discriminative analyses are applied for understanding differences between sample populations without necessarily creating statistical models or evaluating possible pathways that may elucidate such differences. Informative metabolomic analyses are directed at identification and quantification of targeted or untargeted metabolites to obtain sample intrinsic information. Predictive metabolomics help to formulate a predictive model to forecast behaviour of variable in sample analysis (Wishart, 2008).

The blending of modern data handling techniques and metabolomic studies has very good and promising future. This would provide unprecedented opportunities in targeted and untargeted analysis as well as databasing. The instrumental screening of samples of foods of animal origins generates huge amount of data in residue and contaminant analysis. Such data can be easily handed and processed using modern software tools using univariate/ multivariate statistics. Selections of signals can be used for further identification. This approach has been adopted for identification of metabolites in urine of calves treated with the natural prohormone DHEA, elucidation of mechanism of action endocrine disruptors in the human H295R adrenocarcinoma cell line and identification of paracetamol metabolites in urine of humans treated with dosages of paracetamol well below maximum daily allowed dosage (Lommen, 2011).

Metabolomics in food quality

Targeted metabolomics have found great potential for application in monitoring of quality of agricultural and dairy foods. Contamination of food during production, processing and packaging can be easily monitored. Pre-harvest and post harvest contamination can be easily profiled and detected using metabolomics. The recent addition of IMS (immunomagnetic separation and concentration) provides monitoring of food quality parameters by allowing *in situ* automatic sampling. This technology provides new approach for biotechnological food processes in which metabolites are changing with time. Discriminative metabolomics has allowed classification of health supplements based on their quality and origin. Discriminative and predictive metabolomics are promising tools for food quality control and monitoring. Quality parameters, usually individually measured, are complicated and costly protocols. Many of these parameters can be quantified in a single run of informative metabolomics whereas others (e.g. sensory attributes) can be estimated by predictive models based on sample metabolite profile, providing a cost-effective alternative to quality analyses (Cevallos-Cevallos *et al*., 2009).

Metabolomics in food safety

The metabolomics provides an opportunity to develop new targets in order to ensure food safety that is important for human health as well as for the agriculture, food processing and storage. Ensuring food safety in the future will require new methods for identification, monitoring and assessing of food borne hazards during production, storage, delivery and consumption.

Many untargeted discriminative tools have been applied in food safety. Amongst the many techniques, neutral desorption extractive electrospray-ionization MS (EESI-MS) was able to discriminate Escherichia coli-contaminated foods through the presence of unidentified high molecular weight peaks (Chen et al., 2007). Same technique was also adopted to discriminate spoiled fish through the presence of putrescine, cadaverine,

and histamine, showing a great potential of this type of analysis in food safety. The environmental attributes affecting food quality can also be studied by using such techniques. Informative and predictive metabolomics have been used to identify water contamination, temperature stress, and the fish health conditions at the moment of the catch (Samuelsson and Larsson, 2008).

The recent advancement in metabolomics through cutting edge technology had made a breakthrough in food quality analysis. The techniques like IMS , a MALDI-MS equipped with a especially developed software is able to record spectra from thin tissue sections and produces images of the distribution of constituent both small and large molecular species. Imaging mass spectrometer acts as a microscope that simultaneously records the distribution of hundreds of bio-molecules. Currently, the IMS does not give the information about their molecular identity, but this technique is a very efficient tool for imaging of small molecules, such as lipids and drug metabolites. Until now, the application of this technique is limited for molecules with a molecular size up to 20 kDa. IMS is technique with a very high potential for detection and tracing of mycotoxins and other harmful agents in food of animal origin, as well as fruits and vegetables where the imaging of their distribution in tissue is possible. Potential application of this technology and the practical use in near future can be expected, especially for tracing and detection of small molecules such as mycotoxins (Giacometti *et al*., 2013).

The alterations induced during processing of foods can also be detected by using metabolomics. All the physical and chemical processes like heating, cooling, fermentation, pasteurization etc. can be effectively monitored for quality and safe food production. This is also able to detect milk and meat adulteration. The descriptive and informative metabolomics have been employed for process monitoring of a soyabean and rice straw fermented milk using analysis of untargeted metabolites with NMR (Choi *et al*., 2007).

The metabolomics profiling of foods of animal origin can also address several issues related to adulteration and authenticity. The bacterial contamination and presence of toxin are one of major problems in foods of livestock origin. Metabolomics studies discriminating meat at the species level and at the intra-species level as a first step in product authentication, as well as attempts to detect beef adulterated with o?al have been conducted and reported. Food metabolomics study involving rapid detection of microbial spoilage has been undertaken on both poultry and beef using FTIR spectroscopy (FTIR52 and 99101). Focus on acquiring a biochemical fingerprint of metabolites formed as a result of the growth and enzymatic activity of bacteria *in situ* on the meat surface was also accomplished. This have added advantages over traditional approach of separating physically the microorganisms from the substrate (which is generally the case) and undertaking the time-consuming analysis and enumeration of the bacteria themselves. This has enhanced and accelerated the detection of microbial spoilage from hours to seconds and could theoretically be used to detect other forms of contaminating substances or bacteria (Ellis *et al*., 2012). Recently, DESI (Desorption electrospray ionization) has been introduced and its potential for the detection of pesticides, natural toxins, veterinary drugs, food additives, adulteration, and packaging migrant's contamination in foods have been evaluated and established. The power of DESI comes from its ability to be coupled with hand-held or on-site mass spectrometers for screening of contamination during sampling before further detailed studies in food analysis laboratories (Ellis *et al*., 2012). Thus, metabolomics has shown to be a frontier tool for the progress of the main food science areas such as compliance of regulations, processing, quality, safety, and microbiology.

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FS-01

HEAVY METAL (Cd, Cr and Pb) CONCENTRATIONS IN MILK OF DAIRY ANIMALS IN MEHSANA DISTRICT OF NORTH GUJARAT

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The presence of toxic metals in environment is a global threat to public health. Heavy metals are non-biodegradable in nature and are bioaccumulated in food webs. Heavy metals are known to cause neurotoxicity, nephrotoxicity, fetotoxicity and carcinogenicity. The present study was planned to find out the concentrations of heavy metal residues such as cadmium, chromium and lead in milk of dairy animals reared in villages near by industrial area in north Gujarat. A total of 100 milk samples were collected from villages of Kadi Taluka of Mehsana district from cow and buffaloes. This area is industrial area with manufacturing units especially plastic, pigment, dye, batteries and automobile industries. The samples preparation involved wet digestion method of milk samples with TCA followed by ashing and reconstitution. Using ICP-AES, the level of heavy metals in the milk samples were estimated. The average value of cadmium in milk was found 0.045 ppm with a concentration range of 0.040 ppm to 0.068 ppm, which was lower than its maximum residue limit (MRL) value of 1.5 ppm as recommended by FSSAI. The average value of chromium in milk was found as 0.247 ppm with concentration range of 0.091 ppm to 2.288 ppm. The average value of lead in milk was found as 0.314 ppm with concentration range of 0.006 ppm to 2.442 ppm. The level of heavy metals was lower than permissible limits.

FS-02

MONITORING OF RESIDUES OF SULFONAMIDES AND FLUOROQUINOLONES IN MILK IN SELECTED DISTRICTS OF BIHAR

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Indiscriminate use of antibiotics poses a great threat to human population. Their residues in milk over a long time may produce a variety of manifestations like individual drug toxicities including drug allergies, carcinogenicity and most importantly microbial resistance to these drugs. Keeping in view of the above facts, monitoring of residues of sulfonamides and fluoroquinolones (enrofloxacin & ciprofloxacin) was done in five districts (*viz.* Patna, Vaishali, Muzaffarpur, Samastipur and Nalanda) of Bihar with the objectives to estimate the residues of these antibiotics in milk samples. Sample survey was done in organized as well as unorganized dairy sectors. Organized sector included organized dairy farms (both private and government) as well as local khatals. Unorganized sector included the marginal farmers who rear animals in few numbers but not in herds.

A total number of 833 milk samples consisting of 609 from organized and 224 from unorganized sectors were randomly collected out of which 357 from Patna, 108 from Vaishali, 102 from Muzaffarpur, 122 from Samastipur and 144 were from Nalanda districts. The samples were stored in deep freeze till analysis. Samples were processed before high performance liquid chromatography (HPLC) analysis as per standard analytical procedures. Analytical methods for estimation of residues of sulfonamides and fluoroquinolones were standardized. The antimicrobial residues in milk were estimated above MRL values (MRL values in respect of sulfonamides and fluoroquinolones is 0.1 µg/ml). A total of six samples for enrofloxacin residues, one for ciprofloxacin, one for sulfamethoxazole and one for sulfadimidine, were found to contain residues above MRL values. On examination of history of individual animal samples, it was found that samples were contaminated with fluoroquinolones due to the fact that animals were given recent treatment with these antibacterials. However, in case of sulfonamides, the immediate cause could not be established indicating some other sources. Other sources may be feed additive as growth promoters.

FS-03

MULTI-RESIDUE ANALYSIS (GC-ECD) OF SOME ORGANOCHLORINE PESTICIDES IN COMMERCIAL BROILER MEAT MARKETED IN MYSURU CITY

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Organochlorine (OC) insecticides are among the most important organotoxins and make a large group of pesticides. Physicochemical properties of these toxins, especially their lipophilicity, facilitate the absorption and storage of these toxins in the meat thus possesses public health threat to humans. The presence of these toxins in broiler meat can be a quantitative and qualitative index for the presence of these toxins in animal bodies, which is attributed to Waste water of irrigation after spraying the crops, contaminated animal feeds with pesticides, polluted air are the potential sources of residues in animal products. Fifty broiler meat samples were collected from different retail outlets of Mysuru city, Karnataka state, in ice cold conditions and later stored under -20°C until analysis. All the samples were subjected to Gas Chromatograph attached to Electron Capture Detector(GC-ECD, VARIAN make) screening and quantification of OC pesticides *viz*; Alachlor, Aldrin, Alpha-BHC, Beta-BHC, Dieldrin, Delta-BHC, o,p-DDE, p,p-DDE, o,p-DDD, p,p-DDD, o,p-DDT, p,p-DDT, Endosulfan-I, Endosulfan-II, Endosulfan Sulphate and Lindane(all the standards were procured from Merck). Extraction was undertaken by blending fifty grams(g)of meat sample with 50g Sodium Sulphate anahydrous, 120 ml of n-hexane, 120 ml acetone for 15 mins, extract washed with distilled water and sample moisture is dried by sodium sulphate anahydrous, partitioning is done with 25 ml petroleum ether, 10 ml acetonitrile and

15 ml n-hexane shake vigorously for two minutes, sample cleanup was done with florosil column. The reconstituted samples (using n-hexane) (Merck chem) were injected to Gas Chromatograph Electron Capture Detector (GC-ECD). The present study reveals that, among the fifty chicken samples subjected for analysis, 60% (15/50), 32% (8/50), 28% (7/50), 20% (5/50) and 16% (4/50) of samples contaminated with DDTs, Delta-BHC, Dieldrin, Aldrin and Alachlor respectively. DDT metabolites, Delta-BHC were the most frequently detected OC pesticides. The detected levels of the pesticides were below the levels of MRL (according to Export Council of India notification for fresh poultry meat).

FS-04

STANDARDIZATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF DIMINAZENE ACETURATE RESIDUES IN BUFFALO MEAT BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH PHOTODIODE ARRAY DETECTION

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Diminazene aceturate is a commonly used antibabesial agent. It is also used in the treatment of the thileriousis in combination with other antibiotics. It is especially useful for treatment of mixed protozoan infections. The residues of Diminazene in foods of animal origin is a major concern since these residues are harmful to the human health which stresses the need for the development of a specific, precise and simple analytical method for the determination of Diminazene residues in buffalo meat. In the present study, a high-performance liquid chromatography (reversed phase) method was standardized for the determination of Diminazene aceturate residues in buffalo meat below the Maximum Residue Limits (MRLs). The Codex Alimentarius Commission regulatory agency has set a Maximum Residue limit (MRLs) of 500 µg/Kg in cattle muscle for Diminazene aceturate as trypanocide agent. After the meat samples were extracted, the analysis of Diminazene aceturate level was carried out using a RP-18 column at an ambient temperature. The chromatographic separation was accomplished with an isocratic mobile phase consisting of a mixture of phosphate buffer and acetonitrile (85:15, v/v). The flow rate of the mobile phase was maintained at 0.6 ml/min and injection volume was 20 µl. A photodiode array detector was operated at a wavelength of 254 nm. The linearity, recovery, selectivity, detection capabilities and precision of the method were evaluated in buffalo meat samples at drug concentrations ranging from 50 to 500 ng/g. Mean extraction recovery were in the range of 86 to 91% and the limit of quantification was 67 µg/Kg for Diminazene in buffalo meat. The retention time and total run time for the analysis of Diminazene were 7 and 15 min respectively. The proposed method was found to be effective and accurate for routine analysis of Diminazene aceturate residues in buffalo meat.

FS-05

EFFECTS OF OSMOTIC PRESSURE, ACID AND COLD STRESSES ON ANTIBIOTIC SUSCEPTIBILITY OF COAGULASE POSITIVE THERMO TOLERANT *STAPHYLOCOCCUS AUREUS*

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The control of microorganisms is one of the most important aspects of food preservation and their destruction ensure food safety. Prevalence of antibiotic resistance of thermo tolerant *Staphylococcus aureus* isolated from a variety of foods has increased and it is major challenging food safety concern in many countries. *Staphylococcus aureus* has many physiological adaptations enabling its survival under a wide range of environmental stresses. The objective of this study was to evaluate effects of osmotic stress (2, 4, 8, 12% NaCl), pH (6, 4, 2) and cold (4^{O}C) on susceptibility of two isolates of *Staphylococcus aureus* towards 9 antibiotics. The Susceptibility of antibiotics (D-test) was checked against unstressed (control), stressed or post-stressed *Staphylococcus aureus* isolates (an ATCC strain and a dairy food isolate), were determined using the disc diffusion method. Unstressed *Staphylococcus aureus* found sensitive to all tested antibiotics. In general, when *Staphylococcus aureus* cells were exposed to salt, pH and cold stresses, their antibiotic resistance increased as salt concentration increased to 8 or 12%, pH reduced to 4 or 2, and as temperature decreased to 4^{O}C. Results showed that dairy isolate and ATCC reference strain were resistant with Amoxycillin in osmotic pressure, pH and cold stressed or post-stressed; whereas cold and pH stresses developed resistance with Streptomycin and osmotic pressure (4% to 12% NaCl) developed resistance to Gentamicin, in both isolates under stressed or post-stressed situation. The Gentamicin and Getifloxacin showed resistance in acid stressed or post-stressed conditions. Over all 4 antibiotics developed resistance in both isolates under different stresses, suggest that increased use of bacteriostatic (sub-lethal) stress, rather than bactericidal treatments in food processing and preservation systems may stimulate antibiotic resistance responses in *Staphylococcus aureus* strains that may contribute to development and dissemination of antibiotic resistance among food-related pathogens.

FS-06

SURVEILLANCE OF ANTIBIOTIC RESIDUES IN COMMERCIAL MILK COLLECTION ROUTES IN SOUTHERN INDIA

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We estimated the prevalence of antibiotics residues in raw milk collected from two different commercial milk processing companies (CMPCs) each from Tamil Nadu (CMPC-1; study area 1) and Karnataka (CMPC-2; study area 2) states of the southern India. Before sampling, a pilot study was done through various stakeholders to understand the existing functional CMPCs in study area and consequently characterized the organizational

setup (number of farmers, milk procurement capacity and routes and transportation methods) of selected CMPCs before identification of study sites. Milk samples (N=432) were screened by commercial kits for antibiotic residues followed by semi-quantitative confirmation. We also collected milk before and after withdrawal period from penicillin and tetracycline treated cows (N=3) to study the effects of heat treatments and fermentation on its residues level in milk. Screening of 432 milk samples (200 from CMPC-1 and 232 from CMPC-2) collected at each stage (farmers, bulk cans/tank of farmers, entire route pooled samples before and after processing) of two CMPCs revealed that 25% were positive by qualitative analysis. However, only 10% of the total samples were found to be exceeded maximum permissible limits (MRL) of beta-lactams and tetracyclines antibiotics as per Codex commission. The magnitude of antibiotic residues violation in study areas was increased from farmers to processing stage suggested its additive effects. Data from treatment record in the study area 2 revealed that mastitis and other udder related problems (38%) as the major reason for treatment, while tetracycline (41%) and penicillins (26%) were most commonly used drugs. It is concluded that mastitis as most common problem in dairy animals and it was further substantiated by significant prevalence of antibiotic residues in milk from these animals. Therefore, an effective mastitis management programmes are required to control the antibiotic residues in milk in the study area.

FS-07

QUANTIFICATION OF LEVEFLOXACIN RESIDUE LEVEL IN LIVER TISSUE OF DUAL PURPOSE CHICKEN BY LCMS/MS ANALYTICAL TECHNIQUE

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Levofloxacin, a third-generation fluoroquinolone and possesses excellent activity against gram-positive, gram-negative and anaerobic bacteria. There is no MRL level and withdrawal period fixed for the levofloxacin by regulatory agencies for birds. The estimation of the residue levels of Levofloxafin in liver samples of chicken was studied using LC-MS/MS analytical technique and residue level concentration were calculated by using PK solver non compartmental analysis software program. The study was conducted in 30 to 40 day old (n= 210) healthy dual purpose chicken Indian Rock -3 (IR-3), a strain of White Plymouth Rock. The Group I (Control) used for preparation of the blank sample for standardization of the LC-MS/MS equipment, Group- II (n=80) birds were administered at 10 mg/kg bw through oral route for five days. All birds in Group I and II were sacrificed (n= 8/day) by cervical dislocation (exsanguinations), on day 1, 2, 3,4,5,6 7,8,9 and 10 after the administration of the last dose of levofloxacin on day five. The birds were plucked and manually eviscerated then liver samples were collected. The tissue samples were stored at - 45^{0}C until assay for concentrations of levofloxacin in the tissue samples. In the present study, high residue concentration of levofloxacin in the liver tissue was 1428.89 ± 0.93µg/kg was observed day one and there was a decrease in residue concentration to 66.87 ± 0.23 µg/kg on day 10 after final dose administration of levofloxacin in chicken. The gradual decrease in the residue

concentration of drug in the chicken tissues from day one to 10 due to high liphophilicity of the levofloxacin, so drug was slowly eliminated from the body. There is no MRL level and withdrawal period fixed for the levofloxacin by regulatory agencies for birds. This study helps in fixing of MRL (Maximal Residue Limit) and withdrawal period for levofloxacin in liver samples of chicken tissues.

FS-08

MONITORING OF ANTIBIOTIC RESIDUE STATUS OF THREE DRUGS IN CHICKEN MEAT FROM TAMILNADU

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Intensive rearing of chicken leads to diseases of infectious nature and often necessitates treatment with antibiotics. This often leads to prevalence of residues in meat samples. With increasing consumer awareness and international trade restrictions, monitoring becomes a very important activity. Present experiment was performed to study the prevalence of antibiotic residues in chicken meat by screening for Enrofloxacin (ENR), Oxytetracycline (OTC) and Tylosin (TYL). Chicken meat samples were collected from retail consumer points of chicken and farms where broiler chicken are reared. The meats were brought to the laboratory and processed for assay of ENR, OTC and TYL residues. ENR and OTC were assayed using HPLC assay procedures using liquid extraction methods. TYL was assayed using a sensitive ELISA kit. The study was done over a period of three years. Out of the 1168 samples collected from 28 districts of Tamil Nadu, only 11 samples were found to be positive for OTC above MRL values. Otherwise most of the samples remained negative or contained levels below MRL values, for TYL OTC or ENR. This study showed the acceptability of most of the chicken meat samples with respect to the residue status for ENT, OTC and TYL. However owing to increased usage of these antibiotics for control of infections, regular monitoring is very important.

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FS-08

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ISVPT-2016

TECHNICAL SESSION - VII

PHARMACOKINETICS / TOXICOKINETICS

Chairperson : Dr. S. K. Mody

Co-chairperson : Dr. P. Sriram

Rapporteur : Dr. Shraddha Nety

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LEAD-PK-01

EXOSOME NANOPARTICLE: A NOVEL DRUG DELIVERY VEHICLE

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Exosomes are the nano-vesicles (~30-100 nm) released by most of the cell types. They act as the carriers of biomolecules, enclosed within them, to the target cells. They mediate cell-cell communication and regulate the cellular environment. Their role has been implicated in various diseases. The function of exosomes as the natural carriers of biomolecules encourages their use as the drug delivery vehicle for pharmacological applications. In this review, we have discussed various aspects of exosomes as a novel and effective drug delivery vehicle. Various attributes, like long circulating half-life, non-immunogenic, biocompatibility, loading of wide spectrum of drug molecules and targetibility, make them ideal as an effective vehicle for drug delivery. Exosomes can be used as a delivery vehicle for various biomolecules, including nucleic acids, small organic molecules and proteins, thereby providing treatment avenues for a wide variety of diseases. However, improved methods for its large scale isolation and effective delivery are needed, so that they can be used in future for clinical purposes.

Introduction: Organelles are the distinct units present in a cell to perform specific functions. They are usually covered within the lipid bilayer. Exosomes are one of the cell organelles that are secreted out of the cell. Exosomes are the nano-sized vesicles with 30-100 nm in size. They carry the cell-specific load of biomolecules, like miRNA, mRNA, DNA and proteins, to the extracellular space. They are secreted by most of the cell types and present in majority of the extracellular fluids, like milk (Baddela *et al*., 2015), urine, saliva, cerebrospinal fluid, amniotic fluid (Weber *et al*., 2010).

Biogenesis of exosomes*:* The production of exosomes in the cells occurs by the inward budding of membranes within endosomes, leading to the formation of intra-luminal vesicles within multi-vesicular bodies (MVBs). These MVBs, further, have two fates. Either, they can be targeted to lysosomes for proteasomal degradation or they are secreted out of the cell as 'exosomes'. Exosome biogenesis can occur via two mechanisms: firstly, the process involving the endosomal sorting complex required for transport (ESCRT) machinery and alternatively, the ESCRT independent mechanism. In the ESCRT dependent mechanism, four cytosolic major protein complexes known as ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III, are involved in the formation as well as sorting of load into the exosomes (Kowal *et al.,* 2014). The ESCRT independent mechanism involves ceramide for exosome formation as Sphingomyelinase inhibitor, which blocks the synthesis of ceramide and impairs the exosome secretion by the cells (Essandoh *et al*., 2015). Finally, the fusion of plasma membrane and MVBs leads to the release of exosomes into the extracellular space, probably, involving the action of Rab GTPAase proteins.

Contents of exosomes*:* Three major classes of biomolecules are present in the exosomes. They have been characterized as proteins, lipids and nucleic acids (Thery *et al*., 2002). The protein content of exosomes includes the families of cellular proteins, such as the common set of proteins present in most of the exosomes (tetraspanins, heat shock proteins and fusion proteins) and others specific to the cell type from which they are secreted. The majority of the proteins are similar to that are present in the plasma membrane, endosomes and

the cytosol. Exosomes enclose bioactive miRNAs and mRNAs, which were found to be resistant to RNAase digestion (Valadi *et al*., 2007). Recently, it was demonstrated that exosomal miRNAs are transferred in the extracellular space and affect the gene expression in the target cells. This led to the major discovery, resulting in the physiological significance of the exosomes and their role in active cell-cell communication. Lipid content in the exosomes mainly includes sphingomyelin, phosphatidyl serine, cholesterol and saturated fatty acids (Wubbolts *et al*., 2003).

Functions of exosomes*:* Earlier considered as garbage bags, exosomes have now been proved to be active players of cell- cell communication and thus, involved in a wide array of cellular processes, like proliferation, differentiation, immunity, maturation, apoptosis etc. In some cases, the interaction of exosomes and the target cells results in the physiological changes in the cell, like in case of antigen-specific T cells when bind to antigen-presenting cell-derived exosomes, which leads to the presentation of MHC-peptide complexes (Segura *et al*., 2007). In other cases, they are involved in the transfer of cargo enclosed within them to the target cells influencing the cellular environment. Exosomes are taken up by the cells via phagocytosis, which can be receptor dependent or independent depending on the cells (Morelli *et al*., 2004). This naturally occurring phenomenon leads to the new field of research that involves the use of exosomes as a vehicle for delivery of drugs to the target cells. The aim of this review is to explore the potential of exosomes as the new emerging drug delivery vehicle which can revolutionize the pharmaceutical field.

Exosome as a drug delivery vehicle: Exosomes possess various attributes, like long circulating half-life, uptake by most of the cell types, non-immunogenic, biocompatibility, loading of wide spectrum of drug molecules and potential to target specific cells using protein engineering. The potential of exosomes as drug delivery vehicle depends on the optimized methods used for harvesting them as well as methods used for administrating drug loaded exosomes into the target cells *in vitro* and *in vivo.*

Spectrum of therapeutic cargo loaded in exosomes: Exosomes have qualified to carry a wide variety of drug molecules and transfer them to the target cells influencing the cellular environment. Exosome mediated delivery of drugs have been proven to be successful to show its therapeutic effect *in vitro* and *in vivo.* As nucleic acids are relatively unstable, one of the milestone in this field is the successful loading and transfer of nucleic acids (siRNA and miRNA) using exosomes. Successful delivery and therapeutic action of siRNA via exosomes have been demonstrated in peripheral blood mononuclear cells (Wahlgren *et al*., 2012), fibrosarcoma cells (Shtam *et al*., 2013), liver cells (Pan *et al*., 2012) and neurons (Alvarez-Erviti *et al*., 2011). Exosome mediated delivery of miRNA-155 results in functionally more efficient inhibition and less cellular toxicity (Momen-Heravi *et al*., 2014). It has also been found that intravenously injected exosomes delivered let-7a miRNA to EGFR-expressing xenograft breast cancer tissue in RAG2/mice (Ohno *et al*., 2013). Exosomes can deliver anti-inflammatory agents, such as curcumin. Curcumin encapsulated exosomes act as a signal transducer and activator of transcription 3 (STAT3) inhibitor i.e JSI124, which were delivered noninvasively to microglia cells via an intranasal route (Zhuang *et al*., 2011). It has also been found that curcumin encapsulated stem cell exosome mitigated T1DM ischemic injury by alleviating synaptic and vascular mitochondrial dysfunction (Kalani *et al.,* 2015). Curcumin delivered by exosomes is more stable and more highly concentrated in the blood. Incorporation of curcumin into exosomes is found to increase the solubility, stability, and bioavailability of curcumin (Sun *et al*., 2010). Clinical use of doxorubicin is greatly hampered by its dose dependent cardiac

toxicity (cardiomyopathy and congestive heart failure). Exosomes are the first example for targeted delivery of chemotherapeutic drug doxorubicin to solid tumors in mice. The effectiveness of targeted exosomes encapsulated doxorubicin in improving therapeutic index is found to be more relative to free doxorubicin at same dose. Systemic administration of this doxorubicin delivery system significantly inhibited tumour growth while causing no overt toxicity (Tian *et al*., 2010).

Methods of loading cargo into exosomes*:* Efficient delivery of therapeutic cargo to the target cells, in turn, depends on the methods used for the loading of cargo into the exosomes. Various methods have been used to encapsulate the drug into the exosomes which ultimately depends on the type of therapeutic to be loaded and the target. The simplest method to load the drug into exosomes is incubation. Drugs, like curcumin and doxorubicin, can be loaded in exosomes by simple incubation with the suspended exosomes at required temperature (Zhuang *et al.,* 2011). Due to small size and hydrophobicity, they get incorporated into the exosomes and have been proved as the efficient method of drug loading. Electroporation have been used as the efficient to load the therapeutic into exosomes. Application of an electric field in the suspension of exosomes and the drug leads to the formation of pores in the membrane of exosomes resulting in the incorporation of drug in the exosomes (Andaloussi *et al*., 2012*).* Various parameters need to be optimized for using this method for loading, like voltage, pulse width, no. of pulses, siRNA and exosomes concentration. Chemicals like lipofectamine and other transfection reagents have also been used for loading of nucleic acids into the exosomes (Shtam *et al*., 2013). Another popular method includes the transfection of the exosome donor cells to overexpress the therapeutic produced by the donor cell, which will be packaged into the exosomes and released by the donor cells. In addition, drug loaded exosomes can be incorporated *in vitro* via incubation with the target cells and various routes have been used to administer these exosomes *in vivo* like intranasal, intraperitoneal or through oral route. Various aspects of exosomes as a drug delivery system is summarized in figure 1.

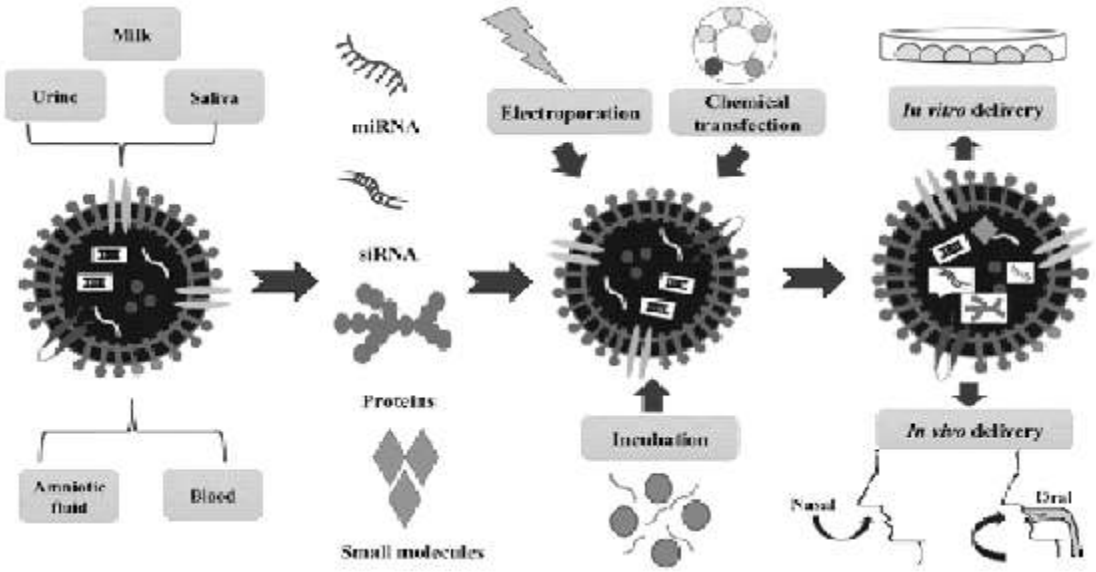

Figure 1: Figure showing (A) the presence of exosomes in different biological fluids, (B) the spectrum of cargo that can be incorporated in exosomes, (C) different techniques which can be used for loading of cargo in the exosomes and (D) *in vitro* and *in vivo* delivery of drug loaded exosomes.

Conclusion: Exosome-based drug delivery has revolutionized the pharmaceutical field as a novel approach, achieving enhanced therapeutic effect via efficient delivery and least side effects. Parameters like loading of wide spectrum of drugs, biocompatibility and stability, have made exosomes as one of the best option to be used as an efficient therapeutic carrier. However, more efforts are needed to standardize the methods for their large scale isolation, loading procedure and increased targetibility along with clinical trials, for their commercial use as an effective therapeutic carrier.

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PK-01

EFFECT OF AMOXICILLIN ON PHARMACOKINETICS OF MEROPENAM IN RATS

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The present study was conducted to evaluate the effect of amoxicillin administration (20 mg/kg) on pharmacokinetics of meropenem after single intramuscular administration (50 mg/kg) in rats. Blood samples were collected at 0 minute (before drug administration), 2, 5, 10, 15, 30 and 45 minutes and at 1, 2, and 4 h. Plasma samples (100 µL) were extracted with solid phase extraction method and residues were dried with nitrogen evaporator at 40^0 C for 1 h. Following intramuscular administration of meropenem, peak plasma concentration C_{max} observed at 0.25 h, were 5.014 ± 0.66 and 4.539 ± 0.24 µg/mL in normal and amoxicillin-treated rats, respectively. After 2 h post administration meropenem was not detected. Mean values of elimination half-life ($t_{1/2\beta}$), mean residence time (MRT), volume of distribution (Vd_{area}), Clearance (Cl_B) and Area under curve (AUC) were 0.6 ± 0.0 h, 0.7 ± 0.0h, 60.11 ± 4.0 L/kg, 73.44 ± 5.6 L/h/kg and 9.4 ± 5.0 g.h/mL,

respectively in normal rats whereas 0.7 ± 0.1 h, 0.8 ± 0.0h, 61.60 ± 8.3 L/kg, 64.97 ± 3.3 L/h/kg and 3.5 ± 0.6 µg.h/mL in amoxicillin-treated rats. Very fast absorption of meropenem in both the groups of rats and slow rate of clearance in amoxicillin-treated rats was observed. Significant changes in pharmacokinetic parameters of meropenem following intramuscular administration have not been observed in amoxicillin-treated rats compared to normal rats.

PK-02

PHARMACOKINETICS OF MARBOFLOXACIN IN SHEEP FOLLOWING INTRAVENOUS ADMINISTRATION

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Marbofloxacin, a fluorinated quinolone, has been introduced for exclusive use in veterinary medicine. Marbofloxacin exhibits high bactericidal activity against most Gram-negative bacteria and some Gram-positive and *Mycoplasma Spp.* This drug is approved in many countries for use in cattle, pigs, dogs, horses and cat for respiratory, urinary tract, soft tissue and dermatological infections. However, there is limited information available on pharmacokinetics of marbofloxacin in sheep. Thus, the present study was conducted to bridge the gap between existing pharmacokinetic data of marbofloxacin in sheep and its clinical prospectus. The pharmacokinetics of marbofloxacin was investigated in six healthy adult male sheep (*Ovis aries*) of Patanwadi breed with body weight ranging between 30 to 38 kgs, given intravenously at the dose rate of 2.0 mg/kg. Periodical blood samples were collected. The plasma concentration of marbofloxacin was assayed using LC-MS/MS method. The plasma marbofloxacin concentration immediately after drug administration was found to be 7.31 ± 0.64 mg/ml at 2 min and same declined to 5.20 ± 0.37 mg/ml at 5 min. Following single dose intravenous administration of marbofloxacin at the dose rate of 2.0 mg/kg, the plasma concentrations of marbofloxacin > 0.04 µg/ml persisted for up to 24 h post drug administration. The mean values of elimination rate constant, half-life, area under curve, mean residence time and total body clearance were found to be 0.28 /h, 3.03 h, 6.87 mg.h/ml, 3.30 h and 0.39 L/h/kg, respectively. The present study demonstrates that single intravenous administration of marbofloxacin in sheep at the dose rate of 2.0 mg/kg led to persistence of effective therapeutic concentration for longer duration of time i.e. up to 24 hours, so it can be effectively employed in treatment of susceptible bacterial infections in sheep.

PK-03

PHARMACOKINETICS OF CEFTIZOXIME IN SHEEP AFTER SINGLE DOSE INTRAVENOUS AND INTRAMUSCULAR ADMINISTRATION

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Ceftizoxime is the third generation cephalosporin, approved for veterinary use. Owing to its broad spectrum of activity, larger volume of distribution and good tissue penetration, it is having bright prospectus for veterinary clinical uses. The pharmacokinetics of ceftizoxime is essential to understand its plasma concentration versus time profile and disposition prior to clinical use in target species. Looking to these facts, the present study was conducted to investigate single dose intravenous (IV) and intramuscular (IM) pharmacokinetics of ceftizoxime at the dose rate of 10 mg kg^{-1} in six Patanwadi sheep aged between 2 to 4 years and weighing between 25 - 35 kg. The blood samples were collected at 0 min (pre-dosing), 2 min (IV only), 5 min, 10 min (IM only), 15 min, 30 min, 1h, 2h, 4h, 6h, 8h, 12h, 24 h, 36 h, 48 h and 96 h. Plasma concentration of ceftizoxime was analysed using UHPLC with UV detector. The pharmacokinetic parameters were calculated using PK solver software. The mean values of elimination half-life, volume of distribution, area under curve, total body clearance were found as 7.31 and 9.97 h, 1.10 and 0.70 L kg^{-1}, 126.29 and 152.32 µg h ml^{-1} and 0.09 and 0.07 L $h^{-1}kg^{-1}$, respectively after IV and IM administration. High concentrations of ceftizoxime ≥ 1.0 µg ml^{-1} were maintained for 24 h after both IV (1.56 ± 0.27 µg ml^{-1}) and IM administration (1.78 ± 0.11 µg ml^{-1}). Intramuscular bioavailability was calculated as 132.12 %.

PK-04

DISPOSITION OF LINCOMYCIN FOLLOWING SINGLE INTRAMUSCULAR ADMINISTRATION IN GOATS

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Pharmacokinetics of lincomycin after intramuscular administration at dose rate 10 mg/kg body weight was investigated in healthy goats. Blood samples were collected from 1 min to 24 h of drug administration. The disposition of lincomycin followed two compartment open model and drug was detected in plasma up to 8 h. The peak plasma concentration (C_{max}) was observed 5.63 ± 2.50 µg.ml^{-1} at 0.20 ± 0.16 h (T_{max}) after IM injection of lincomycin. Absorption half-life ($t_{1/2ka}$) in the present study was very short (0.05 ± 0.01 h) denoting faster absorption of drug *via* IM route. Lincomycin was slowly distributed from blood to the tissue, as evidenced by the low value of the distribution coefficient (1.50 ± 0.36 h^{-1}). The high AUC (33.8±7.68 µg.h/mL) indicated good antibacterial activity of lincomycin in healthy goats. The elimination half-life, volume of distribution and total

body clearance were 6.19±0.25 h, 2.95±0.50 L/kg and 0.52±0.24L/h/kg, respectively. The long elimination half-life indicated drug retain for longer period in body. Based on results, lincomycin is suggested to be repeated following IM route in healthy goats at 12 h interval for that organism which are sensitive to lincomycin having MIC up to 0.6 µg/mL and at 8 h interval for bacteria having MIC of 1 µg/mL.

PK-05

DISPOSITION KINETICS AND DOSAGE REGIMEN OF MOXIFLOXACIN IN COW CALVES FOLLOWING SINGLE INTRAVENOUS ADMINISTRATION

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Investigation was carried out to study the disposition kinetics of moxifloxacin at the dose rate of 5 $mg.kg^{-1}$ after a single intravenous administration in cow calves and an appropriate dosage regimen was calculated. At 1 min after injection, the concentration of moxifloxacin in the plasma was 11.6 ± 0.21 $\mu g.ml^{-1}$, which declined to 0.12 ± 0.005 $\mu g.ml^{-1}$ was detected at 12 h. The low value of distribution half-life ($t_{1/2\alpha}$) of moxifloxacin (0.160 ± 0.005 h) indicated the rapid distribution of drug from central to peripheral compartments in cow calves. The rapid distribution of moxifloxacin further confirmed by the high value of K_{12}/K_{21} ratio (1.42 0.037). The value of Vd_{area} in cow calves was 1.35 ± 0.028 $L.kg^{-1}$ indicated high volume of distribution of drug into various body fluids and tissues. The high value of AUC_{0-8} (13.36 ± 0.286 $\mu g.ml^{-1}.h$) and AUMC (39.02 ± 0.985 $\mu g.ml^{-1}.h^2$) reflected that a vast body area is covered by drug concentrations in cow calves. The total body clearance (Cl_B) of moxifloxacin, was 0.375 ± 0.008 $L.kg^{-1}.h^{-1}$ in cow calves. The elimination rate constant of moxifloxacin from central compartment (K_{el}) and its half-life ($t_{1/2\beta}$) in cow calves were 0.752 ± 0.008 h^{-1} and 2.50 ± 0.011 h, respectively. The values of MRT of moxifloxacin was 2.92 ± 0.02 h in cow calves. The most appropriate priming and maintenance doses of moxifloxacin to be administered by i.v. route to maintain the MIC of 0.50 $\mu g.ml^{-1}$ in cow calves would be 6.21 $mg.kg^{-1}$ b.wt. followed by 5.53 $mg.kg^{-1}$ b. wt. at 8 h intervals.

PK-06

PHARMACOKINETICS OF VERBENONE IN WISTAR ALBINO RATS

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Verbenone (4,6,6-trimethylbicyclo(3,1) hept-3-en-2-one) is a bicyclic ketone monoterpene. Essential oils containing verbenone have been reported to have antibacterial, acaricidal, and anti-inflammatory properties. A new HPLC method was standardized for detection of verbenone with a mobile phase of Acetonitrile: Water (60:40), flow rate of 1 ml/min and detected at 250nm using PDA detector with a mean retention time of 5.35

min. The plasma extraction with acidified acetonitrile gave a recovery percentage of 89.8 to 95.79% with a mean error of 3.03 to 0.05 for concentrations of 0.001 to 0.1. The compound was tested for acute oral toxicity studies in wistar albino rats as per OECD 425 guidelines and was found to be safe for oral administration. Eight adult male wistar albino rats were given 200 mg/kg of the drug orally and the blood was collected periodically and extracted with acidified acetonitrile and injected in to HPLC, and the plasma concentration were calculated from 0.25 - 24 hours. The plasma concentration data were analyzed for one compartmental model using PKSolver. It was found to have the following kinetic parameters A= 26.37 ± 0.69 mg/ml, Ka= 0.86 ± 0.002 /hr, $t_{1/2}$ = 0.806 ± 0.002 hrs, T_{max} = 1.18 ± 0.003 hrs, C_{max} 0.332 ± 0.002 mg/ml, $AUC_{0\text{-}inf}$ = 1.067 ± 0.007 ?g/ml*h and MRT of 2.37 ± 0.007 hrs.

ISVPT-2016

TECHNICAL SESSION - VIII

EDUCATION IN VETERINARY PHARMACOLOGY & TOXICOLOGY

Chairperson : Dr. S. Ramesh

Co-chairperson : Dr. S. K. Jain

Rapporteur : Dr. S. Palai

NAVSARI AGRICULTURAL UNIVERSITY

LEAD EVPT-01

TEACHING METHODOLOGIES IN VETERINARY PHARMACOLOGY AND TOXICOLOGY

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The primary aim of veterinary education is to produce highly skilled and enlightened manpower needed for the improvement of livestock health and production, socio-economic transformation and development of our country. Veterinary education has many general objectives such as training in skills used in scientific research, communication, information, management, problem solving and independent learning. These can be accomplished without adding independent subjects to the curriculum but by building their pursuit into other courses, using innovative teaching methods. Presently, there is an urgent need to consciously move away from didactic teaching and look for new instructional approaches like learners focused instructions, problem solving and experimental learning.

The teachers not only need to learn the subject matter content, but also the techniques of teaching, social values and philosophy of education and psychology, intellectual level, socio-economic status, pre-university background of education, age, sex and aptitude of learners.

The desirable characteristics of a graduating veterinary student are knowledge base of veterinary and comparative biomedical sciences; understanding of principles of biological sciences; problem solving and critical thinking skills; thoroughness, reliability, efficiency and critical analysis; skills in finding, managing and using information; skill in oral and written communication; skill and desire for sustained scholarship and lifelong commitment to learning and professional development.

Possible modes of instruction with these criteria in mind are didactic lecture; independent study; group study; information management experience with computer; clinical instruction; evaluation/ feedback experiences; research experience; advising, mentorship and role modelling. Of all of the above modes of instruction, the lecture mode offers the least opportunity for active learning. There is a little doubt that, as far as 'covering the material', the lecture is the most common form of delivery in veterinary colleges as it is one of the most efficient ways to convey information.

Veterinary Pharmacology is a specialized field whose focus of concern is ultimately the application of pharmacologic methods and knowledge to the resolution of the health problem of animals. Veterinary pharmacologists serve the purposes and needs of the veterinary medical profession. In this service, veterinary pharmacology is a broad discipline whose distinguishing characteristic is an understanding of the characteristics of species differences in the effects of drugs. This is necessary because our profession is concerned with the treatment of diseases occurring in all the animals with the exception of human beings (Davis, L.E. ,1984, 1989).

Veterinary pharmacology is a broad discipline which includes veterinary comparative pharmacology (basic pharmacology), veterinary clinical pharmacology and veterinary toxicology. Veterinary pharmacology is a specialized division of pharmacology which is concerned with the discovery and application of knowledge of

the effects of the drugs in animals to the use of drugs in the practice of veterinary medicine. The science of toxicology is based on principles and facts normally taught in biochemistry and pharmacology courses, but also rely to some degree on knowledge of pathology (Davis, 1988)

A student at B.V.Sc. & A.H level as per VCI guidelines, 2008 has to offer 178 credit hr. (102 theories & 76 practical) with a total of 254 contact hr. Pharmacology & Toxicology comprises of only 10 credit hr. (only 5.62% of entire course curriculum) and 12 contact hr (only 4.72% of entire course curriculum). In the light of excessive information explosion, it is impossible to cover the entire course during the time allotted as per the comprehensive text book Veterinary Pharmacology & Therapeutics (Rivere & Papich, 2009). The entire course has to be delivered by lecture and distributing handouts with little time for questions and discussions. Due to the objective type of questions students rely on 'spoon feeding' of voluminous information. The students are loaded with information without having knowledge to assimilate. The students in this system are evaluated for their 'cramming' power. There is an urgent need to decrease memorization and increase learning which they could apply in clinics. Student evaluation should be based upon ability to think critically, solve problems and communicate efficiently. If the students are given the fundamentals, that basic foundation for step by step learning, and if concepts are emphasized, the students will be able to make rational decisions.

The objective of teaching Pharmacology & Toxicology are: to gain a general understanding of all aspects of the science of Pharmacology & Toxicology; to learn the vocabulary related to Pharmacology & Toxicology; to understand (i) action(s) of toxicants on an animal's system and the mechanism of the action (ii) the action of the animal's body upon the toxicant; to gain an understanding of how actions and interactions of drugs & toxicants relate to physiological principles.; to learn to relate the altered physiology of the system(s) involved in a given poisoning condition(s) to setting forth the objectives of the therapy; to learn to summarize the different therapeutic means, based upon drug mechanisms of actions of toxicants to accomplish the objectives of therapy ; to understand how to properly set up a drug regimen based upon all of the above, to include i)drug or drug combinations ii) dosage and route of administration iii) frequency and duration of dosing; iv) proper withdrawal times in food producing animals and v) consideration of all precautions, side effects, adverse effects and possible drug interactions of drugs being used (Upson, 1990)

Besides what has been outlined above, a solid understanding of basic pharmacological concepts is critical. Physiochemical characteristics; mechanisms of pharmacological action; principles of absorption, distribution, metabolism and excretion; pharmacokinetic properties; mechanisms of adverse reactions; pharmaceutical considerations; drug delivery systems and epidemiological concepts are essential components. We must prepare our students so that they can keep up with any future developments on their own.

The students at both graduate and post graduate level need to be sensitized and made aware on various issues as follows:

1. Basic concepts in clinical pharmacology

Clinical pharmacology is Pharmacology applied in clinical setting. It involves basic understanding of pathophysiology, pharmacokinetics, pharmacotherapy interaction of xenobiotics, surgery and anaesthesia, nutrition and management. Besides students need to be made well aware about pharmacovigilance, ADR, pharmacoepidemiology and bioequivalence.

2. Drug residues and food quality

The presence of drug residues and toxicants is an important issue to be addressed. There is an urgent need to avoid residues in food animals and must be addressed in pharmacology & Toxicology courses at length. Graduates should be made more aware of this important facet of veterinary practice.

3. Development of drug resistance

This issue is of paramount importance considering the development of resistance against antibiotics. The onus being shifted on Veterinarians owing to the indiscriminate and off-label or extra-label use of drugs specially antimicrobials. The graduates need to be sensitized on this problem.

4. Animal Ethics

The animal ethics is now influencing the teaching of veterinary pharmacology & Toxicology because of ban on use of animals in Practical classes. Computer simulated experiments have come up to replace the animal experimentation. However, my own experience is that students appreciate the live experiments more than computer simulation.

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LEAD-EVPT-02

TEACHING OF VETERINARY PHARMACOLOGY AND TOXICOLOGY IN VETERINARY COLLEGES

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Currently Veterinary Council of India is the apex national body that frames and makes the Veterinary institutes execute the curriculum for graduate degree program whereas post graduate program is governed by the Indian Council of Agricultural Research.

The syllabi for these are modified recently; the coverage of the subject, evaluation pattern have been finalized after lot of deliberations and are satisfactory. Besides teaching, the veterinary institutes are also expected to contribute to research and extension program. Hence the discussion pertinent to teaching can be viewed at different levels

Undergraduate Teaching

For undergraduate program, courses in Pharmacology are offered in third professional year. The Science of Pharmacology has developed by leaps and bounds in the last four decades. There has been tremendous rise in the number of drug molecules poured in the market, their dosage forms and combinations. Parallel development in the science of biochemistry, genetics enabled to elucidate the mechanisms of drug action / toxicity at molecular level. However, if existing system of conducting examination is considered, this growth of science has compelled the students to memorize minute details of drug monograph in order to score in the examination.

Books of Pharmacology are the sources of information for pharmacists, medical students, dentists and veterinary students. Each of these students should be able to read between the lines and concentrate on the information practically more relevant to his/ her field.

While playing the role of paper setter, teachers should also make an attempt to design concept oriented question paper close to day to day field/ clinical situation that student is likely to face in the future. And to achieve this, pharmacology teachers should also have some exposure to the veterinary clinics. The instructions issued to the paper setter should not be very rigid and he/ she should be availed enough freedom to set interesting patterns of question.

Post- graduate and Ph.D teaching

Post graduate students and Ph. D. scholars are working hands of the department that need firm, mature guidance from a mentor. At M.V. Sc. level student is introduced for the first time to the field of research.

He should be made to understand the difference in writing the notes, to which he is used to, during his graduation tenure, against writing the scientific statements supported by evidence. Running of Journal club of staff and students can help to build the habit of critical evaluation of the research work. An orientation regarding 'do's and don'ts of thesis writing also helps to avoid common mistakes committed while writing thesis. Students should also learn that hypotheses can go wrong during the research and that in no way would make the research quality inferior.

Review of the research carried out in last two decades reveals that most of the work is carried out in laboratory animals. Increased job opportunities in the R and D sector and the time taken for the approval of research project involving use of large animals by CPCSEA are the two major reasons behind it. Considering the latter, preparing a repository of proposals and submitting it regularly to CPCSEA can be thought of as a solution.

In the post graduate curriculum, Biostatistics is not offered and it is felt that, the syllabus should be modified with its inclusion as non credit compulsory course.

Trainings for strengthening competency of veterinary graduates

It is not unusual to find the job description of the post quite different than the things learnt during graduate studies. The short term trainings or diploma courses of different duration can be offered by the universities to fill this gap Viz. Regulatory toxicology, Analytical chemistry etc.

Continuing education

The continuing education to the field veterinarians contributes bidirectionally. The faculty shall make the field veterinarians conversant with latest development in the subject visa vis there would also be sharing of field experiences from the participants.

Training to the Society

In many states, Veterinary Colleges have changed their affiliation from Agricultural universities to Veterinary Universities. This has enabled to expand the target of extension beneficiaries from farmers, livestock owners to pet owners, industries, science graduates etc. The training can be undertaken for the society in different areas Viz., Diploma in Toxicology for science graduates, Residue awareness program, Basics of basic research etc.

SWOT analysis of teaching and curriculum of VPT

Strengths

- Availability of option to the student to choose career in basic or clinical area depending on inclination
- Discipline directly linked to major industry

Weakness

- Veterinary pharmacy is in infancy as far as curriculum is concerned
- Alternative medicinal treatments is untapped area
- Less emphasis given on Clinical pharmacology
- Deficiency of books on poultry Pharmacology

Opportunities

- Establishment of alternative medicine departments/institutes
- Liaison with pharmaceutics departments for new drug development
- Validation of alternate therapies on modern principles

Threats

- Overlapping of interest in areas of research from other faculties

EVPT-01

PERPETUAL ADVANCES IN COMPUTER AIDED DATABASE AS AN ALTERNATIVE TO ANIMAL MODELS BOOSTING ADVANCED RESEARCH IN THE FIELD OF VETERINARY PHARMACOLOGY

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Advanced knowledge in the field of pharmacology and toxicology encompasses principles of pharmacokinetics and pharmacodynamics, chemotherapy, toxicological (OECD, ICH, FAO guidelines) research, industrial pharmacology, clinical trials, drug development protocols, OMICs studies, bioassay (*Invitro, Invivo, Exvivo*) techniques, regulatory veterinary medicines and post marketing surveillance. Computer models and cell cultures are good for experimental screening and are used frequently. Such models cannot replicate complicated interactions in the whole system. Final testing depends on studies in animals; sometimes it is required by law. Animal and non-animal models used in conjunction achieve the best answer. "Alternatives" or "Substitutes" is defined as anything from absolute to partial replacement of live animals in biomedical research and testing *viz.,* Physico-chemical techniques (e.g. GCMS, Chitosan films etc.), microbiological systems (e.g. Ames Test, use of fungus to detect metabolism of drugs), tissue/organ culture preparation (e.g. human dopaminergic neurons as substitute for animal models of Parkinson's disease and for transgenic models with modified expression of PARK genes), computer or mathematical analysis (*in silico* testing), epidemiological surveys (e.g. epidemiology has linked smoking to cancer; high cholesterol to heart disease; and folic acid deficiency in pregnancy to *spina bifida*), and plant analysis (e.g. toxicity assays in plants). Wet lab Research methods using animals to extrapolate the pathological conditions of humans are stem cells (*in-vitro* model of human cardiac tissue), microdosing, DNA chips, microfluidics chips, isolated human tissue (Alzheimer's and Parkinson's diseases), new imaging technologies [Magnetoencephalography (MEG), magnetic resonance imaging (MRI), functional MRI (fMRI), magnetic resonance spectroscopy (MRS), positron emission tomography (PET), single-photon emission computed tomography (SPECT), event-related optical signals (EROS) and transcranial magnetic stimulation (TMS)], and post-marketing drug surveillance. Biological database covers genomics, proteomics, metabolomics, microarray gene expression and phylogenetics. Advantages associated with these methods are, time efficiency, requires less man power, and cost effectiveness. Easily accessible tool in alternative study is computer based software databases for e.g. Discovery studio® for insilico drug designing, preADME® for PK and PD studies, TEST® (Toxicity Estimation Software Tools by USEPA), ToxCalc™ (environmental toxicity testing), Prolis lab Softwares (statistical package for environmental toxicity testing) are various software tools used to estimate the toxicity of chemicals using QSAR's methodologies. Free access to existing (proprietary) animal test data, availability of validated alternative methods and a practical implementation of conceptual approaches such as the Adverse Outcome Pathways and Integrated Testing Strategies were identified as major requirements towards the successful development and implementation of alternative approaches in veterinary research and education.

ISVPT-2016

TECHNICAL SESSION - IX

TOXICOLOGY OF XENOBIOTICS

Chairperson : Dr. A. M. Thaker

Co-chairperson : Dr. Hitesh B. Patel

Rapporteur : Dr. M. Usharani

NAVSARI AGRICULTURAL UNIVERSITY

LEAD-TOX-01

PESTICIDES INDUCED IMMUNOTOXICITY AND ITS PHYTOREMEDY

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INTRODUCTION

To the consequence of indiscriminate use of various agrochemicals such as organophosphates, carbamates, organochlorines, synthetic pyrethroids, nicotinamides, etc for the protection of crops, seeds and food grains; and release of toxic chemicals as industrial effluents such as PAH, phenols, nitrophenols, heavy metallic compounds etc and other automobile exhausts into air, soil and water; they are either distributed in soil, water or air are transmitted and deposited in vegetation and subsequently taken up directly into body systems of man and animals. Accumulation of residues of these environmental pollutants produce adverse effect on various body functions, immune system in particular, making man and animals more vulnerable and susceptible to infectious, non-infectious and neoplastic diseases. The immune system is responsible for modulating host defences against a range of human diseases. The successful development and functioning of the immune system require recognition and response to a range of cellular and circulating signals acting through endocrine, autocrine, and paracrine mechanisms. These complex control systems offer multiple opportunities for disruption by xenobiotics including agrochemicals. The immune system is known to be involved in the etiology as well as pathophysiological mechanisms of many diseases. Ayurveda gives emphasis on promotion of health- a concept of strengthening host defences against different diseases. The natural resistance of the body may be enhanced by herbal drugs. There is an upsurge of clinical usage of indigenous drugs as they are free from serious side effects. Development of agents capable of restoring patients' immune system from a state of immune deficiency to a more functional one would likely to have a significant impact on disease and patient ill effects (Gaur *et al.,* 2009).

IMMUNOTOXIC EFFECTS

As a result of persistent exposure, pesticides or their metabolites such as pesticides accumulate in body tissues leading to alterations in the normal immunological response of the body. Immunotoxic effects may occur when the immune system is the target of chemical insults, resulting in immunosuppression or immunostimulation. Suppression in immune response can result in decreased resistance to infection, occurrence of various forms of neoplasia, or immune dysfunction evidenced by vaccine failure and increased incidence of epidemics making animals more susceptible to infectious and neoplastic diseases. Immunostimulation on the other hand may exacerbate allergy or induce autoimmunity in response to exposure of certain drugs and chemicals.

Consequences of immunosuppression include recurrence of bacterial and viral infections and frequent occurrence of neoplasm. The respiratory tract infections are most common in immunodeficient patients. The

pathogens most frequently encountered in such patients include the bacterial agents *such as Staphylococcus aureus, Streptococci, Escherichia coli, Pseudomonas aeruginosa, Listeria monocytogenes, Mycobacterium tuberculosis* and atypical mycobacteria. Herpes virus, cytomegalovirus, Epstein-Barr virus, and human papilloma virus are the eading viral infections in immunosuppressed patients. Fungal infections include those induced by *Candida, Aspergillus,* and *Cryptococcus* species (Frattini and Trulock, 1993).

Immunostimulation also results in a number of adverse effects such as influenza-like reactions, facilitation or exacerbation of underlying diseases, and inhibition of hepatic drug metabolism. The reaction usually develops within hours after taking an immunostimulant drug and the patient recovers within a few hours. Stimulation of the immune system has also been shown to inhibit oxidative drug metabolism by the hepatic cytochrome P450 system. Activation of macrophages resulting in the release of IL-1 and IL-6 has been suggested to be involved in such conditions (Luster *et al*., 1987).

MECHANISMS OF IMMUNOTOXICITY

The adverse immunotoxic effects of xenobiotics on immunocompetent cells and organs include damage such as necrosis and histopathological effects on the immunocompetent cells like neutrophils, T and B lymphocytes, macrophages etc. and organs like thymus, lymph node and bone marrow. Functional alterations of immunocompetent B-lymphocytes are generally classified as humoral mediated immunity (HMI) interfering antibody production and cellular mediated immunity (CMI) altering functions of T-lymphocytes and macrophages. Interaction of xenobiotics with immune system leads to an impairment of CMI and/or HMI causing weakened host resistance to viral and bacterial infection and parasitic infestation and increased incidence of cancer (Luster *et al.,* 1992). The immune system is highly complex and its maturation is controlled by endogenous substances such as lymphokines, cytokines etc. and exogenous mediators such as bacterial products. These mediators possess activation, growth promotion and differentiation properties and act under the control of various regulators. Second messengers such as tyrosine phosphorylation, cAMP and calcium and their associated signaling pathways have been identified as the primary targets for a number of diverse classes of chemicals. Alteration in the normal physiological role of these signaling pathways of immunocompetent cells including T and B-lymphocytes, macrophage, NK cells etc. results in consequences ranging from changes immune function to marked immunosuppression and may even induce apoptosis of these cells (Luster *et al.,* 1987; Selegrade *et al.,* 1995).

ASSESSMENT OF IMMUNE FUNCTION

Routine toxicity evaluation data on body weight and growth rate, change in the morphology and weight of adrenals, thymus, spleen indicate imunotoxicity. Alteration in weight and morphology of adrenal, thymus, spleen and lymph nodes may be the indication of stress mediated changes in glucocorticoid hormones which subsequently lead to induction of lymphopoenia, lymphoid depletion in thymus causing alteration in its size and weight or thymic atrophy particularly in growing animals. Reduction in the weight and size of gut associated lymphoid tissue (GALT) and changes in the cellular component of blood especially leucocyte count in chronic studies predict immunotoxic potential of the chemicals under investigation (Holesapple *et al.,* 1996).

For evaluation of immunotoxicity, a two tier approach has been suggested. Tier I usually screens for potentially immunotoxic chemicals and includes assays to evaluate immunopathology, humoral and cellular

immunity (HMI and, CMI). Tier II consists of specific confirmatory immune tests and also include challenge with bacterial, viral, parasitic agents. Thus, to assess the immunotoxic potential of a chemical in animals, both humoral and cellular responses should be examined. For humoral immunity, bone marrow derived B-lymphocyte are responsible for producing antibodies against any antigenic substance. For assessment of humoral immunity, agglutination titre, haemagglutination titre, ELISA tests etc. are used to measure the antibody titers. (WHO, 1996).

The cellular immunity can be assessed by the determining the following parameters

1. *Neutrophil* adhesion test

After total leukocyte counts (TLC), differential leukocyte counts (DLC) is done by fixing blood smears and staining with Field stain I and II- Leishman's stain. After initial counts, blood samples are incubated with 80mg/ml of nylon fibers for 15 min at 37°C. The incubated blood samples are again analyzed for TLC and DLC. The product of TLC and % neutrophil gives neutrophil index (NI) of blood sample. Percent neutrophil adhesion can be calculated by the formula as

Neutrophil adhesion (%) = NI u NI t x 100, where NI u = Neutrophil index of untreated blood sample and NI t = Neutrophil index of treated blood sample.

2. *Macrophage migration index*

The test may be performed according to the method of Saxena *et al.* (1991). Peritoneal exudate cells (PEC) collected in a haematocrit capillary tubes are allowed to migrate in migration chamber filled with complete Roswell Park Memorial Institute (RPMI-1640) medium. The area of PEC migration is marked on Whatmann filter paper using Camera Lucida and weighed separately. The ratio of the migration area of PEC from of animals of different groups can be compared and expressed as macrophage migration index.

3. *E. coli* induced abdominal sepsis model

Experimental animals are injected with 0.2 ml suspension of E. coli ($1x10^8$ cells) i.p. and then observed for 16 hours to find mortality if any. Blood samples are then collected into heparinized capillary tubes. Blood samples are analyzed for total and differential WBC count. The immunosuppressed animals show reduction in number of WBC and neutrophil. The acute peritonitis is also evident and there is presence of bacteria in the sterile peritoneal fluids.

4. Phagocytic index by carbon clearance test

The test can be performed according to the method of Gonda *et al.* (1990). The test animals are injected carbon ink suspension (Pelican ink, Germany) via the tail vein and blood samples are collected. The blood (25µl) is dissolved in 0.1% sodium carbonate (2 ml) and absorbance is determined at 660 nm. The phagocytic index K is calculated by using following equation:

$K= (\ln OD_1 - \ln OD_2) / (t_2-t_1)$, where OD_1 and OD_2 are the optical densities at times t_1 and t_2, respectively.

5. Lymphocyte stimulation test (LST)

The evaluation of the activity of T and B lymphocytes is determined by the method as explained by Rai-el-Balhaa *et al.* (1985). For the stimulation of T cell blastogenesis, Con-A is used as the mitogen; whereas lipopolysaccharide (LPS) for for B-cell blastogenesis. Lymphocytes are separated from the blood and cultured in RPMI-1640 cell culture media (Boyse *et al*., 1964) and incubated for 72 hrs Blastogenesis is measured by

ELISA plate reader to read the optical density (OD) at 570 nm and difference with the control is expressed as ΔOD.

6. Macrophage function test (MFT)

The metabolic activity of macrophage was measured employing nitro blue tetrazolium (NBT) dye reduction (Talwar, 1983). The macrophage functions test is employed to determine the phagocytic and bactericidal activity of macrophages. For this purpose macrophages and polymorphonuclear cells are collected from peripheral blood and subjected for MFT.

7. Delayed type hypersensitivity reaction (DTH) test

Delayed type hypersensitivity reaction to dinitrochlorobenzene (DNCB) is determined by method of Phanuphak *et al.* (1974). Experimental animals are sensitized to DNCB and then challenged after 14 days with CDNB and thickness of skin is measured. Change in the thickness is correlated with hypersensitivity.

8. Estimation of cytokines

The cytokines especially IL-2 and INF-α indicates the immunomodulation by ELISA method.

PHYTOREMEDIAL APPROACH FOR TO RESTORE IMMUNE FUNCTION

Various plants have been reported to have immunomodulatory properties. *W. somnifera* is used as a general tonic to increase energy and prevent diseases. The plant thus has reported immunomodulatory property of ashwagandha. Glycowithanolides and sitoindosides isolated from *W. somnifera* were found to have immunmodulatory action by mobilization and activation of peritoneal macrophage, increase in phagocytosis and increase in the activity of lysosomal enzymes in mice and rats (Ghosal *et al.,*1989).

The mechanism underlying immunostimulatory effect of *W. somnifera* is an increase in nitric oxide (NO) production from macrophage by inducing nitric oxide synthatase enzyme at transcriptional level. *W. somnifera* markedly increased the chemotactic activity of the macrophages, interleukin-1 (IL1) and tumor necrosis factor alpha (TNF-α) production in mice treated with carcinogen ochratoxin (Dhuley, 1997; Agarwal *et al.,* 1999). Davis and Kuttan (1999) assessed the myeloproliferative immunopotentiating effect of *W. somnifera*. It enhanced the cytokine (IFN-α, IL-2, and GM-CSF) production, stem cell proliferation and its differentiation in normal and cyclophosphamide-treated mice.

Withanolides (isolated from *Ajuga bracteosa*) were found to possess both immunosuppressant and immunostimulant properties. The immunosuppressive activity of withaferin A is evident from its ability to inhibit adjuvant arthritis in rats and the graft versus host reaction in chicks. The substance A was demonstrated to inhibit proliferation of murine spleen cell culture. The glucowithanolides and sitoindosidewere shown to exhibit adaptogenic and immunostimulating activities (Camps *et al.,* 1987).

Nisar *et al.* (2014) investigated the immunomodulatory effects of *Ajuga bracteosa* on systemic Th1/Th2 immunity in SRBC challenged mice. Treatment with ethanolic extract demonstrated biphasic effector T-helper immunity and also increased antibody titers, DTH responses and $CD4^+/CD8^+$ T-cell percentages indicated maximal activation and proliferation of T and B lymphocytes (Nisar *et al.*, 2014).

Ocimum basilicum was evaluated for immunomodulatory effect. It showed increase in body weight in *Ocimum basilicum* treated animals than the control animal. Administration of aqueous extract of *Ocimum basilicum* @ and 200 mg/kg produced a dose dependent significant increase in antibody titre value as compared to control

Fresh leaves of *Ocimum sanctum (OS)* showed increased humoral immune response in albino rats which may be due to enhanced antibody production, release of mediators of hypersensitivity reactions and tissues responses to immunomediators in the target tissue. *OS* seed oil modulated both humoral and cell-mediated immune responsiveness and GABAergic pathways which mediate immunomodulatory effects (Mukherjee *et al.,* 2005).

A significant increase in body weights, larger thymi, higher natural killer cell (NK) activity, cutaneous basophillic hypersensitivity (CBH) response, percent phagocytic macrophages, and number of SRBC/phagocytic macrophage and secondary anti-SRBC antibody response was observed in leghorn chicks reared on *Spirulina* supplementation (Qureshi *et al*., 1994).

CONCLUSION

Prolonged exposure of pesticides and other environmental pollutants results in alteration in the immune function, Immunosuppression, in particular, increases the susceptibility of an individual to infectious and neoplastic diseases. Second messenger such as tyrosine phosphorylation, cAMP and calcium linked signaling pathways have been identified as target sites of most of the xenobiotics resulting in interference in proliferation, maturation and activation of the immunocompetent cells and organs and thus warrants the evaluation of their immunotoxic potential in man and animals. For assessment of immune status, both cellular and humoral immune responses are determined to evaluate immunotoxic potential of a compound. Phytoremedial approach has been successful and can be instituted without side effects to restore the immune function.

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LEAD-TOX-02

MYCOTOXICOSIS IN LIVESTOCK OF KARNATAKA: AN UPDATE

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Mycotoxins are produced mainly by the mycelial structure of the filamentous fungi, commonly referred to as moulds. Moulds use the same general pool of low molecular weight intermediates during grow-associated primary metabolism as the majority of other living organisms. Most of the known mycotoxins have been recognized as secondary metabolites of *Fungi imperfect,* particularly of the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Claviceps*, *Alternaria*, *Neotyphodium*, *Stachybotrys*, *Myrothecium*, *Phoma* and *Diploidia*. The term secondary metabolite designates compounds that are not indispensable for the growth and metabolism of the fungi as such, and in contrast to primary metabolites, such as amino acids, fatty acids, saccharides, nucleic acids and proteins, are not essential for living.

Mycotoxicoses produce a variety of clinical signs, depending on factors such as type and concentrations of mycotoxin, the duration of exposure, and the species, gender, age, and health status of the animal. Prolonged exposure results in an increased susceptibility to secondary diseases caused by viruses, bacteria, and fungi. Frequently, there are synergistic effects between several mycotoxins multiplying adverse influences on the animal's health and production potential. Today, even though there is a great experimental evidence characterizing the potential of mycotoxins and mouldy feedstuffs to cause animal disease, definitively linking a disease outbreak in the field to a specific mycotoxin can be very difficult. Briefly, most of the time, mycotoxins often do not cause acute disease and, when they do, there are often multiple interacting factors that can modify the expression of toxicity.

Each year million tones of forage consumed by animals are contaminated with fungal species invading forage plants prior to harvest or during storage as hay, straw or silage. Many of these mycotoxins display overlapping toxicities. The primary storage methods for forage are drying (hay) and ensiling (silage). These methods of storage utilize the principles that growth of undesirable organisms is retarded in hay by low moisture content and in silage by a low pH and absence of air. In poorly stored forages, molds are potential spoilage organisms, which not only cause deterioration but can also produce mycotoxins. Therefore, maintaining proper preservative conditions are critical in ensuring quality forages.

Many episodes of myotoxicosis have been investigated during the disease investigation process under the project entitled “Obscure Diseases of Cattle and Buffaloes of Karnataka: Studies on Cause and Cure” and they are listed. Usually more emphasis is given to aflatoxicosis in poultry, but information available about the ruminant mycotoxicosis eventhough it is rampant. Hence, an effort has been made to focus on few mycotoxicosis incidences and its investigations in the present context. The updates of mycotoxicosis in livestock are given below;

1. Mycotoxicosis in cattle due to ingestion of fungal affected ground nut hay

Since 2005, in Sira Taluk of Tumkur district, Karnataka, cattle were dying after showing clinical signs of epistaxis, hemoglobinurea, anorexia, anemia and blood oozing out all over the body on pricking with a needle or fly bite. Laboratory findings of blood smears and lymph node biopsy sample did not reveal any specific pathogens. The blood smears were also negative for any hemoprotozoan parasites. The histological study of lungs revealed severe pneumonic changes, kidney had nephritis and glomerulonephritis. Liver tissue had lot of accumulation of inflammatory cells. Petecchial hemorrhagic lesions in brain were prominent.

A preliminary study was conducted to evaluate the toxic feature of the fungal infected ground nut hay in rats and mice. The study revealed monsoon harvested groundnut hay was susceptible for fungal contamination due to improper drying and humid environmental conditions. Fungal contaminants identified from infected hay were *Fusarium, Macrophomina, Puccinia, Cercospora, Aspergillus* and *Rhizopus spp*.

2. Mycotoxicosis in cattle and buffaloes due to ingestion of fungal affected sorghum straw

During 2003, there was a drought in various districts like Haveri, Gadag and Belgaum of North Karnataka. The farmers fed the dried sorghum fodder of the previous years to their cattle and buffaloes. More than 125 animals were examined. The affected animals were fed with fungal affected sorghum straws since two months. The animals were exhibiting the clinical signs like of paraplegia, salivation, staggering gait and dyspnoea. Buffaloes were highly susceptible followed milking cross bred cows. Bullocks were also affected but the incidence was less. Few animals were

Due to drought in 2003, in Northern Karnataka, the farmers fed the dried sorghum fodder of the previous years to their cattle and buffaloes. Similarly fungal affected paddy grass also caused paralysis, colic and nervous disorders in buffaloes of southern part of Karnataka. The buffaloes and cattle ingesting such stale sorghum fodder showed clinical signs of paraplegia, salivation, staggering gait and dyspnoea. Buffaloes were highly susceptible to the mycotoxicosis. The fungi identified and isolated were Rhizoctonia, Rhizopus, *Macrophomina phaseolina*, *Aspergillus niger* and Penicillium species from various grasses. The dominant fungi in sorghum grass was *Rhizoctonia leguminicola* which was reported to cause toxicity in animals. There was a significant increase in blood serum aspartate aminotransferase (AST), and alanine aminotransferase (ALT) values in ailing cattle and buffaloes compared to normal cattle and buffaloes of other regions in Karnataka. There was no change in haematological parameters like haemoglobin, total and differential leucocyte count and blood serum calcium, magnesium, bilirubin, blood urea nitrogen and creatinine concentrations. After treatment with atropine (2 g/kg i.m twice a day for 5 days), these clinically ailing cases recovered. The response to atropine therapy suggested the possible contribution of the cholinomimetic mycotoxin slaframine produced by the dominant *Rhizoctonia. Rhizoctonia leguminicola* causes "Slobbers Syndrome," a salivation syndrome in livestock due to effects of its two known biologically active alkaloids, slaframine as well as swainsonine. The downer animals responded well to the therapy with intravenous potassium acitate 10 mg/kg as 2.5% solution.Later a study was under taken to investigate the toxic features of fungal isolates from the affected sorghum straw in rats, mice and calves.

It was concluded from the study that, the culture filtrate containing the secondary metabolites of *Aspergillus wentii strain 2 was more toxic to mice when compared to rats. The calves were also susceptible to the toxicity of the culture filtrate of Aspegillus wentii strain 2.*

3. Toxicity of fungal infected paddy straw suspected to cause necrosis of extremities in buffaloes

During the disease investigation process, peculiar symptoms were noticed in buffaloes at Yedur village of Hosanagar taluk, Shimoga district (Karnataka). The buffaloes died after exhibiting the clinical signs of tail and ear necrosis, wounds on entire body and brisket edema. Laboratory findings of blood smears did not reveal any specific bacterial or haemoprotozoan parasites. Blood serum analysis revealed an increase in Aspartate amino transferase (AST) and Alanine amino transferase (ALT) values suggestive of liver damage. The detailed clinical investigation and history revealed that the paddy straw fed to these animals had blackish spots or specks indicative of fungal infection. *Aspergillus terreus* and *penicillium digitatum* and *Cladosporium oxysporum* fungi have been isolated from the fungal infected paddy straw. A study was undertaken to find out the toxic nature of the fungal affected paddy straw in rats and mice.It was concluded from the observations made in the study that the fungal culture filtrate has shown toxicity in rats and mice, attributing to the presence of toxic principle. Further study is necessary to isolate the active mycotoxins responsible for the toxicity.

4. Toxicity of fungal infected paddy straw caused death in cattle and buffaloes in South Canara district

A study was undertaken to confirm a disease especially in buffaloes of Hiriyadka region in Udupi district of Karnataka, characterized by clinical signs of knuckling of hock joint, colic, tenesmus, rumen atony and anorexia. Serum analysis revealed increase in alanine aminotransferase (ALT) values indicating liver damage. These animals were fed mainly on fungal affected paddy straw and very little green grass and other fodder crops. The main fungi isolated from the paddy straw were *Rhizopus* and *Aspergillus*. *Aspergillus ornatus, Aspergillus niger, Aspergillus glaucus, Penicillium resticulosm, Penicillium aurantiogrisium* and *Rhizopus oryzae have* been isolated from the fungal infected paddy straw. Hence, a study was planned to evaluate the toxic nature of the fungal affected paddy straw in rats, mice and calves. The culture filtrate had shown toxicity feature to certain extent in rats and mice indicating the presence of toxic principle. Same was not appreciable in calves which might be because of the species variation, the dose and route of administration and the duration of treatment.

5. Toxicity of fungal infected maize hulls caused death in cattle and buffaloes

In Sirsi of Uttara Kannada district of Karnataka there were 25 deaths in cattle in the month of September 2006, after ingestion of fungal infected maize hulls. The ailing cattle, 10 cross breed and 4 local cattle exhibited the clinical signs of severe convulsion, opisthotonus, nystagmus, bloat salivation, respiratory distress and staggering gait. There was decrease in concentration of Hb, PCV and total erythrocyte count with no change in TLC or DLC. There was increase in concentration of AST without any change in serum creatinine and urea nitrogen concentration. The gross pathology revealed haemorrhages in liver heart and mucosa of GI tract. The histopathology of liver revealed swollen hepatocytes, accumulation of erythrocytes in perilobular space and inflammatory changes in cardiac and small intestine muscles. *Aspergillus clavatus*, *Aspergillus flavus* and *Syncephalastrum racemosum* fungi have been isolated. A study was undertaken to find out the toxic nature of the fungal affected maize hulls in rats and mice. It is concluded from the observations made in the present study that the fungal culture filtrate has shown toxicity in rats and mice, attributing to the presence of toxic principle.

6. Series of fracture incidence due to consumption of fungal contaminated maize straw

Series of fractures were noticed in a village Shiraguppi of Hubli taluk, Dharawad district, Karnataka state during March 2008. During the visit, 4 bullocks were examined suffering front legs lameness and by palpation it was diagnosed as humerus fracture. The characteristic feature of episode was, only humerus bone fracture was observed in all the affected bullocks. However, there was no incidence of fracture in buffaloes or cows. The fracture was observed only in well built draught bullocks. The grazing area of the animals was observed and it was found that in the cotton field sorghum was grown in between the crop. There was a history of recent unseasonal raining immediately after harvesting the sorghum. The remnant of the sorghum crop with fresh blades was fed to all the animals. During the observation, it was found that the remnant of the sorghum was infected with fungus. In the slaughtered bullocks, there was a clear cut spiral type of fracture on the left side of humerus. Blood and serum samples were collected from the affected animals as well as the normal animals and there was no change in the haematalogical parameters (Hb,TC, DC, E, B and N) and biochemical parameters (AST, ALT, BUN and CRT) and in the calcium to phosphorus ratio. The affected fodder sample was also collected and submitted to Plant Pathology Department GKVK, Bangalore who identified the species of the fungi as *Fusarium, Rhizopus* and *Penicillium.* From the investigation, it may be concluded that all the fractures could be attributed to the mycotoxicosis due to consumption of fungal infected fodder by the animals. Only humerus fracture was noticed in bullocks which might be attributed to much weight bearing capacity of the humerus bone during ploughing and the bone as such is porous. The mycotoxin/s might have caused disturbance in the bone structure. However, there were no fracture incidences in the buffaloes and cows. This might be due to non use of these animals for ploughing. The normal calcium and phosphorus ratio might be attributed to the homeostasis mechanism. Further study is required to explore exact species of fungi and the mycotoxins responsible for the fracture incidence.

7. Mycotoxicosis due to consumption of fungal contaminated maize straw

During the disease investigation process, peculiar symptoms were noticed in cattle at Yellodu and other villages of Gudibande and Bagepalli taluks of Chikkaballapur district, Karnataka state. All the affected animals were exhibiting the clinical signs of loss of body condition, ruminal atony, severe anorexia, recumbency, hyperapnoea, abdominal type of respiration, dry muzzle, nasal discharge and abortions in pregnant cows. There were haemorrhagic spots on facial region. Laboratory findings of blood smears did not reveal any specific bacterial or haemoprotozoan parasites. Blood serum analysis revealed an increase in AST and ALT values suggestive of liver damage. The detailed clinical investigation and history revealed that the dry maize stalks were fed to these animals had blackish spots or specks indicative of fungal infection.

A systematic study was conducted. Repeated dose 28-day oral toxicity study was conducted in 16 groups of rats consisting of 6 rats of either sex. The fungal culture filtrates of the three isolated fungi *F. oxysporum, F. subglutinans and A. niger* was administered to induce the toxicity in rats in the dose range of 0.5, 1, 2 ml/ 100 g. Two groups consisting of 6 rats were administered with mixed proportion of all the three isolated fungi *F. oxysporum, F. subglutinans and A. niger*. During the study period, animals were observed for clinical signs of toxicity. Body weight was measured and blood samples were collected on day 0, 14 and 28 of the study. Biochemical parameters like BUN, creatinine, AST, ALT and haematological parameters like TEC, TLC, PCV, Hb were analysed. The fungal infected wheat material was analyzed for the presence mycotoxins by LC-MS/MS

method. The *F.oxysporum* infected wheat material showed the presence beauvericin (11300µg/kg) and *F.subglutinans (*87470µg/kg). The *A.niger* infected wheat material showed the presence of aflatoxin B1, aflatoxin B2, aflatoxin G1 and aflatoxin G2. There was significant (P>0.001) change in serum biochemical and hematological parameters, when compared with control group rats. The gross and histopathological changes revealed hepatotoxicity, cardiotoxicity and nephrotoxicity of the fungal culture filtrate. Thus it is concluded from the observations made in the present study that the fungal culture filtrate had shown toxicity in rats, attributed to the presence of toxic principle.

8. Mytoxicosis in goats of Bantwal after consumption of fungal infected paddy straw

During the year 2007, sheep and goats were dying in Bantwal of Dakshina Kannada District. All sheep and goats were belonging to single owner. Owner's history revealed that, since 2-3 months, goat and sheep were dying at constant interval. During investigation, it was observed that, the owner has reared all these animals in the 4 acre land and was fenced. After complete grazing, the owner used to feed the sheep and goats with the stored paddy straw during night time. After through examination of the animals it was felt that, most of the animals were weak, debilitated and exhibited sort of hind limb paralysis. The paddy straw fed to these animals was heavily infected with fungus and black specks were visible on the paddy straw. The farmer procured this particular fodder 5 months back and the problem in the herd was traced after using this particular fodder. All the affected animals were treated with dextrose normal saline and Vit B complex Inj. The owner was advised to stop feeding the fungal infected paddy straw. After one weak, there was much more improvement in the health status of the animals with no any observed deaths. The fodder sample was containing the fungi belonging to *Rhizopus* and *Fusarium* species. Further study is necessary to induce the toxicity in laboratory animals to identify and isolate the exact fungi and mycotoxins.

9. Mycotoxisosis of fungal infected medow grass toxicity

In Siddapur taluk of Uttara Kannada district, in Dhannalli , Nilkund Village, during January 2005, total 17 animals, among them 9 were buffaloes were affected and showing the clinical signs of posterior paralysis, downer cow syndrome, normothermia, keratomalacia of the tail. The food and water intake was reduced. There was rumen impaction and the rumen movements were 1-2 per minutes. Rectal temperature, respiration, heart rate, was in the normal range. All the animals were stall fed and dried meadow grass fodder was the major food served to the animals with very little concentrated food. Postmortem of died buffalo heifer conducted. The gross lesions were degenerative changes in liver, congestion and haemorrhages. The spleen was moderately enlarged. Haemorrhages were present in the heart and lungs. There were no significant changes in the biochemical parameters when compared to normal animals except there was significant increase in SGPT values indicating the liver damage.This was confirmed by histopathological examination. All the blood smear samples were negative for the presence of haemoprotozoan parasites or pathogenic bacteria. The meadow grass fodder was containing the fungi belonging to *Penicillium* spp. Mycotoxicosis due to the presence of *Penicillium* and other species of fungi in the fodder might be responsible for the condition. Hence in future, such fungal contaminated meadow grass feeding for prolonged period should be discouraged especially in buffaloes since these are more susceptible to the condition. Potassium acetate therapy 20mg/kg as 2% solution slow i/v once in two days followed by oral potassium therapy 60 mg / kg gave the encouraging results. Supportive therapy of administering 10% dextrose about 1-2 liters with B-complex vitamins favored

the early recovery. Other fungal species are yet to be identified. An effort to induce the toxicity in laboratory animals like rats, rabbits and guinea pigs after administering the fungus culture to them is being done.

A study was undertaken to confirm the ailments in cattle and buffaloes which had occurred after feeding with the fungal infected meadow grass. The fungal isolates were identified as *Trichoderma harzianum, Penicillium citrinum* and *Aspergillus versicolor.* The fungal inoculated wheat cultures were subjected to LCMS/MS multi-mycotoxin analysis which confirmed the presence of citrinin, aflatoxins and trichothecenes. Sub acute toxicity study of the culture filtrates were conducted using Wistar albino rats. The animals were gavaged at three different dose levels daily for 28 days. During the study period animals were observed for clinical signs of toxicity. Biochemical and haematological parameters also were analysed. There was a significant (p?0.001) increase in the serum concentrations of ALT, AST, BUN and creatinine, indicated hepatic and renal damage which got confirmed by histopathology. Also there was significant ($p<0.001$) decrease in haematological parameters such as TEC, Hb and PCV. Hence it could be concluded that the present study indicated the toxic features of the culture filtrate isolated from the infected meadow grass in rats

In conclusion, the fungal infected fodder or feed should not be fed to the animals as it is a one of the important cause of many obscure diseases with unappreciable clinical signs. The fungal infected fodder or feed should not be fed to the animals. During unavoidable circumstances, where there is severe scarcity of fresh fodder, the fungal infected fodder can be dried in sunlight after spraying with 1% salt solution. This fodder can be mixed with equal amount of good quality forage or feed and may be used for feeding the animals along with good concentrate. Awarness should be created among the farmers to store the fodder properly to avoid fungal infection. The treatment is symptomatic and administration of fluids, B-complex vitamins and supplementation of essential minerals will help the recovery of the affected animal. The mycotoxins isolated from the various fungi were aflatoxins, rubratoxins, sporidesmin and sterigmatocystin, ochratoxin, citrinin, zearalenone and trichothecenes etc.The field veterinarians are instructed to co-operate by the way of reporting such incidences to the authors for further studies to identify the fungi responsible for the mycotoxicosis and further study is necessary to identify the related mycotoxins.

TOX-01

CLINICAL IMPACT OF ORNIDAZOLE ON PLASMA BIOCHEMISTRY IN SHEEP

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A safety evaluation of ornidazole was carried out to study the alterations induced by its intravenous administration on various biochemical parameters in sheep. Six healthy male sheep were treated with ornidazole @ 20.0 mg/kg body weight intravenously. The blood samples before and after intravenous drug administration at different time intervals were collected and plasma were separated. The plasma biochemical analysis was done using by Automatic Biochemistry Analyzer, Pictus 400 (Diatron, Germany). The average value of blood biochemical parameters viz. alanine aminotransferase (ALT), aspartate aminotransferase (AST), acid phosphatase (ACP), alkaline phosphatase (ALP), creatine kinase (CK), lactose dehydrogenase (LDH), creatinine (CR), total bilirubin (T-BIL), direct bilirubin, blood glucose, blood urea nitrogen (BUN) and total protein were measured/estimated before and after single dose intravenous administration of ornidazole on 6 h, 12 h, 1st, 2nd, 3rd, 4th, 5th, 6th and 7th day. The values of all parameters observed at 0 day (before drug administration or pre-dose) were compared with values observed on subsequent days (after drug administration or post dose). The values of AST (6th day), AKP (2nd and 6th day), direct bilirubin (6 h) and blood glucose at (3rd day) were found be significantly different when compared to pre-dose value of same parameters ($p<0.05$). All the animals were clinically normal with respect to body temperature, respiration rate and heart rate. Form the findings of present study, it is concluded that single intravenous dose of ornidazole @ 20.0 mg/kg in sheep does not produce any clinically relevant alterations in the values of biochemical parameters for up to 7 days.

TOX-02

EFFECT OF ORAL EXPOSURE OF IMIDACLOPRID AND ITS AMELIORATION BY RESVERATROL IN 42 DAYS TRIAL IN MALE RATS

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Imidacloprid, a first generation neonicotinoid systemic insecticide, acts on nicotinic acetylcholine receptors of insects. The aim of the present study was to investigate the nature of toxicity produced by imidacloprid and its amelioration by a natural polyphenol, resveratrol in male Wistar rats. Apparently healthy adult male rats weighing 100 - 120 g were randomly divided into six groups consisting of 6 animals in each group. First group served as naive and were given only 2% gum acacia suspension, which served as vehicle for imidacloprid and resveratrol. Second group animals were given resveratrol @ 2 mg/kg. Third and fourth group animals were

given imidacloprid @ 185 mg/kg (MTD/10) and 92.5 mg/kg (MTD/20), respectively. Animals in fifth and sixth group were given treatment similar to third and fourth group and additional treatment with resveratrol @ 2 mg/kg was also given. Both imidacloprid and resveratrol were given orally once daily for 28 days. All the animals were weighed on day 0 and then at an interval of two days till completion of trial. All the animals were sacrificed on day 43 by using ether overdose and weights of different organs (liver, kidney, spleen, testis, heart) were recorded. The results were analysed by ANOVA followed by Tukey's multiple comparison post-hoc test. Both imidacloprid and resveratrol separately reduced the absolute (g) and relative (g/100 g) body weight gain significantly. Imidacloprid at both doses significantly increased relative kidney and resveratrol treatment decreased it towards normalcy. MDA levels were increased significantly in liver, testis, brain and plasma which were reversed to normal values by resveratrol treatment in these tissues. A significant increase was observed in MPO levels of liver and testis. Similarly, increased protein carbonyl levels were recorded in liver and testis on imidacloprid exposure, which were reversed significantly by resveratrol treatment. No significant change was observed in testis, brain and plasma levels of catalase, GSH and GST following imidacloprid exposure. But significant increase in liver catalase level was recorded. Imidacloprid significantly increased plasma levels of liver function biomarkers (ALT, AST, GGT), but no significant change was observed in kidney function biomarkers (BUN, creatinine) and total plasma protein. Histopathological evaluation revealed that imidacloprid exposure resulted in mild congestion and gliasis in brain tissue, cloudy swelling and perivascular reaction in liver parenchyma, degeneration of seminiferous tubules and shrinkage of spermatic vesicles in testicular tissue. These alterations were reverted back to normalcy by resveratrol treatment.

TOX-03

ASSESSMENT OF ACETAMIPRID INDUCED GENOTOXIC EFFECTS IN SOMATIC CELLS OF MALE MICE

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Acetamiprid is the most highly effective neonicotinoid insecticide used worldwide for crop protection. Acetamiprid is highly selective and provides outstanding control over sucking pests such as aphids and white flies in vegetables and highly effective as an ectoparasiticide in veterinary medicine. The study was carried out to assess the genotoxic effects of acetamiprid in somatic cells of male Swiss albino mice by micronucleus test (MNT) and chromosomal aberration studies (CAs). Two dose levels of acetamiprid (4.6 and 2.3 mg/kg/day) along with 3% gum acacia as negative control were administered i.p. to mice daily for 30, 60 and 90 days and subsequent mutagenic effect was assayed by MNT and CAs. Results were compared with animals receiving cyclophosphamide as positive control for 5 days. At the end of dosing bone marrow was excised, processed and stained with Giemsa stain and then evaluated for presence of micronuclei and chromosomal aberrations. Genotoxicity was measured using the micronucleus (MN) assay, scoring 500 polychromatic erythrocytes (PCEs) per animal for bone marrow and 100 well spread metaphase were carefully observed at random for the

various type of chromosomal aberrations. Genotoxicity was assayed in bone marrow by calculating the ratio of PCEs to normochromatic erythrocytes (NCEs) and counted the number of micronuclei in PCEs and NCEs. In MNT, there was significant difference in P/N ratio which was observed at 4.6 mg/kg dose level daily administered for 30, 60 and 90 days, however significant difference was observed in PCE with MN at 4.6 mg/kg dose level daily administered for 90 days. The main aberration scores included chromatid and chromosome breaks, endoreduplication and gaps (chromatid and isochromatid). In CAs, a significant difference was observed in number of metaphase cells showing various types of chromosomal aberrations at 4.6 mg/kg/day dose level daily administered for 60 and 90 days. On the basis of above results of MNT and CAs it may be concluded that acetamiprid is a weak mutagen at a dose level of 4.6 mg/kg b.wt administered i.p. daily for 90 days.

TOX-04

EVALUATION OF GENOTOXICITY OF KARANJIN, ISOLATED FROM *PONGAMIA PINNATA*

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Pongamia pinnata, is a commonly used plant in traditional medicine and all parts of the plants are used therapeutically. The seeds of this plant are reported in Ayurveda and Siddha medicine for its usefulness in treating bronchitis, chronic fever and rheumatism. The seed extracts have been reported to possess anti-inflammatory, anti-oxidative, analgesic, hypoglycaemic, anti-ulcerogenic, antihelmintic properties. Karanjin is the major bioactive furano-flavonoid obtained from the seeds of *Pongamia pinnata*. The genotoxicity of this bioactive molecule have not been reported. The objective of this study was to evaluate the mutagenic potential of Karanjin by *in vitro* Bacterial Reverse Mutation Assay (Ames test) and by *in vivo* rodent micronucleus assay. Karanjin at the concentrations of 50, 100, 200, 450 and 900 µg/plate were tested for the mutagenic potential by Ames test using five histidine deficient (his-) tester strains of *Salmonella typhimurium* (viz., TA98, TAIOO, TAI02, TA1535, TAI537), in the presence or absence of S9 mixture. Micronucleus assay was conducted in Swiss albino mice at the dose levels of 100, 300 and 1000 mg/kg body weight. In the Ames test, the Karaj in at the concentration tested both in the absence and presence of S9 mix did not increase the number of histidine revertant colonies and the number of histidine revertant colonies were. Within the range of spontaneous revertants control reported for each strain. The results of the *in vivo* rodent micronucleus assay showed no significant increase in the micro nucleated polychromatic erythrocytes (MNPCE) and total micronuclei per cent at all dose levels compared to negative control. There was no significant decrease in the polychromatic erythrocyte / normochromatic erythrocyte (PCE / NCE) ratio in the bone marrow cells of mice. Karanjin is non-mutagenic at the concentrations tested as evidenced by the results of AMES and *in vivo* rodent bone marrow micronucleus assay.

TOX-05

A STUDY ON SEROLOGICAL AND HORMONAL PROFILE OF OFFSPRING BORN TO CHRONIC CADMIUM EXPOSED RATS

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This experiment was carried out to study the serological and hormonal profile of offspring born to chronic cadmium exposed rats and to evaluate protective role of green tea if any. Twenty four weaned *Sprague dawley* male rats were randomly divided into 4 groups of 12 rats in each (6 males+6 females). Group 1 served as Sham control, Group 2 was treated with $CdCl_2$ @5mg/kg b.wt. per orally for 3 months, Group 3 was treated with Green tea (1.5%) and Group 4 with $CdCl_2$ + green tea. In all the groups, females were mated at the end of three months with male rats belonging to respective groups/treatments and the treatment was continued till 17th day of gestation and the rats were allowed to normal delivery. The pups of F1 generation from all the groups were kept till weaning (post-natal day 21) and were subjected to sero biochemical (serum glucose, albumin, globulins, total proteins, total cholesterol and HDL cholesterol) and thyroid hormone profile were estimated. There was increased serum glucose, total proteins, albumins, globulins, total cholesterol and HDL-cholesterol in group 2 as compared to control. The concentration of T_3 in group 3 offspring was significantly ($p<0.05$) higher than the groups 1, 2 and 4. The concentration of T_4 in group 2 offspring was significantly ($p<0.05$) lower than the groups 1 and 3. Treatment with green tea significantly ameliorated ($p<0.05$) toxic effects of $CdCl_2$ by restoring biochemical and hormonal profile to normal. It is concluded that green tea exhibits protective property in $CdCl_2$ induced toxicity.

TOX-06

AMELIORATING EFFECT OF *ECLIPTAALBA* AGAINST ARSENIC INDUCED EFFECTS ON REPRODUCTIVE PARAMETERS IN WLH COCKERELS

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The present study was designed to assess the ameliorating potential of dried powder of *Ecliptaalba* plant (DPEA) on reproductive parameters against arsenic induced toxicity in white leghorn cockerels. The dried powder of *Eclipta alba* (DPEA) was prepared from the whole plant. Arsenic was given in the feed @100ppm and DPEA was given in two doses @ 1000 ppm and 2000 ppm in feed for 90 days to evaluate its protective efficacy by determining reproductive parameters. Gross, microscopic and electron microscopic examination of testes

was done to get the pathological details. Thirty five cockerels were divided randomly and equally into five groups viz. Groups I as control, II for arsenic only, III for arsenic+ silymarin, IV for arsenic + DPEA @1000ppm and V, arsenic+ DPEA @2000ppm. The reduction in mass motility and decrease in sperm concentration was significant with an increase in percentage of dead sperms. On histopathological examination, testicles showed damaged seminiferous tubules and degeneration. The electron microscopic examination of the testes revealed degeneration of seminiferous tubules which supported arsenic as reproductive toxicant. $DPEA_{2000}$ showed normal histological structure of most of the seminiferous tubules. Normal spermatogenic cells were noticed with intact systolic tight junctions. These microscopic lesions were attenuated by treatment with $DPEA_{1000}$, $DPEA_{2000.}$ It is concluded from this study that *Eclipta alba* produced protective efficacy against arsenic induced toxic effects on reproductive system in cockerels.

TOX-07

SUBACUTE TOXICITY OF THIACLOPRID AND ITS AMELIORATION BY RESVERATROL IN MALE RATS

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Thiacloprid, a neonicotinoid insecticide is known to target the nicotinic acetyl choline receptors (nAChRs) in insects, and potentially in mammals. Present study was undertaken to ascertain the effects of subacute toxicity of thiacloprid in 28 days trial and its amelioration by resveratrol in male Wistar rats. Male rats (100-120 g) were divided into six groups of six animals each. Group I rats were given 3% gum acacia suspension and served as naive control. Group II rats were given resveratrol @ 2mg/kg. Group III and IV rats were given thiacloprid @ MTD/10 (34.50 mg/kg) and MTD/20 (17.25 mg/kg), respectively. Group V and VI rats were given similar treatment as group III and IV respectively, but additionally resveratrol co-treatment was given @ 2mg/kg in both groups. Thiacloprid and resveratrol exposure was given orally once daily for 28 days. All animals were sacrificed on day 29 and organs (liver, kidney, brain, spleen) and blood were collected for different assays. Results were analysed using ANOVA followed by Duncan's multiple comparison post-hoc test.

Thiacloprid treatment produced significant changes in oxidative stress markers viz. malondialdehyde, myeloperoxidase, catalase, reduced glutathione, glutathione peroxidase, nitric oxide, total thiols and superoxide anion radical generation which were significantly restored by resveratrol co-treatment. Liver and kidney function tests viz. ALT, AST, GGT, BUN, creatinine, were significantly increased while reduction in total protein levels were recorded from thiacloprid treatment which were significantly restored with resveratrol co-treatment. Thiacloprid treatment resulted in a significant increase in serum T_3, and T_4 levels and reduction in serum TSH levels which were significantly restored by resveratrol co-treatment. A decline in Hb, TEC, HCT and MCV and increase in ESR and TLC due to neutrophilia and lymphocytosis was observed. Histopathological alterations were observed in kidney, liver, brain and spleen due to thiacloprid exposure which were attenuated by resveratrol co-treatment. The study revealed that subacute oral exposure of thiacloprid produces mild to moderate toxic effects on liver, kidney, brain and blood of adult male rats. Resveratrol possesses potential to sufficiently ameliorate the toxicity produced by thiacloprid and as such do not have any toxic effect at therapeutic doses in adult male rats.

TOX-08

AMELIORATION OF CARTAP-INDUCED LIVER AND KIDNEY TOXICITY IN RATS BY GALLIC ACID IN WISTAR RATS

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Cartap is a first synthetic organonitrogen insecticide belonging to thiocarbamate group. Cartap is extensively used in agriculture to control rice stem borer and several other pests. The present study was undertaken to explore possible protective effect of Gallic acid against Cartap-induced hepato and renal toxicity in rats. Male wistar rats were divided in six groups containing control, gallic acid control and treatment groups. Rats in treatment gropus were exposed orally to Cartap alone at different doses i.e. 30, 15 and 7.5 mg/kg body weight, respectively for 60 consecutive days. In amelioration group, gallic acid (100 mg/kg b.w.) was administered 1 h prior to administration of highest dose of Cartap (30 mg/kg b. w.). Cartap exposure caused dose dependant and significant increase in biochemical markers of liver (AST, ALT, ALP) and kidney (Creatinine and BUN) function, lipid peroxidation and prominent histopathological alterations; while level of antioxidant enzymes (SOD, Catalse) was severely decreased in liver and kidney tissues. Prior administration of Gallic acid in Cartap exposed rats resulted in restoration of hepato and renal biochemical toxicity markers, decrease in lipid peroxidation and significant increase in antioxidant enzymes along with improved histoarchitecture of liver and kidney. Thus, the results of the present study demonstrated ameliorative effect of Gallic acid in Cartap-induced hepato and renal toxicity.

TOX-09

ACUTE AND SUB-CHRONIC EFFECTS OF 3,4-DICHLOROANILINE ON EMBRYO, SAC-FRY AND JUVENILE ZEBRAFISH, *DENIO RERIO*

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Zebrafish (*Danio rerio*) is commonly used as a test organism in ecotoxicology, developmental biology, genetics and drug discovery. 3,4-dichloroaniline (DCA) has been used for the synthesis of herbicides (propanil and diuron) and insecticide (diazinon). It is major residue in the effluents from textile and pharmaceuticals industries. In the present study, the effect of DCA was evaluated on various developmental stages and on growth of juvenile zebrafish. For fish embryo acute toxicity test (FET; OECD 236) and fish, short-term toxicity test on embryo and sac-fry stages test (FST; OECD 212), fertilized 1 and 1.2 hour-post-fertilization (hpf) zebrafish embryos were used, respectively. For fish juvenile growth test (FJGT; OECD 215) juvenile zebrafish were used. The embryo and juvenile fish were exposed to different concentrations of DCA along with control. The 96 h LC_{50} value for FET was 2.97 mg/L and 10 day LC_{50} value for FST was 1.11 mg/L. The 96 h EC_{50} value for FST for hatching of embryo was 2.83 mg/L. For FST, the no observed effect concentration (NOEC) and lowest

observed effect concentration (LOEC) over the 10 days exposure period were 0.09 and 0.19 mg/L, respectively. For FJGT, the NOEC and LOEC up to 28 day exposure period were 0.016 and 0.031 mg/L, respectively. The EC_{50} values of DCA for average specific growth rate between days 0 and 14; 14 and 28; and 0 and 28 were 0.144, 0.178 and 0.257 mg/L, respectively. Results of series of studies on zebrafish developmental stages indicated that DCA is teratogenic compound and growth suppressor. The study demonstrated a higher sensitivity to DCA in embryo and sac-fry stages compared to only embryonic stages.

TOX-10

SUBACUTE TOXICITY OF THIAMETHOXAM AND AMELIORATIVE EFFECT OF QUERCETIN ON PLASMA HORMONAL LEVELS OF ADULT FEMALE RATS

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Thiamethoxam belongs to newer class of insecticide called neonicotinoids used primarily as plant systemic. Subacute toxicity of thiamethoxam and ameliorative effect of quercetin (Qu) on hormonal levels were studied in adult female rats. Rats were divided in 6 groups, each comprising of 10 rats for T3, T4 and TSH and 12 animals for oestrogen and progesterone hormone. Thiamethoxam (TMX) was administered orally daily at dose rate of 7.5% and 15% of maximum tolerated dose (3700 mg/kg b.wt.). Quercetin (100 mg/kg) was administered orally daily at an interval of 12 hours. Half no. of rats from each group were killed on 15^{th} and 29^{th} days of treatment to collect the blood and then plasma was separated and stored at 20° C. T_3, T_4, TSH, oestrogen and progesterone hormone levels were estimated using a double- antibody sandwich enzyme-linked immunosorbent assay (ELISA). T_3 levels were increased significantly in plasma of 7.5%, 15%TMX and 15%MTD of TMX+Qu -treated groups as compared to control group in 14 days treatment schedule. In 28 days schedule, T_3 levels were observed to be increased significantly ($p \leq 0.05$) in TMX-treated groups at both doses (7.5% MTD of TMX and 15% MTD of TMX) in dose dependent manner as compared to control and quercetin groups. There was significant decrease in T_3 level in 7.5% MTD of TMX +Qu and 15% MTD of TMX +Qu-treated groups as compared to 7.5% MTD of TMX and 15% MTD of TMX treated group, respectively. T_4 levels did not vary significantly in plasma of TMX and TMX+Qu-treated groups as compared to control and quercetin treated groups in 14 days schedule. In 28 days schedule, T_4 level were observed to be increased significantly ($p \leq 0.05$) in 15% MTD of TMX-treated groups as compared to control and quercetin treated groups. TSH level did not vary significantly at any of dose levels and treatment schedules. Estrogen levels did not change significantly at any of dose levels and treatment schedules. Progesterone level in 15% MTD of TMX treated group animals decreased significantly as compared to control in 14 days schedule, whereas, in 28 days schedule, progesterone level were observed to be decreased significantly in TMX-treated groups at both doses (7.5% MTD of TMX and 15% MTD of TMX) in dose dependent manner as compared to control and quercetin treated groups. It is concluded that the level of T_3 was decreased significantly by quercetin treatment in 28 days treatment schedule. The changes in levels of T4 and progesterone hormones were not significant in TMX+Qu treated groups as compared to respective thiamethoxam treated groups.

TOX-11

SUBCHRONIC TOXICITY OF THIAMETHOXAM AND ITS AMELIORATION BY QUERCETIN ON BODY WEIGHT, ORGAN WEIGHT AND DIFFERENTIAL LEUCOCYTIC COUNT IN MALE WISTAR RATS

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Insecticides are agrochemicals having significant importance to mankind which hitherto exposes nontarget organism such as human and animals to some deleterious effects. The aim of this study is to evaluate subchronic toxicity of thiamethoxam and amelioration potential of quercetin in male Wistar rats. Male Wistar rats were used in this research where their weights were recorded starting at day 0 and on every alternate day till completion of oral gavage of thiamethoxam and quercetin. The rats were sacrificed after 60 and 90 days treatment and vital organs viz. liver, heart, spleen, brain, kidney and testis were collected and weighed individually. The relative body weight gain and relative organ weight were expressed in g/100g bwt. Blood samples were taken intracardially after ether anaesthesia in heparinized vials for the determination of hematological parameters. Thiamethoxam administered at the dose rate of 2.5% and 5% of maximum tolerated dose (MTD = 4200 mg/kg b.wt.) significantly ($p \leq 0.05$) decreased the relative body weight gain and relative organ weight of some organs such as liver and kidney; the relative organ weight of other organs was not affected significantly. White blood cell (WBC) count decreased significantly in both thiamethoxam treatment groups of rats as compared to control. Differential leucocytic count (DLC) indicated significant ($p \leq 0.05$) increase in neutrophils, eosinophils, basophils, monocytes count in 60 and 90 days treatment schedule. There was decrease in differential lymphocytic count at higher dose in 60 days treatment schedule and at both doses in 90 days treatment schedule. The ameliorative effect of quercetin was exhibited in relative body weight gain at 2.5% MTD + quercetin treatment in 90 days treatment schedule. Qu showed non-significant ameliorative effect on relative weight of liver at both doses and treatment schedules. The ameliorative effect of Qu on WBC count was observed at 2.5% MTD + Qu treatment in 60 days treatment schedule. The ameliorative effect was not observed in differential leucocytic counts in any of the treatment groups.

TOX-12

AMINOGUANIDINE-HEMISULPHATE AMELIORATES KIDNEY FUNCTION AND OXIDATIVE-STRESS IN AMIKACIN TREATED WISTAR-RATS

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Amikacin, a semi-synthetic aminoglycoside participate in amikacin-induced toxicity characterized by proximal tubule injury which impairs kidney function. Aminoguanidine-hemisulfate is a free radical scavenger inhibits the formation of advanced glycation end products by reacting with reactive carbonyl groups of proteins to form relatively non-toxic adducts. It inhibits inducible nitric oxide synthase in a selective and competitive manner, leading to decreased generation of nitric-oxide and free-radicals. Therefore, the present study was aimed to investigate the effect of aminoguanidine-hemisulphate on amikacin-induced alterations of kidney function and antioxidant enzymes in wistar-rats. Healthy wistar rats of either sex (150-200gms) were divided in 4 groups with 6 rats in each group. Group-I rats served as control. Group-II and Group-III rats were treated with amikacin (@15mg/kg) and aminoguanidine-hemisulphate (@20mg/kg) body weight, intra-peritoneally for 28-days, respectively. In Group-IV both amikacin and aminoguanidine-hemisulphate were co-administered @15 and 20 mg/kg B.W. intra-peritoneally for 28-days, respectively. In amikacin treated Group-II rats, a significant increase of plasma urea nitrogen and plasma creatinine level were found compared to control group-I. However, a non-significant change in plasma urea nitrogen and plasma creatinine levels were observed in aminoguanidine treated Group-IV rats. In amikacin-treated group-II, a significant increase of lipid-peroxidation was found indicating oxidative damages and significant decrease of antioxidant biomarkers such as GPx, SOD, catalase and GSH has been noted compared to control group. In co-administered group-IV rats, aminoguanidine-hemisulphate significantly decreases lipid-peroxidation and significantly increases the GPx, SOD, catalase and GSH compared to control group. In Present study, aminoguanidine-hemisulphate an iNOS-inhibitor significantly improves kidney function and oxidative stress condition induced by sub-acute-administration of amikacin.

TOX-13

ACUTE ORAL TOXICITY STUDY OF AQUEOUS AND ALCOHOLIC EXTRACTS OF *MORINGA OLEIFERA* IN RATS

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The acute oral toxicity studies of aqueous and alcoholic extracts of *Moringa oleifera* were carried out as per Organization for Economic Cooperation and Development (OECD) guideline No. 423. The present study was conducted on eighteen (18) male Albino Wistar rats dividing them in three groups having six rats in each group.

Rats of Group I served as vehicle control and given only water during the study period, while rats of group II and III were given aqueous and alcoholic extracts of *M. oleifera* at first day of study @ 2000 mg/kg, orally. The treated rats were monitored for signs of toxicity and mortality for the first critical 4 hours and then after daily up to 14 days for general clinical signs and symptoms, as well as mortality. On 14th day of study, animals were subjected to blood collection; blood and serum sample were analyzed for hematological and serum biochemical parameters respectively. At the end of study period, animals were sacrificed and necropsy was performed; tissues (liver, kidney, spleen, pancreas, heart and intestine) were collected for histopathological studies. During 14 days of study period rats of both group did not show any noticeable clinical signs and there was no mortality in any group. There was no significant difference in hematological parameters like Hb, RBCs, PCV, MCV, MCH, MCHC, TLC and serum biochemical parameters like SGPT, SGOT, TC, LDH, CK, BUN, liver glycogen, albumin and total protein as compared to vehicle control group. No appreciable gross and histopathological alterations were found in any of the organs of aqueous and alcoholic extracts treated groups as compared to vehicle control group. Reported studies also provides critical evidence for ascertaining the safety of standardize (LD_{50}>2000 mg/kg) that can be used as medicine along with absence of untoward effect. Upon acute oral toxicity testing, aqueous and alcoholic extracts of *M. oleifera* pods were found safe.

TOX-14

HEMATO- BIOCHEMICAL ALTERATIONS FOLLOWING ORAL ADMINISTRATION OF AQUEOUS EXTRACTS OF *MORINGA OLEIFERA* IN DIABETIC RATS

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The present study was conducted on forty two (42) male Albino Wistar rats dividing them in various groups having six rats in each group. Group I served as vehicle control and received 0.5 % solution of sodium bicarbonate in normal saline orally once daily for 28 days. Group II served as diabetic control, group III served as standard treatment control and treatment groups IV, V, and VI received streptozotocin @ 60 mg/kg body weight, by dissolving it in 50 mM citric buffer (pH 4.5) solution as a single intraperitoneal injection for induction of diabetes. Group III received glibenclamide @ 5 mg/kg of body weight (p.o.) once daily after establishment of diabetes. Group IV, V and VI received aqueous extracts of *M. oleifera* pods @ 100 and 200 and 400 mg/kg orally once daily, respectively. Whereas group VII served as plant extract control were administered aqueous extracts of *M. oleifera* pods @ 200 mg/kg orally once daily. On 29th day of study, animals were subjected to blood collection; blood and serum sample were analyzed for haematological and serum biochemical parameters respectively. Blood glucose was estimated by one touch select simple glucometer on day 0 and weekly for 28 days. Upon acute oral toxicity testing, aqueous extracts of *M. oleifera* pods were found safe. Administration of aqueous extracts of *M. oleifera* pods @ 100, 200 and 400 mg/kg body weight and glibenclamide at 5 mg/kg body weight in diabetic rats for 28 days showed significant ($p<0.05$) reduction in the elevated level of blood glucose and TLC and significant ($p<0.05$) increase in the reduced level of Hb, RBCs, PCV, MCV, MCH and MCHC in dose- dependent manner as compared to rats of diabetic control group. Similarly produced significant ($p<0.05$) reduction in the elevated level of SGPT, SGOT, TC, LDH, CK and BUN and significant ($p<0.01$) increase in

the reduced level of liver glycogen, albumin and total protein in dose- dependent manner compared to rats of diabetic control group. Aqueous extracts of the *M. oleifera* pods @ 400 mg/kg body weight showed better effect than dose rate of 100 and 200 mg/kg body weight. The findings of present study suggest that aqueous extracts of *M. oleifera* has significantly altered hemato-biochemical parameters in streptozotocin induced diabetic rat model.

TOX-15

CADMIUM PRODUCES DOSE-DEPENDENT DIFFERENTIAL EFFECTS ON RAT MYOMETRIUM

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In vivo modulation of myometrial spontaneity by cadmium (Cd) and its regulatory pathways were studied in rat myometrium following exposure of rats to 3, 10 and 30 ppm Cd in drinking water for 28 days. Cadmium and Ca^{2+} levels in uterus and blood were measured by atomic absorption spectroscopy. Isometric tension in myometrial strips, under a resting tension of 1g, mounted in organ bath containing Ringer-Locke solution continuously aerated with carbogen, was measured using data acquisition system-based physiograph and Lab Chart Pro V7.3.7 software. There was dose-dependent increase in levels of cadmium in both blood and uterus, however rise in calcium was observed only in uterus and it was not statistically significant. Increasing concentrations of cadmium resulted in significant increase in absolute tension and mean integral tension while the increase in frequency of myometrial contraction was non-significant. Cadmium decreased the contractile effect of calcium chloride, 80mM KCl, histamine and oxytocin at lower doses (3 ppm) while increased at higher doses (10 and 30 ppm), as compared to control. Contrary to the effect on spasmogens, cadmium potentiated the relaxant response of phenylephrine at lower dose (3 ppm) while inhibited the relaxant response at higher dose (30 ppm). Thus based on the results of our limited study, it may be inferred that following *in vivo* exposure of rats to cadmium, cadmium accumulates in uterus and differentially alter the effect of uterotonics and tocolytics on rat myometrium at-lower and higher doses, and thus has the potential to adversely affect female reproduction; however, underlying mechanism is yet to be elucidated.

ISVPT-2016

TECHNICAL SESSION - X

MOLECULAR & NEUROPHARMACOLOGY

Chairperson : Dr. C. C. Barua

Co-chairperson : Dr. Usha P.T.A.

Rapporteur : Dr. Arpita Shrivastava

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LEAD-MNP-01

ROLE OF TRPV4 CHANNELS IN PULMONARY VASCULATURE

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Introduction

The pulmonary artery is formed by three layers such as inner intima (pulmonary arterial endothelial cells), media (pulmonary arterial smooth muscle cells) and outer adventitia (fibroblasts). Pathogenesis of pulmonary arterial hypertension is attributed to the effects of vascular remodeling, vasoconstriction, *in situ* thrombosis, and arterial wall stiffening all of which attributes to an increase in peripheral vascular resistance leading to right heart failure (Morell *et al.,* 2009). The features of pulmonary vascular remodeling in pulmonary arterial hypertension include medial and intimal cell layer thickening and lesions that occlude the artery. Vasoconstriction is an increase in tensile force which translates to narrowing of the lumen of the vessel. The sustained vasoconstriction in pulmonary arterial hypertension is caused by an increased cytosolic Ca^{++} in pulmonary arterial smooth muscle cells, which leads to smooth muscle contraction by both Ca^{++}-dependent and Ca^{++}-independent mechanisms by directly activating the myosin light chain kinase. Also, an increase in cytosolic Ca^{++} stimulates Ca^{++}-dependent signal transduction proteins (Fernandez *et al.,* 2012).

Pulmonary arterial hypertension

Pulmonary arterial hypertension is a severe chronic disorder that affects the pulmonary circulatory system characterized by an increase in pulmonary vascular resistance which involved proliferation and migration of pulmonary arterial smooth muscle cells. Endothelial dysfunction is considered to play an important role in pulmonary hypertension. It is hemodynamic irregularity that may be heritable, idiopathic or secondary to other diseases such as chronic obstructive pulmonary disease. Pulmonary arterial hypertension develops when the mean pulmonary artery pressure is sustained at an elevated level =25 mmHg at rest or 30 mmHg during exercise and is associated with a progressive increase in pulmonary vascular resistance (Firth et al., 2007). Increased pulmonary vascular resistance may be caused by sustained pulmonary vasoconstriction and considerable obstruction of the lumen of small arteries caused primarily by excessive proliferation of pulmonary artery smooth muscle cells in the vascular wall. An increased pulmonary vascular resistance results in increased right ventricle afterload which leads to right heart failure and eventually death (Firth *et al.,* 2010).

Transient receptor potential channels (TRP)

TRP channels belong to the superfamily of cation channels formed by tetramers of six transmembrane domains subunits. But unlike voltage-gated ion (Ca^{2+} and K^{+}) channels, TRP subunits do not possess a voltage sensing moiety, making their activity insensitive to changes in membrane potential, therefore functioning as voltage-independent, non-selective cation channels which are permeable to Na^{+}, K^{+}, Li^{+}, Ca^{2+}, and Mg^{2+}. TRP subunits are split into several subfamilies according to their activation stimuli and the presence of regulatory domains on the cytosolic N and C-terminals. TRP channels play an important functional role in the regulation of vascular tone (Firth et al., 2007). TRP channels contribute to mechanosensation and G protein-coupled receptor-initiated signaling pathways that modulate vasoconstrictor and vasodilator activity and cellular

proliferation. In vascular endothelial cells, Ca^{++} entry through TRP channels is an important contributor to endothelium-dependent vasodilatation, vascular wall permeability, and angiogenesis. Altered TRP channel function has been commonly linked to vascular disorders, including hypertension, vascular occlusive disease, inflammation and pulmonary edema (Jia and Lee, 2007).

Transient receptor potential vanilloid channels (TRPV4) in pulmonary vasculature

The mRNA of TRPV1, TRPV2, TRPV3, TRPV4, and TRPV6 of the TRPV family were detected in pulmonary arteries. Among the TRPV channel subfamily, TRPV4 predominantly is expressed in pulmonary arterial smooth muscle cells and other smooth muscle. The endothelium in pulmonary blood vessels regulates the vascular tone by release of several vasoactive factors that include both vasodilators such as nitric oxide, endothelium-derived hyperpolarizing factors and prostacyclin as well as vasoconstrictors like thromboxane A2, leukotrienes, endothelin and superoxide anions (Addison *et al*., 2016). Transient receptor potential vanilloid (TRPV4) channels are non-selective cation channels permeable to Ca^{++}, Na^{+}, and Mg^{++} ions. These channels are widely expressed in the cardiovascular system including endothelial cells, cardiac fibroblasts, vascular smooth muscles, and perivascular nerves. Therefore, TRPV4 channels play a pivotal role in the maintenance of cardiovascular homeostasis. These channels mediate hypoxia-induced increase in proliferation and migration of pulmonary artery smooth muscle cells and progression of pulmonary hypertension. TRPV4 channels maintain flow-induced vasodilation and preserve vascular function by directly activating Ca^{++}-dependent K_{Ca} channels. Furthermore, these may also induce vasodilation and maintain blood pressure indirectly by evoking the release of NO, CGRP, and substance P (Randhawa and Jaggi, 2015). Mainly TRPV1 and TRPV4 are expressed in pulmonary artery smooth muscle cells and implicated in the remodeling of pulmonary artery, a landmark of pulmonary hypertension (Penumatsa *et al*., 2014). TRPV4 channels are present both in endothelial and vascular cells and are implicated in the regulation of vascular tone. It has been observed that the interplay between endothelial cells (ECs) and the underlying vascular cells plays a critical role in determining vascular tone, regional blood flow and arterial pressure in different vascular beds. Vasoactive agonists like acetylcholine, adenosine triphosphate, bradykinin and mechanical stimuli, such as flow and shear stress cause a rapid increase in intracellular Ca^{++} leading to synthesis and release of endothelium-derived vasodilators like nitric oxide (NO), endothelium derived hyperpolarizing factor (EDHF) and prostacyclin (Sukumaran *et al*., 2013). Recently, our laboratory revealed that pharmacological activation of TRPV4 channels with the selective agonist GSK1016790A caused robust relaxation of endothelium-intact rat pulmonary artery rings. The selectivity of TRPV4 channel antagonism by a selective TRPV4 channel inhibitor was confirmed by the observation that the antagonist did not modify the endothelium-independent relaxation produced by sodium nitroprusside in the rat pulmonary artery. Further, we confirmed that endothelial TRPV4 channels are present in the endothelium of the rat pulmonary artery and their activation causes endothelium-dependent relaxation through nitric oxide and endothelium-derived hyperpolarizing factor. However, it remains to be seen whether any endogenous agonist or blood flow in pulmonary circulation would activate these channels to regulate the pulmonary arterial tone in health and diseases (Sukumaran *et al*., 2013). Recently we have explored that nitric oxide synthesis must be intact for the expression of the endothelium-derived hyperpolarizing factor mediated by TRPV4 channels in the rat pulmonary artery. Once nitric oxide inhibits by L-NAME, it leads to attenuation of endothelium-derived hyperpolarizing factor response mediated by GSK1016790A-induded relaxation

mediated by TRPV4 channels but presence of blockage of thromboxane unmasks the endothelium-derived hyperpolarizing factor pathway. Further, a mechanistic study is required to rule out the appearance of endothelium-derived hyperpolarizing factor in presence of thromboxane synthesis inhibition (Addison *et al*., 2016).

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MNP-01

UPREGULATION OF LPAR1 AND LPAR6 mRNA IN EARLY PREGNANT BUFFALO ENDOMETRIUM

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The purpose of the present study was to demonstrate the presence of the lysophosphatidic acid (LPA) receptors, and compare their expression in nonpregnant and early pregnant buffalo uterus. Nonpregnant (NP) luteal (n=6) and early pregnant (P) uterine tissues (n=6) were collected from a local slaughter house within 20 minutes of exsanguination. Early pregnancy (29-42 days) was detected on the basis of crown-rump length of the foetus. Total RNA was isolated from the endometrium and cDNA was synthesized by using commercially available kits following manufacturer's instructions. PCR was carried out to amplify the nucleic acid fragments of all the six LPAR mRNAs in both nonpregnant and early pregnant endometrial tissues. The primers for the purpose were predesigned using IDT software from predicted buffalo LPAR sequences available at NCBI website. These mRNA fragments were sequenced and submitted to NCBI Genbank (KU684452, KU543688, KU684453, KU684454, KU684455, KU543689). Comparative profiling of each mRNA by SYBR Green-based real-time PCR demonstrated a significant increase (P<0.05) in LPAR1 (1±1.24 in NP vs 23.58 ±1.27 in P) and LPAR6 (1±1.02 in NP vs 27.09±1.15 in P) mRNA expression in early pregnant endometrium compared to nonpregnant endometrial tissues. On the other hand, LPAR2 (1±1.25 in NP vs 7.36±2.3 in P), LPAR3 (1±1.29 in NP vs 9.78±1.67 in P), LPAR4 (1±1.15 in NP vs 0.37±1.42 in P) and LPAR5 (1±1.39 in NP vs 2.15±1.73 in P) mRNA expressions were not altered (P>0.05) in early pregnancy. The results of the present study suggest that 1) mRNA of all the six receptors of lysophosphatidic acid are expressed in buffalo endometrium, 2) Out of the six, LPAR1 and LPAR6 mRNA are upregulated during 29-42 days of early pregnancy.

MNP-02

VASORELAXANT EFFECT OF QUERCETIN MAY BE MEDIATED BY NO AND PGI_2 PATHWAYS IN GOAT PULMONARY ARTERY

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Quercetin, a flavonoid present in apples , onions, citrus fruits, berries, red grapes, red wine, broccoli, bark roots, flowers, and tea. It possesses antihypertensive effect which could be mediated via EDRF/EDHF pathways. Such effect of Quercetin in goat pulmonary artery (GPA) could be useful in controlling pulmonary hypertension. The present study examines the possible mechanisms of vasorelaxation of Quercetin in GPA. 5HT (5-hydroxy tryptamine, 10µM)-induced a contractile response of 1.0 gm ± 0.2 gm attained in 8-10 minutes. ACh (1nM-0.1mM) and Quercetin (1nM-0.1mM) were added cumulatively in 5HT precontracted GPA

rings. To examine the role of EDRF / EDHF, vasorelaxation of ACh and Quercetin was elicited either in ED- rings or in presence of L-NAME (10µM), Indomethacin (10µM) , L-Arginine(10µM) in ED + rings . The Ach induced concentration related vasorelaxation in ED+ rings was shifted to right with decrease in I_{max} 23.38± 1.5 % and $IC_{5:}$ 7.13 ± 0.09 µM. Quercetin induced vasorelaxation with I_{max} 33.83± 1.62% and IC_{50} 6.99±0.05 µM in ED+ rings was significantly inhibited with decrease in I_{max} 24.87±1.49% and IC_{50} 7.68±0.14 in ED- rings. L-Arginine did not poteintiate vasorelaxation of Quercetin. Indomethacin attenuated the vasorelaxation of Quercetin with rightward shift of vasorelaxation curve with significant decrease in I_{max} 19.50± 2.47%. In contrast, L -NAME moderately inhibited the quercetin induced vasorelaxation with right ward shift of vasorelaxation curve with decrease in I_{max} 26.49± 4.2% and IC_{50} 6.26±0.52 µM.In conclusion, the secondary branch of GPA is moderately relaxed with ACh suggesting that NO mediates the vasorelaxation. The vasorelaxation of Quercetin induced may be mediated in part by EDRF via eNOS and PGI_2 pathways.

MNP-03

STUDY OF INDIGENOUS PLANT PRODUCT ON EXPERIMENTALLY INDUCED NEUROPATHIC PAIN IN RATS

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Neuropathic pain is defined as pain arising as a direct consequence of a lesion or disease affecting the somatosensory nervous system. Neuropathic pain can persist without an obvious injury. Hyperalgesia and allodynia is the primary sign of the neuropathic pain. Inspite of intensive research findings, the therapeutic approach remains least satisfactory. In the present study we aim to confirm whether capsaicin can reduce the pain and stress condition in neuropathic birds induced by sciatic nerve crush. In this study ducks were exposed to sciatic nerve crush. Capsaicin was given at the dose rate of 1 µg /kg body weight of at the surgical site in the treated group by local infiltration. The growth rate was observed by measuring the weight of the birds and examining posture and gait. Pain perception to cold stimulation was measured from 0 to 4th week of the experimental group by submerging the foot in ice cool water ($4^0C \pm 0.5^0C$). Lipid peroxidation, reduced glutathione and serum cortisol level were measured in all groups of birds to study role of oxidative stress. Thiobarbituric acid and trimethylamine were assessed to evaluate the quality of meat. The sham operated group of birds behaved normally throughout the study period. There was significant rise in the body weight in capsaicin treated ducks as compared to neuropathic birds. There was significant increase in pain threshold by giving cold stimuli in capsaicin treated birds, compared to untreated birds. Malondialdehyde and reduced glutathione increase significantly suggesting the aspect of oxidative stress in neuropathic pain. One of the

biomarker of stress is serum cortisol. It increased significantly in neuropathic birds than the other groups, whereas the concentration recorded was significantly decreased in capsaicin treated birds. Thiobarbituric acid concentration was more in treated birds as compared to neuropathic birds. The TMA concentration was significantly elevated in neuropathic birds suggesting stress could affect the quality of meat. Thus, our present findings indicate that capsaicin did ameliorate the pain and stress condition generated by neuropathic pain, hence suggesting that it can be used as a potential drug in treating the neuropathic pain.

MNP-04

HYPERCHOLESTEROLAEMIA SUPPRESSES THE EXPRESSION OF CONTRACTION-ASSOCIATED PROTEINS IN LATE PREGNANT MOUSE UTERUS

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The purpose of the present study was to examine the effect of hypercholesterolaemia on contractile proteins expression in late pregnant mouse uterus. Apparently healthy female Swiss albino mice were fed with high fat high cholesterol diet (0.5% sodium cholate, 1.25% cholesterol and 15% fat) for 6 weeks. The animals were then kept for mating with males at a ratio of 1:1. The day of observing vaginal plug was considered as day 1 of gestation. High fat high cholesterol diet was continued throughout the mating and gestation period. On day 19 of gestation, serum cholesterol level was increased (P<0.05) more than 3 fold (272.4± 22.04 mg/dl, n=7) as compared to control animals (84.51 ± 4.95 mg/dl, n=7) fed with a standard chow. Animals were sacrificed on day 19 and uterine tissues were subjected to membrane protein extraction, SDS-PAGE and subsequent Western blotting for detection and quantitation of oxytocin receptor (OTR), Gq/11, and Rho A proteins. The primary antibodies to OTR (Rabbit monoclonal, 1:1000), $G_{q/11}$ (Rabbit polyclonal, 1:200), Rho A (Goat polyclonal antibody, 1:500) and GAPDH (Rabbit polyclonal, 1:5000, Santa Cruz Biotechnology, Inc., CA, USA) were used to detect the respective proteins. Densitometric analysis of the Western blot data by Image J software revealed a significant reduction (P<0.05) in the OTR protein expression in HFHC diet-fed mice compared to control mice. Control animals showed GAPDH normalized value of 8.65 ± 1.21 (n=6) as compared to 4.66 ± 0.61 for HFHC animals (n=6). Similar were the Gq/11 (3.36 ± 0.87, n=4 vs control, 6.38 ± 0.28, n=4; P<0.05) and RhoA (0.07 ± 0.02, n=3 vs control, 0.28 ± 0.02, n=3; P<0.05) protein expressions which showed significant decrease in reference protein normalized values in HFHC-fed animals. The results of the present study suggest that hypercholesterolaemia suppresses the expression of contraction-associated proteins in late pregnancy.

MNP-05

VASORELAXATION MECHANISMS OF EUGENOL IN MIDDLE UTERINE ARTERY OF NON-PREGNANT *CAPRA HIRCUS*

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Hypertensive disorders during pregnancy include gestational hypertension and preeclampsia which are the major causes of maternal and fetal morbidity and mortality, affecting 510% of pregnancies. Eugenol (4-Allyl-2-methoxyphenol) an essential oil and chief component of clove oil, could be a possible agent to produce antihypertensive effect with vasorelaxant action property mainly used in food industry. Our objective is to investigate the endothelium dependent or /and independent vasorelaxation mechanisms of eugenol in middle uterine artery (MUA) of non pregnant goat (NPG). Eugenol (1nM-1mM) induced vasorelaxation was elicited in phenyephrine (PE, 10µM) precontracted MUA rings (1.5-2 mm) adopting standard functional experiments using an automatic organ bath. The isometric contraction was recorded with the help of Lab chart 7 pro software (AD Instrument software, Australia). Eugenol caused a concentration related vasorelaxation (R_{max} 50% and -Log IC_{50} 3.87) in PE precontracted ED+ MUA rings. The R_{max} of eugenol was reduced to 36% by endothelium denudation, increased to 60% by L- arginine with non- significant alteration in -Log IC_{50}. Similarly, -Log IC_{50} of eugenol was significantly ($p<0.001$) increased to 6.99 and 6.68 with little change in R_{max} by L-NAME and indomethacin, respectively. Vasorelaxation to eugenol was significantly attenuated with decrease of R_{max} by 4-AP (31.8%), barium (25.2%), ouabain (32%), glibenclamide (18%). Eugenol did not inhibit the KCl-depolarization evoked sustained contraction. In conclusion, eugenol induced vasorelaxation in MUA of NPG could be mediated by activation of EDHF (Na^+- K^+ ATPase, K_{ir} ,K_V and K_{ATP}) and K_{ATP} channels being the major contributor for vasorelaxation.

MNP-06

PHARMACOLOGICAL STUDIES ON CHARACTERIZATION OF STORE-OPERATED CALCIUM CHANNELS (SOCC) IN MYOMETRIUM OF BUFFALOES

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Present study unravels the existence and functional involvement of store-operated calcium channels in myometrium of non-pregnant buffaloes. Uteri along with ovaries were collected from nondescript adult cyclic

buffaloes immediately after their slaughter from the local abattoir and transported to laboratory in chilled (4.0 ± 0.5°C) Ringer-Locke solution (RLS) having pH of 7.4. Isometric tension was recorded in isolated myometrial strips of non-pregnant buffaloes mounted in a thermostatically (37.0 ± 0.5°C) controlled organ bath containing Ca^{2+} free RLS. Under a resting tension of 2 gm, effect of $CaCl_2$ in the absence and presence of different blockers/modulators of calcium regulatory pathways was recorded. $CaCl_2$ produced concentration-dependent contraction and the DRCs of $CaCl_2$ were significantly ($P < 0.05$) shifted to right in the presence of nifedipine (1 µM), nifedipine (1 µM) + CPA (10 µM) and nifedipine (1 µM) + CPA (10 µM) + 2-APB (10 µM). After incubation of myometrial strips with nifedipine + CPA in Ca^{2+} free RLS (-Ca^{2+}), histamine was added to the tissue bath to allow the release of Ca^{2+} from SR while having already blocked the SERCA by CPA (10 µM) to prevent the Ca^{2+} reuptake into SR and nifedipine was used to prevent entry of Ca^{+2} from VDCC when calcium chloride was added and 2-APB (10 µM) was used as a non-specific blocker of SOCC. In the presence of nifedipine + CPA + 2-APB, calcium chloride produced contractile effect and the maximal contraction observed was only 0.62 ± 0.14 g (n=6) which was significantly ($P<0.05$) lower compared to that of 1.20 ± 0.10 g (n=6) in the presence of nifedipine + CPA in normal Ca^{2+} free RLS (-Ca^{2+}). This observation indicated that after depletion of SR, SOCC got activated and in the presence of 2-APB, response was significantly reduced. Thus, implying the functional involvement of store-operated calcium channels in myometrium of non-pregnant buffaloes.

ISVPT-2016

TECHNICAL SESSION - XI

ANIMAL WELFARE AND GOOD LABORATORY PRACTICES

Chairperson : Dr. N. Prakash

Co-chairperson : Dr. Sudhirkumar Tiwari

Rapporteur : Dr. Neetu Rajput

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LEAD-AW-01

ETHICS AND ANIMAL WELFARE IN ANIMAL EXPERIMENTATION

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It is the duty of every citizen of India, 'to protect and improve the natural environment including forests, lakes, rivers and wild life, and to have compassion for living creatures'

- Constitution of India (Article 51 A (g)

Need for laboratory animal:

The importance of animal experimentation need not be overemphasized. There are many an important discoveries in the field of medicine that have been largely possible due to experimentation with laboratory animals. Laboratory animals play a crucial role in the drug development process. Since they possess similar organ systems, metabolism, enzyme systems they are a suitable alternative to the human beings and the results obtained are extrapolatable to man. They are small in size, reparable in controlled environment and are highly prolific. High level of genetic purity is possible thus making it yield reproducible reliable results.

Need for Animal Welfare:

The use of animals for experimentation has been a subject of great debate for many decades now. While there have been lots of discussions on the propriety of use of animals to benefit human race, the use of animals for experimentation with adequate care and compassion for the animals used seems to be a well-accepted norm.

Goals of animal welfare:

Ethical goal:

To meet the noble desire to treat all living beings with care and compassion

When a man has pity on all living creatures then only is he noble. --Buddha

he love for all living creatures is the noblest attribute of man. -- Charles Darwin

Research goal:

To provide ideal conditions of animal rearing so that the animals are devoid of stress, pain and suffering during the study to achieve results that are reliable and reproducible.

Legal goal:

Keeping up the spirit of constitution the Government of India has laid down the principles of animal welfare in the Prevention of Cruelty to Animals Act, 1960. Sec 15 of the Act provides for the formation of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Under this the first set of guidelines called the Breeding of, and experiments on animals (control and supervision) rules were laid down. Further amendments were brought in 2001 and 2006. As a result the rearing of experimental animals and experiments on animals in the entire country has been brought under the ambit of CPCSEA.

Some of the important points in the guidelines:

- No establishment can breed experimental animals or trade animals for experiments unless registered

- All institutions using experimental animals for research / education should register with the CPCSEA
- Proper care of animals during and after experimentation by trained staff
- Constitution of IAEC is a pre-requisite for registration
- All experiments on small animals need approval of IAEC
- Register of experiments carried out is to be maintained for inspection
- Experiments on large animals are to be approved by the sub-committee for large animals
- Animal experiments are to be carried out by qualified personnel or under supervision by trained personnel
- Experiments are to be carried out with humane care
- Animals are looked after with care before and after experimentation
- Wherever necessary appropriate anesthesia should be performed
- If recovery after experimentation involves sever pain or injury, perform euthanasia
- Experiments are not to be performed for gaining manual skills or for public demonstration, except in schools, colleges and recognized training institutions
- No experiments can be carried out without justification
- The institution / IAEC shall maintain records of animals in its control / custody, and provide whatever information needed by the committee

The main functions of CPCSEA are:

The CPCSEA ensures that all the experiments involving animals in our country are carried out in animal houses approved by CPCSEA, with qualified persons and with due approval for each and every project. The CPCSEA lays strict emphasis on the quality of animal house with assurance of 'five freedoms' for the animals and also strict adoption of '3Rs' while deciding on the permission to conduct animal experiments.

FIVE FREEDOMS: The concept of five freedoms is generally applicable to all animals, whether they are in farm or performing animals or animals for research.

1. Freedom from hunger and thirst
2. Freedom from environmental discomfort
3. Freedom from external injury, pain and disease
4. Freedom from stress and mental distress
5. Freedom to move and express its normal behaviour

Institutional Animal Ethics Committee (IAEC)

In every institute where animal based research is planned, the institute should mandatorily have a committee called the Institutional Animal Ethics Committee (IAEC), which screens all the research projects and sanctions the animals required for the research. It consists of members from biological scientists, socially aware members and nominees of CPCSEA. No animal experiment is permitted without the approval of the IAEC.

Procedure of Animal Experimentation Clearance:

- A proposal entertained only when the Animal House Facility is approved
- The approval for experimentation on small animals by IAEC.
- Approval for experimentation on Large Animals will be considered by Sub Committee on the recommendation of IAEC

- The decision in the IAEC would normally be taken by consensus. However, in case of dissent by the CPCSEA nominee, the proposal with the dissenting note submitted to Large Animal Sub-committee for taking a final decision

3R principle: They were first described by W. M. S. Russell and R. L. Burch in 1959.

The 3Rs are:

1. Replacement: methods which avoid or replace the use of animals in research
   Refers to 'replace with less sentient species'
   Refers to 'replace in vivo with in vitro tests'
2. Reduction: use of methods that enable researchers to obtain comparable levels of information from fewer animals, or to obtain more information from the same number of animals.
3. Refinement: use of methods that alleviate or minimize potential pain, suffering or distress, and enhance animal welfare for the animals used.

The adoption of 3R is expected in any research work involving animals. The committee carefully screens the projects submitted and accepts or rejects the proposals taking the 3R principle into consideration.

LEAD-AW-02

LABORATORY ANIMAL WELFARE: IT'S INFLUENCES AND ASSESSMENT

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Introduction

Laboratory animals can be considered the life-line of biological research and drug discovery programmes. The use of animals is increasing for testing the antisera, new drug entities, vaccines as well as for basic research. It is obligatory to understand the behavior of laboratory animals to handle them gently and humanely, reduce unnecessary pain and unwanted usage of animals by enforcing good welfare, ethical consideration and fulfilling their micro and micro environment.

The animal welfare is defined as "*the avoidance of abuse and exploitation of animals by humans by maintaining appropriate standards of accommodation, feeding and general care, the prevention and treatment of disease and the assurance of freedom from harassment, and unnecessary discomfort and pain*". A sound understanding of laboratory animal welfare should be grounded in a general knowledge of some of the major events, organizations, and philosophies that have shaped how animals are used and cared for since the industrial revolution. It includes the evolution of animal protection and advocacy movements, the development of professional animal science associations, and the creation of laws and regulations governing the use of animals in research.

History of Welfare of Animals used in experimentation

The use of animals in research in the 1600s in Europe expanded as people were searching for greater

knowledge. In the 1800s, many people were exploring a variety of medical related conditions. Since that time, the numbers and types of animals used in the search for cures to medical diseases and conditions have expanded greatly. Until the late 1800s, there were no laws anywhere that governed how animals shall be used in research or vaccine production.

Since 1822, when Irish MP Richard Martin brought the "Cruel Treatment of Cattle Act 1822" through Parliament offering protection from cruelty to cattle, horses, and sheep. Martin was among the founders of the world's first animal welfare organization, the Society for the Prevention of Cruelty to Animals, or SPCA, in 1824. One of the first national laws to protect animals was the UK "Cruelty to Animals Act 1835". In 1837, the German minister Albert Knapp founded the first German animal welfare society. The first national law to regulate animal experimentation was passed in Britain in 1876the Cruelty to Animals Act of 1876. The Animal Welfare Act of 1966 set minimum standards for the handling, sale, and transport of cats, dogs, non-human primates, rabbits, hamsters, and guinea pigs held by animal dealers or pre-research in laboratories. Improved Standards for Laboratory Animals Act as part of the Food Security Act of 1985 for expanding the regulation for care of animals in research laboratories and prevention of misconduct on research animals.

Regulations, Policies, and Guidelines Impacting Laboratory Animal Welfare in India

The first law for prevention of animal cruelty were came in existence first time in India by 1960, which was known as the Prevention of Cruelty Animals Act,1960. Additional laws were implemented in 1965 to prevent the cruelty of animal used in transportation and implemented as the Prevention of Cruelty to Draught and Pack Animals Rules, 1965. In 1968, A specific law were prepared to control the animal experimentation and prevent unnecessary suffering of animals which was known as "The Experiments On Animals (Control And Supervision) Rules, 1968 and further amended in 1998 & 2006. In 1973, another laws were implemented to prevent the cruelty of animals performing in the circus, which is known as the Performing Animals Rules, 1973 and later on amended in 2001 as "The Performing Animals (Registration) Rules, 2001". In 1978, laws were prepared and implemented for preventing the cruelty while transportation of animals, which known as "The Transport of Animals Rules, 1978". In 2001, in addition to amendment of performing animals protection law, additional law were prepared and implemented for preventing cruelty while and before slaughtering of animals, which known as "The Prevention of Cruelty to Animals (Slaughter House) Rules, 2001"

What is good state of animal welfare?

Terrestrial Animal Health Code of World Organization for Animal Health defines animal welfare as "how an animal is coping with the conditions in which it lives". An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behavior, and if it is not suffering from unpleasant states such as pain, fear, and distress. Good animal welfare requires disease prevention and veterinary treatment, appropriate shelter, management, nutrition, humane handling and humane slaughter/killing. Animal welfare refers to the state of the animal, the treatment that an animal receives is covered by other terms such as animal care, animal husbandry, and humane treatment (OIE, 2011). This is a complex issue and part of a continuing scientific and philosophical debate.

Welfare may be considered to be a subjective experience, it has a biological function that is related to the

fitness and survival of the animal, and researchers have suggested that welfare is compromised when the animal's evolutionary fitness is reduced. If animal's welfare is compromised, the animal fitness is compromised in terms of animal physiology which eventually leads to compromised experimental outcome. Animal experiments should only be performed when no alternative is available and when the benefit of the experiment outweighs the suffering of the animal.

Assessment of Welfare of Laboratory Animals

Assessing welfare is also a complex problem, and numbers of approaches have been tried to address it. The question is whether the animals are physically and psychologically healthy and whether they get what they want. In respect to health, early warning signs are obviously important. Needs can be defined as fundamental biological requirement of the animal, to obtain a particular resource or respond to a particular environmental or stimulus, whereas 'wanting' is related to an incentive motivation. Accordingly, the veterinary profession has great responsibility and tremendous opportunity to work with people and animals to ensure animals' good welfare. The effective assessment of pain, suffering, and distress in laboratory animals is an important issue at present (Baran et al., 2010), largely because it is a crucial step for reducing animal suffering. The ability to know signs of distress as rapidly as possible means that proper action may be taken to alleviate the suffering. Reducing suffering and improving welfare is also extensively recognized to be an important component of science (Kelly, 2010). In laboratory animals, number of clinical signs which indicates moderate to severe suffering, such as hunched posture, piloerection, laboured respiration, vocalisation, and self-mutilation. For instance, changes in fecal pellet consistency, stiff movements, reduced alertness, or reduced nest-building behavior can indicate milder levels of suffering which are likely to alter animal physical and physiological behavior; hence such observation can be acted upon in good time.

Many criteria for the assessment quality of welfare of animals have already been put forward (Barnard and Hurst 1996, Morton, 1997) including physiological effects (e.g. growth, reproduction, longevity, immune suppression, corticosteroid levels, disease, and injury) and behavioral responses (e.g. preferences, stereotypies, anxiety). However, it is unrealistic to include them all in any programme of routine monitoring because of time and labour related constraints. Therefore, the challenge is to select measurable biological parameters that will cover most of these criteria, while enabling the monitoring of a large number of animals. A number of methods have been explored for monitoring the health and welfare of laboratory animals using score sheets (MUAWC, 1999). Approaches for assessment of laboratory animals' welfare should be naturalistic, functional and subjective. It should be assessed by observing the behavior, physiological, health and production aspects of animals. An assessment of animal welfare must be made through different measures in addition to health indices.

Behavioral responses: The choice of animals should be taken care by the person involved in handling of animals. If environment and material of choice is provided to animals, the level of stress will be reduced. The assessment of correlation of enriched environment provided and behavior performed by the animals in housing system must be compared with behavior in normal condition. Enrichment of the animal's environment can be focused on both the social environment (social partners, including human beings) and the

physical environment consisting of sensory stimuli (auditory, visual, olfactory and tactile) and nutritional aspects. More precise welfare need to contemplate specific behavior of particular species facing environmental challenges. Continuous monitoring by scientist having expertise in assessment of behavior of particular species of animal should be followed.

Physiological responses: Physiological changes include weight loss, reduced food intake, diarrhoea, respiratory and cardiovascular signs, and changes in stress-hormone levels and immunological parameters. The health monitoring of animals in laboratory facility is an essential process by which one can know the actual physiological status of animals by virtue of hematological and biochemical parameters. Post-mortem parameters are valuable in assessing animal welfare retrospectively, with the results being beneficial for the surviving animals; examples of postmortem parameters include fatty deposits, organ size, infections, stomach ulcers and dehydration. The health monitoring must be carried out at regular interval based on changes of breeding lines or nucleus of animals in production or any new line is introduced in facility.

Health status/responses: Maintaining normal health without pain, discomfort or disease state of animal is considered an important challenge against campaign for welfare of animals. Important criteria for assessment of animal welfare related to health is prevalence and intensity of certain health problems in animal colony. Providing hygienic feed, water and bedding material will enhance the reliability of micro environment of housing system of animals which may reduce the health related problems in facility. Individually ventilated cage can improve protection of the animals against micro-organisms at cage level, protection of the animal-care staff against allergens, the improved microclimate and the reduced need for cage cleaning.

Production Influence/ response: The macro and micro environment provided to animals may affect the growth of animals. It has been studied by various scientist and us also that the enrichment provided to rats boost the growth of animals due to enhanced wellbeing (feeling well) affected physiological changes. The good microenvironment provided to animals may also enhance the productivity of animals as well as mortality of newly born animals. Need based nutrient added feed is also a key factor for maintaining the productivity of animals.

Environments surrounding Animals and its Impact on Welfare

Proper housing and management of animal facilities are essential for animal well-being quality research data as well as health and safety of personnel. A good management program includes the appropriate micro and macro environment, spacious housing, proper nutrition and care that permit animals to grow, mature, reproduce, and maintain good health; provides for their well-being; and minimizes variations in outcome of research results. The micro environment of an animal is the physical environment immediately surrounding it such as primary enclosure with its own temperature, humidity, and gaseous and particulate composition of the air. The macro environment is physical environment of the secondary enclosure such as a room, a barn, or an outdoor habitat.

Euthanasia and Laboratory Animal Welfare

Veterinarians performing euthanasia must evaluate the potential for animal suffering due to physical discomfort, abnormal social settings, novel surroundings, pheromones or odors from nearby or previously

euthanized animals, or other factors including choice of euthanasia agent, time as well as process taken for loss of consciousness. To prevent/alleviate animal suffering, all laboratory animals must be euthanized in a timely manner, either as described in the approved protocol according to established time points, or as soon as necessary if established criteria or humane endpoints are reached. Human safety is of utmost importance, and appropriate safety equipment, protocols, and knowledge must be available before animals are handled. Once euthanasia has been carried out, death must be carefully verified. All laws and regulations pertaining to the species being euthanized, the methods employed, and disposal of the animal's body must be scientific.

Animal welfare by Veterinary profession

The American Veterinary Medical Association (AVMA) offered the following eight principles for developing and evaluating animal welfare policies.

- The responsible use of animals for human purposes, such as companionship, food, fiber, recreation, work, education, exhibition, and research conducted for the benefit of both humans and animals, is consistent with the Veterinarian's Oath.
- Decisions regarding animal care, use, and welfare shall be made by balancing scientific knowledge and professional judgment with consideration of ethical and societal values.
- Animals must be provided water, food, proper handling, health care, and an environment appropriate to their care and use, with thoughtful consideration for their species-typical biology and behavior.
- Animals should be cared for in ways that minimize fear, pain, stress, and suffering.
- Procedures related to animal housing, management, care, and use should be continuously evaluated, and when indicated, refined or replaced.
- Conservation and management of animal populations should be humane, socially responsible, and scientifically prudent.
- Animals shall be treated with respect and dignity throughout their lives and, when necessary, provided a humane death.
- The veterinary profession shall continually strive to improve animal health and welfare through scientific research, education, collaboration, advocacy, and the development of legislation and regulations.

Important challenges in Animal Welfare

Hosing (light and noise) environment: Human interaction and physical environmental factors are part of the stimuli presented to laboratory animals every day, influencing their behavior and physiology and contributing to their welfare. Certain environmental conditions and routine procedures in the animal facility might induce stress responses and when the animal is unable to maintain its homeostasis in the presence of a particular stressor, the animal's wellbeing is threatened (Castelhano-Carlos and Baumans, 2009).

Hygienic environment: A high level of hygiene and cleanliness must be kept at all times by providing the clean bedding material, water, feed to animals. Regular changing of cages or cleanliness provide good the environment to animals & affects the behavior as well as health of animals which ultimately affect research output.

Pain management after surgical intervention: Animal post-surgical pain is likely undertreated, but the literature is not sufficiently detailed. Pain management still awaits systematic analysis of how much and in what direction pain and pain relieving drugs might be use without interfering the outcomes of experimental results.

The scientific assessment of animal welfare: It is practically difficult to screen the status of animal welfare scientifically in each and every animal facility due to involvement of various factors. Some measures of animal welfare involve assessing the degree of impaired functioning associated with injury, disease, and malnutrition

In conclusion, the state of animal welfare and its scientific assessment is the key to ensure a benefit to experimental animals. The scientific evaluation of animal welfare may be the crucial step to reconcile the predicted and the measured values in an overall assessment of animal welfare.

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LEAD-AW-03

SKIN SENSITISATION ALTERNATIVE TEST METHODS AND APPROACHES

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Over the past 40 years the 3Rs, Replacement, Reduction and Refinement alternatives are widely accepted as ethical principles in animal studies. In case of skin sensitisation, animal models like Guinea Pig Maximization Test, Buehlers test, Local Lymph Node assays are employed to study induction and elicitation phases of skin sensitisation. Due to the complexity of biological mechanism, there is no single alternative non animal test

method which is formally validated and regulatory adopted for skin sensitization. Instead a combination of methods addressing key mechanisms of the sensitization will be needed to achieve full replacement.Mechanistically relevant non-animal test methods, like Direct Peptide Reactivity Assay (DPRA), KeratinoSen™ assay, USen assay, hCLAT assay are used in combination to predict the skin sensitisation outcome and thus to reduce and replace the use of laboratory animals for skin sensitisation testing. In chemico based, DPRA addresses the process of haptenation, i.e. the covalent binding of haptens (low-molecular weight substances) to proteins. This is considered to be the molecular initiating event of the skin sensitisation. The KeratinoSens™ addresses the activation of the antioxidant/electrophile response element (ARE)-dependent pathway in keratinocytes mimicking a biological response. Assays like USen and hCLAT are used to study cellular response of monocytes and dendritic cells by measuring surface markers like CD86 and CD54. These studies provide mechanistic information considered relevant for the assessment of the skin sensitisation potential of chemicals. For this study chemicals with different sensitisation categories were used, which includes in vivo extreme sensitizers (2,4-Dinitrochlorobenzene, Oxazolone), in vivo strong sensitizer (Formaldehyde), in vivo moderate sensitizer (Benzylideneacetone), in vivo weak sensitizers (Farnesal, 2,3 - Butanedione) and in vivo non-sensitizers (1-Butanol, 6- Methylcoumarin, Lactic acid, 4-Methoxyacetophenone). All these test items were analyzed for depletion with synthetic peptides of Cysteine and Lysine in DPRA assay. The results have predicted the expected outcome validating the importance of DPRA assay in skin sensitisation.

AW-01

EFFECTS OF RUBBER MAT BEDDING ON PRODUCTION PERFORMANCE AND WELFARE OF CROSSBRED COWS

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Present study was conducted on twelve lactating crossbred cows, maintained at Bhestan, Surat. These animals were divided randomly into two groups i.e., concrete floor without rubber mat (control) and concrete floor with rubber mat (treatment) during the winter, summer and monsoon season. The microclimatic observation taken during the experimental period: maximum temperature was 30.00^{0}C, 33.72^{0}C and 31.24^{0}C during winter, summer and monsoon season respectively. Average morning (7:30PM) THI was found to be 69.44, 79.43 and 80.71 in winter, summer and monsoon season, respectively. While in afternoon (2:30PM) values were 78.46, 84.70 and 83.78 respectively. Average monthly milk yield was 202.67±11.95 kg & 160.46±10.82 kg during winter and summer seasons, respectively in control group whereas, in treatment group average monthly milk yield was 224.63±13.72 kg & 177.47±13.19 kg. We observed that the monthly mean milk yield and its compositions were not significantly different ($P<0.05$) with treatment group animals. The average hock and knee lesion score (control vs. treatment) were 1.333±0.098 vs. 0.458±0.104, 1.375±0.101 vs. 0.625±0.101 & 1.875±0.125 vs. 0.917±0.133 during winter, summer and monsoon season, respectively. Further, it was observed that there was significant difference ($P< 0.05$) in the hock and knee lesion score between the two groups of animals. Hence, it could be concluded that the concrete floor with rubber mat improves the welfare in-house animal round the year.

AW-02

EFFECT OF USING AGRONET AS SHADE MATERIAL ON PHYSIOLOGICAL AND OXIDATIVE STRESS PARAMETERS DURING HEAT STRESS IN SURTI BUFFALO

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Heat stress may create imbalance between reactive oxygen species production and body's antioxidant defenses leading to oxidative stress subsequently affecting buffalo's health and performance. Changes in body antioxidants' reserve such as superoxide dismutase (SOD) and reduced glutathione (GSH) and markers of oxidative damage such as MDA production as an appropriate index of lipid peroxidation (LPO) serve to evaluate and measure the impact of heat stress on buffalo. A simple shade over an animal exposed to a hot environment and direct solar radiant energy from the sun cuts the radiant heat load on that animal by about 45%. However the micro climate underneath covered area depends on type of shade material. Therefore the present study was conducted to assess impact of using green Agronet as shade material on oxidative stress parameters during heat stress in Surti buffalo. Forty surti buffaloes were selected and divided into two groups of twenty each (control- open paddock unshaded and treatment-open paddock shaded with 75% Agronet). Floor temperature, ambient temperature and humidity were recorded during the study. Blood samples were collected on 0, 21 and 42 days and analyzed for oxidative parameters of LPO, SOD and reduced GSH. The mean temperature humidity index was 82.51±0.30, 85.17±0.17 for control and 82.31±0.40, 85.72±0.15 for treatment and floor temperature was 47.92±0.97, 47.60±0.91 for control and 35.67±0.0.75, 37.70±0.37 for treatment group at 21 and 42 day respectively. The oxidative stress parameters' values for control and treatment group at 0, 21 and 42 days was 2.63±0.12, 2.93±0.16, 2.29±0.18 and 2.78±0.47, 2.39±0.16, 2.54±0.15 for SOD (U/mg Hb); 5.76±0.46, 8.62±0.15, 8.13±0.28 and 4.44±0.45, 6.80±0.29, 7.02±0.20 for LPO (nmole of MDA/ml of cells) and 9.58±0.56, 7.75±0.15, 7.07±0.20 and 8.13±0.38, 8.09±0.23, 7.54±0.10 for GSH (mg/dl). Thus it was concluded that heat stress due to significantly ($P<0.01$) higher floor temperature in control group during both the duration led to significantly ($P<0.05$) increased SOD at day 21 and significantly ($P<0.01$) increased LPO at both day 21 and 42.

ISVPT-2016

TECHNICAL SESSION - XII

CLINICAL REGULATORY PHARMACOLOGY & TOXICOLOGY/ NUTRITIONAL PHARMACOLOGY

Chairperson : Dr. S. C. Parija

Co-chairperson : Dr. Thakur Uttam Singh

Rapporteur : Dr. R. D. Singh

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LEAD-CRPT-01

ALTERNATIVE METHODS TO ANIMAL TESTING AND COSMETIC PRODUCTS' SAFETY-AN OVERVIEW

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Cosmetics are personal care products used by millions of consumers around the world who expect them to be safe. These products are formulations comprised of specific combinations of substances which are usually referred as ingredients. The cosmetics industry needs to be innovative approaches, products being fast moving and consumers expect variations frequently. The industry is hence dependent on new ingredients and new uses of existing ingredients to provide new products that meet consumer demand. In order to market cosmetics following their foreseeable use, without any safety concerns, the cosmetics industry follows a robust scientific safety evaluation process at the same time meeting regulatory compliance.

The safety of cosmetic products is mainly based on the safety of the individual ingredient and the rationale comes from the fact that there are thousands of different cosmetic products on the market and they are all derived from a limited number of ingredients. The risk assessment typically follows a process starting with the hazard assessment of ingredients, dose response assessment, exposure assessment of ingredients and finally risk characterization on the ingredients and the whole product. The hazard characterization is done for local skin effects (dermal irritation, dermal sensitization, photo-toxicity etc.) as well systemic effects (reproductive toxicity, carcinogenicity, mutagenicity etc.). The first two steps invariably involve use of animals to evaluate the Tox end points.

Since the mid-20th century, ethical considerations have been emerging concerning animal experimentation. The cosmetic industry was the first to come under purview of restrictions on animal experimentation. Although the movement was initiated almost few decades ago, the actual ban was implemented for animal experimentation with regard to ingredients and finished product in Europe in 2013. This was followed by other countries, such as Norway, Israel, and New Zealand. India has followed the suit with regulations coming in force in 2014-15. As a result, a lot of recent changeovers have been observed in the field of toxicology including consideration of alternative methods. These changes, although, have put forth several challenges to conduct risk assessment, it has also provided opportunities to develop innovative alternatives for evaluating different toxicology endpoints. These alternatives are helping to come out of toxicity evaluation based on animal experimentation.

Some alternatives to the use of animals in testing include:

- *In vitro* methods and models based on human cell and tissue cultures
- Animal cell lines and tissues
- Stem cell and genetic testing methods
- Genomics
- Computerized patient-drug databases and virtual drug trials

- Computer models and simulations; *In-silico* predictions
- Non-invasive imaging techniques such as MRIs and CT Scans
- Micro dosing (in which humans are given very low quantities of a drug to test the effects on the body on the cellular level, without affecting the whole body system).

These non-animal methods enjoy several advantages but also suffer from certain shortcomings. After use of human cell lines/ tissues, they overcome issues of extrapolation of animal data owing to species differences that make applying animal test results to humans difficult. Plus they are also advantageous being faster and economical. Mainly they cover the animal ethics concern very well. On the contrary they lack the intact animal biological response and are semi quantitative in estimates of toxicity. Another challenge is , for complex toxicity end points there cannot be one method that can be used. There might be several mechanisms involved to exhibit any adverse outcome and each would require a separate model/method and one would require following an integrated approach to arrive at a conclusion. Implementing an alternative from idea to acceptance can take years. The developing of an alternative method is a multi-step process involving Defining, Developing, Validating and Accepting. One cannot use these alternatives unless they are completely validated. The validation itself frequently depends on animal data.

For cosmetics and consumer products, currently the validated alternative methods are available for skin corrosivity, skin irritation, eye irritation, skin sensitization, photo toxicity and genotoxicity. Plus *in vitro* skin absorption methods help in exposure assumptions. All these alternate tests have been developed, standardized and validated by scientific communities in close partnership with Industry for more than a decade. Skin sensitization has three validated methods and it is required to have favorable results in two out of three tests using integrated approach to conclude the result. However, there are few critical toxicological endpoints where alternate methods are still under development viz., reproductive toxicity, carcinogenicity, and repeat-dose systemic toxicity.

Research into alternative test methods has so far resulted in the incorporation of a range of new cell and tissue culture systems into the repertoire of alternative methods. Although the efforts in researching alternatives to animal testing methods over the years have produced a number of successful results, a great deal still needs to be done before it will be possible to eliminate animal testing completely. This will require consistent use of the most advanced research methods in the areas of molecular biology and computer technologies. Till such time a pragmatic approach is warranted.

LEAD-CRPT-02

OVERVIEW OF ENDOCRINE DISRUPTOR SCREENING PROGRAMME AND ITS KEY ELEMENT

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Man-made pollutants can affect the endocrine system and interfere with important developmental processes in human and wildlife. The presence of such chemicals in our environment raises concern because of the

harmful effects that have been observed on reproduction, growth, and development in some species of wildlife, aquatic, terrestrial, and laboratory animals. To screen these chemicals for their potential to produce effects similar to that produced by the hormones (estrogen, androgen) in humans, based largely on the EDSTAC (EDSTAC, 1998) recommendation, EPA developed a two-tiered framework for the Endocrine Disruptor Screening Program (EDSP): Tier 1 and Tier 2. Tier 1 screening data is used to identify substances that have the potential to interact with the endocrine system. Chemicals that go through the Tier 1 screening, if found to exhibit the potential to interact with the estrogen, androgen, or thyroid hormone systems, will proceed to the Tier 2 for testing. Five *in vitro* and six *in vivo* rodent assays, one assay in frog and fish are included in the Tier 1 Screening Battery. Tier 2 is designed to evaluate adverse effects for potential endocrine-active compounds identified in Tier 1 as well as to generate dose response data for use in the risk assessment. Tier 2 testing data identify any adverse endocrine-related effects caused by the substance, and establish a quantitative relationship between the dose and the adverse effect. In Tier 2 three *in vivo* assays are included. When performing the EDSP, the complexity of this study should not be underestimated and experienced testing laboratories with sufficient resources and historical control data for all parameters are essential.

LEAD-CRPT-03

REPRODUCTIVE TOXICOLOGY IN NON-CLINICAL SAFETY

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The occurrence of biologically adverse effects on the reproductive systems of females or males that may result from exposure to medicines and chemicals. The toxicity may be expressed as alterations to the female or male reproductive organs, the related endocrine system, or pregnancy outcomes. Reproductive toxicology aims to predict effects of medicines and chemicals on the ability of man to reproduce by assessing effects in animals. Reproduction is a moving target and requires special non-clinical toxicology studies to assess effects at specific points (such as pre-mating to conception, conception to implantation, implantation and organ formation, organ formation to end of pregnancy, birth to weaning and weaning to sexual maturity) in the reproductive cycle. Effects seen in man are likely to be retrospective and may not be detected until considerable damage has been done e.g. Thalidomide. In non-clinical testing, adverse effects of treatment, dose relationships, evidence of different sensitivity of reproductive organ systems compared to other systems, differential effects in mother / babies and NOAEL values are determined. Animal models available for reproduction toxicology testing are rat, rabbit, mouse, mini-pig, ferret, primate and dog.

CRPT-01

INFLUENCE OF PARENTERAL ADMINISTRATION OF VITAMIN E AND SELENIUM DURING PERIPARTURIENT PERIOD ON THYROID (T_3 & T_4) PROFILE IN SURTI BUFFALOES

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The research work was carried out on twenty Surti buffaloes during their transient period maintained at the LRS, Navsari Agricultural University, Navsari, Gujarat. The experimental animals were divided into two groups comprising of ten animals in each group as: Group-I: Treatment group of Surti buffaloes treated with Inj. Vitamin E and Selenium (E-CARE Se) on 60th, 45th, 30th and 15th day before expected date of parturition and after parturition on 15th, 30th day IM and Group-II: Control group of Surti buffaloes given Inj. Normal Saline (IM) as placebo treatment. Blood samples were collected on same days before injection as well as on the day of parturition, 45 and 60 days postpartum in serum clotting vaccutainer. The mean serum tri-iodothyronine (T_3) concentration was found to be significantly ($p<0.05$) lower at 60th day prepartum in treatment than control group and thereafter it was non-significantly differed at 45th day, 30th day and 15th day prepartum but significantly higher values were observed on the day of parturition and significantly lower values at 30th day and 60th day postpartum, while, non-significantly lower values at 15th day and higher at 45th day postpartum. The mean serum thyroxine (T_4) concentration was found non-significantly lower at 60th day prepartum in treatment than control group, but it was significantly higher at 45th day and non-significantly higher at 30th day and again significantly higher at 15th day before parturition and on the day of calving and thereafter fluctuated non-significantly higher at 15th day, lower at 30th day, higher at 45th day and non-significantly lower at 60th day after parturition. The mean serum T_3 and T_4 concentration did not differ significantly between pregnant and non-pregnant groups at any of the days studied, although the overall pooled mean T_4 level was non-significantly higher in the pregnant group as compared to non-pregnant group. It can therefore be concluded that after a repeated administration of selenium and vitamin E combination during the peripartum period, positive effect of increased selenium intake results on higher T_3 concentrations. Further, the decreased level of T_4 might support the mammary gland in partitioning of nutrients between mammary and non-mammary tissue at the onset of lactation.

CRPT-02

A COMPARATIVE STUDIES ON HORMONAL AND BIOCHEMICAL PROFILES OF NORMAL CYCLIC AND ANOESTRUS SURTI BUFFALOES

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A comparative studies on BCS (Body Condition Score), hormonal and biochemical profiles was carried out on twenty four (24) lactating Surti buffaloes, comprising of normal cyclic (n=12) and anoestrus (n=12) Surti buffaloes, maintained at Livestock Research Station, Navsari Agricultural University, Navsari, Gujarat. The BCS was non-significantly differed between normal cyclic and anoestrus Surti buffaloes. The mean serum T_3 (Tri-iodothyronine) and T_4 (Thyroxine) levels were found significantly ($p<0.05$) higher as 3.39±0.14 vs. 2.76±0.23 and 63.72±1.60 vs. 48.39±4.11 ng/ml, respectively whereas, mean serum cortisol levels differed non-significantly in between normal cyclic and anoestrus Surti buffaloes. The mean serum total cholesterol level was found significantly ($p<0.05$) higher as 88.79±1.51 vs. 82.40±2.55 mg/dl in normal cyclic as compared to anoestrus buffaloes however, the levels of mean serum BUN (Blood Urea Nitrogen) and creatinine were differed non-significantly between normal cyclic and anoestrus Surti buffaloes.

CRPT-03

EVALUATION OF GENOTOXICITY OF MELOXICAM AND KETOPROFEN IN WISTAR RATS

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The present work is intended to evaluate the genotoxicity of meloxicam and ketoprofen in rats. Because of no single *in vitro* assay is capable of detecting all the types, a battery of tests is recommended. i.e. Gene mutation and chromosomal aberration tests, Bone marrow micronucleus test (MNT) and Single Gel Electrophoresis (Comet Assay). Along with genotoxicity, some of the subacute toxico-pathological parameters such as haematology, gross pathology and histopathology were also evaluated. Study was conducted on 6-8 weeks old 48 wistar rats (in two phases). Bodyweight of the rats were between 120-180 grams. Meloxicam with the dose rate of 8 mg/kg and 16 mg/kg BW were administered orally for 28 days in phase I. In the II phase, ketoprofen orally administered at the dose rate of 5 mg/kg BW and 10 mg/kg BW. Cyclophosphamide (20 mg/kg) was used as a positive control for genotoxicity administered before 24 hours of sacrificing the test animals. After 28 days of oral administration of meloxicam and ketoprofen, blood was collected and the rats were sacrificed. Bone marrow flushing was taken for performing chromosomal aberration and micronuclei assay. From series of

genotoxic tests, it was found that oral administration of 8mg/kg BW of meloxicam did not cause genotoxicity, while the 16 mg/kg BW caused significant genotoxicity but it was not upto the extent of cyclophosphamide damage at @ 20mg/kg (positive control). Stomach and intestines were found as the most affected target organs of meloxicam intoxication in rats. Meloxicam produced the serious gastrointestinal toxicity, such as inflammation, bleeding and ulceration occur at any time, with or without warning symptoms in rats. Ketoprofen at the dose of 5mg/kg and 10mg/kg BW caused significant genotoxicity, but they were not upto the extent of cyclophosphamide @20mg/kg (positive control). Severe form of gastro-intestinal lesions was found by ketoprofen intoxication. Ketoprofen produced ulcerative erosions and severe haemorrhages in stomach and intestine. Meloxicam and ketoprofen are the non-steroidal anti-inflammatory drugs, which caused genotoxic changes at higher concentrations. Its doses should be carefully monitored in order to avoid its accumulation in the body of animals which may cause genotoxic mutations.

CRPT-04

COMPARISON OF FACE MASK AND ENDOTRACHEAL TUBE FOR ISOFLURANE ANAESTHESIA IN RABBITS

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An experimental study was conducted in 12 male New Zealand White rabbits after obtaining the permission from IAEC to compare the face mask and endotracheal intubation techniques for maintenance of anaesthesia. Premedication was done with Butorphanol @ 0.1 mg/kg IM, followed by combination of ketamine (@ 20 mg/kg IM) and midazolam (@ 1mg/kg IM) for induction of anaesthesia and maintained by using isoflurane with oxygen (0.5-3.0 % with 1.5 LPM O_2) in both the groups (n=6, each) up to 30 minutes. The rabbits were observed for righting reflex, corneal reflex, palpebral reflex, ear-pinch and toe-pinch reflexes and the jaw muscle tone. Rectal temperature and blood pressure decreased non-significantly, however, heart rate was increased non-significantly, respiration rate was decreased significantly ($P<0.01$) in group II. Saturation of peripheral oxygen increased non-significantly at 10 minutes, decreased non-significantly at 20 minutes, however, increased significantly at 30 minutes between the groups. Hb, PCV and TEC showed a decreased trend. TLC decreased non-significantly, while DLC increased non-significantly in group I & II, except for monocytes, which increased significantly ($P<0.05$) at 10 minutes. Glucose increased non-significantly at 10 minutes and decreased at 20 & 30 minutes. ALT increased in group I but decreased in group II; however, AST showed an increasing trend in both the groups. The maintenance of anaesthesia using facemask was easier as compared to intubation.

LEAD-NP-01

QUERCETIN: A NUTRACEUTICAL INGREDIENT OR DRUG?

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Introduction

Many nutraceutical products have been investigated to have preventive role in various diseases process with no or little adverse effect. Some of the nutraceuticals are also genuinely researched and offer novel ingredients that can bring about health benefits quicker than would normally be the case through eating conventionally healthy foods alone. The oxidative damage created by free radicals has been associated with several degenerative diseases. Plants have also developed methods of stopping free radical damage. Flavonoids present in plants have strong anti-oxidant properties which stabilize the free radicals and reduce degenerative diseases (Machlin and Bendich 1987). Quercetin, a potent antioxidant flavonoid, is found in red wine, onions, green tea, apples, berries and cruciferous vegetables. Quercetin-incorporated collagen matrix could be a novel dressing material for wound healing. Quercetin in the human diet has been shown to inhibit platelet aggregation. Consequently, it has been proposed that quercetin may contribute to the protective effects against cancer, atherosclerosis, aging, stroke and chronic inflammation (Murota and Terao, 2003).

Chemistry

Flavonoids occur as aglycones, glycosides and methylated derivatives. The flavonoid aglycone consists of a benzene ring condensed with a six membered ring, which in the 2-position carries a phenyl ring as a substituent (Narayana *et al*., 2001). One of the best described flavonoid is quercetin. It is more specifically classified as a flavonol which is a plant-derived and used as an ingredient in supplements, beverages or foods. It has shown several pharmacological activities including antioxidant, anti-in?ammatory, anticancer and antiviral effect. Different types of flavonoids with their availability in food are depicted in Table 1 (Groot *et al*., 1998).

Pharmacokinetics

Quercetin is generally believed to be poorly absorbed (25%) from small intestine. It is found in plasma as conjugates with glucuronic acid, sulfate or methyl groups. After getting absorbed in small intestine, quercetin is transported to liver via portal circulation, where it undergoes first pass metabolism. Quercetin and its metabolites are distributed to various tissues in the body. It is strongly bound to the albumin in plasma. Peak plasma level reaches in 0.7 to 7.0 hours following its ingestion. The elimination of quercetin was significantly delayed after its application with fat-enriched diets (Lesser, 2004).

Table 1: Types of flavonoids with their food source

Groups	Compounds	Food sources
Flavanones	Naringenin, Eriodictyol Hesperetin, Dihydroquercetin, Dihydroofisetin, Dihydrobinetin	Orange juice, Grapefruit juice, lemon juice
Anthocyanins	Cyanidin, Delphinidin, Malvidin Pelargonidin, Peonidin, Petunidin	Blueberry, Black grapes, Cheery, Rhubarb, Plum, Strawberry, Red cabbage
Flavonols	Quercetin, Kempferol, Myricetin, Isorhamnetin, Querctagetin	Yellow onion, Curly kale, Cherry, Tomato, Apple, Green and black tea, Black grapes, Blueberry
Catechhins (Proanthocyanidins)	Catechin, Gallocatechin Epicatechin, Epigallocatechin, Epicatechin 3-gallate	Chocolate, Beans, Apricot, Cherry, Grapes, Peach, Cider, Green tea, Black tea, Blackberry
Flavanols	Silibinin, Silymarin, Taxifolin, Pinobanksin	Cocoa, Cocoa beverages, Chocolates
Isoflavones	Daidzein, Genistein, Glycitein	Soy cheese, Soy flour, Soy bean, Tofu
Flavones	Tangeretin, Heptamethoxyflavone	Parsley, Celery, Capsicum pepper

Pharmacological activities

Antioxidant activity

Quercetin is considered to be a strong antioxidant due to its ability to scavenge free radicals and bind transition metal ions. These properties of quercetin allow it to inhibit lipid peroxidation (Hollman and Katan 1997). Liu et al. (2012) evaluated the effect of quercetin on myocardial oxidative stress (Ischemia) and immunity function impairment induced by isoproterenol in rats. It was found that the levels of blood AST, creatine kinase, NO, NOS, IL-10, IL-1, IL-8 and lactate dehydrogenase in isoproterenol-treated rats were significantly increased. Administration of quercetin significantly ameliorated myocardial oxidative injury and immunity function impairment induced by isoproterenol. Pretreatment with quercetin significantly reduce ethanol-induced gastric damage and malondialdehyde levels, and increased antioxidant enzyme activities (Coskun *et al.,* 2014).

Antidiabetic activity

Studies carried out in cultured cells have shown that one of the mechanisms of action by which quercetin improves glycaemic control is the reduction of intestinal glucose absorption at the level of glucose transporters (Aguirre *et al.,* 2011). It has been also postulated that quercetin blocks tyrosine kinase (Rosen, 2007). Strobel et al. (2005) reported inhibitory effect of quercetin on glucose uptake was due to a direct action on the transporter GLUT4. Vessal et al. (2003) observed dose-dependently decreased the plasma glucose level in diabetic rats treated with quercetin. Plasma cholesterol and triglycerides were reduced significantly, while their hepatic glucokinase activity was significantly increased upon quercetin treatment. They reported that quercetin regenerated the pancreatic islets and probably increased level of insulin in streptozocin-induced diabetic rats. It also prevented the alloxon and glucocorticoid-induced hyperglycemia in rats (Lukacinova *et al.*, 2008; Hoda *et al.,* 2012)

Anti-inflammatory and other activities

Treatment with quercetin reduced the raised systolic blood pressure and high plasma concentrations of triglycerides, total cholesterol, free fatty acid, and insulin found in obese zucker rats (Rivera *et al.,* 2008). It increased the plasma concentration of adiponectin, reduced NOx levels in plasma, and lowered VAT (visceral adipose tissue) TNF-alpha production in obese rats. Quercetin reduced levels of inflammatory cytokines and macrophage accumulation in the skeletal muscle of the HFD-fed obese mice Le *et al*. (2014).

Effect of quercetin on cardiovascular system

Quercetin can alleviate LPS-induced cardiac dysfunctions in mice to increase their survival rate following LPS challenge (Li *et al*., 2015). It attenuates LPS-induced increment in myocardial iNOS expression and decrement in eNOS level as well as serum NO level.

Effect of quercetin on blood biochemistry

Petruska *et al*. (2013) reported non-significant changes in WBC, lymphocytes, granulocytes, RBC, Hb and PCV in rabbits treated with quercetin. The values of other haematological parameters (LY%, GR%, MCV, MCH, MCHC, PLT) were not influenced after quercetin treatment. Quercetin could be able to normalize the polychlorined biphenyls-induced high levels of AST, ALT, ALP, and GGT in rats (Selvakumar *et al*., 2013)

Therapeutic uses

Quercetin can be useful for the treatment of arthritis and cancer as it inhibits cyclooxygenase, lipoxygenase (Kim *et al*., 1998) and reactive oxygen and nitrogen species (Knekt *et al*., 1997). Quercetin intake protects against coronary heart disease (CHD), caused by oxidized LDL. It was also shown to be effective inhibitor of platelets aggregation in dogs and monkeys (Osman *et al*., 1998). Quercetin has been found to be an inhibitor of aldose reductase, which plays a role in converting glucose to sorbitol in the body. Thus, it may be beneficial in the nutritional management of diabetes (Costantino *et al*., 1999). According to a study conducted by researchers at Cornell University in New York, a potent antioxidant (quercetin) in apples and in vegetables appear to protect brain cells against oxidative stress, a tissue damaging process associated with Alzheimer and other neurodegenerative disorders (Heo *et al*., 2004). Quercetin seems to play a very important role in the prevention and treatment of peptic ulcer as it promotes mucus secretion, thereby serves as gastroprotective agent (Martin *et al*., 1988). In conclusion, quercetin may be useful as a drug for prevention and treatment of various ailments rather than its use as ingredient of nutraceutical products.

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LEAD-NP-02

ANDROGRAPHOLIDE: PHARMACOLOGICAL AND TOXICOLOGICAL PROFILE

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INTRODUCTION

Over the past twenty years, interest in medicinal plants has grown enormously from the use of herbal products as natural cosmetics and for self-medication by the general public to the scientific investigations of plants for their biological effects in human beings. India is blessed with varieties of aromatic and medicinal plants. Among them, *Andrographis paniculata* (kalmegh/ kariyatu) have been used in Indian and Chinese herbal medicine from ancient times. It is also known as Bhui-neem, meaning "neem of the ground", since the plant, though being a small annual herb, has a similar strong bitter taste as that of the large Neem tree (king of bitter). It contain Major active principle andrographolide was first isolated by Boorsma from different parts of *Andrographis paniculata*. It is grouped as diterpene lactone with a very bitter taste and colourless crystalline in appearance with the molecular formula of $C_{20}H_{30}O_5$. Minor includes Neoandrographolide, deoxyandrographolide, deoxy-dihydroandrographolide, deoxy-oxoandrographolide. In traditional chinese medicine, *Andrographis paniculata* is used in the treatment of anti-inflammatory conditions like pneumonia, tonsillitis, gastroenteritis, pyelonephritis and hepatoprotective. Beside these it possess anticancer, antibacterial and antiviral activity. Andrographolide found to have prominent anti-inflammatory and immunomodulatory effect as it inhibitory effect on iNOS and COX-2 expression in macrophages, attributable to the modulation of transcription factors AP-1 and STAT3. AP-1 and STAT3, which are important for the production of pro-inflammatory cytokines such as IL-1β, IL-6 and IL-10

PHARMACOLOGICAL ACTIVITY

Pain management is most critical criteria in the management of veterinary patient. Systemic inflammation was suggested to be associated with increased risk of chronic diseases such as hepatitis, nephritis, cardiovascular disease and cancer and insulin resistance

In *ex vivo* condition andrographolide inhibits LPS-induced iNOS, COX-2 proteins expression, TNF-α, nitrite and PGE_2 production in RAW 264.7 cells. In in vivo condition andrographolide (30 mg/kg) nearly abolished (92.5% inhibition) the net increase in TNF-α concentration in the bronchial lavage fluid in mice. The anti-edematogenic activity of andrographolide behave in a dose dependent manner, with activity at 10 and 25

mg/kg doses of andrographolide starting 3 hr after their administration, while the onset for 50 mg/kg andrographolide was observed after 2 hr of its administration.

Andrographolide shown to possess heptoprotective against ethanol and carbon tetrachloride- (CCl_4) induced hepatotoxicity in mice with an equivalent efficacy of silymarin. Morover, it was observed to possess choleretic effects of andrographolide in conscious rats and anesthetized guinea pigs. It was more potent than silymarin against acetaminophen-induced reduction of the volume and contents of bile.

In addition to this andrographolide also possess antioxidant action and manifested by decreasing malondialdehyde (MDA) formation via lipid peroxidation and increase of hepatic oxidative enzymes and antioxidants such as TBRAS, glutathione peroxidase (GPX), glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD) and glutathione S transferase (GST).

It also retain antibacterial activity in MIC range (100-350 µg/ml) on *Salmonella typhimurium, Escherichia coli, Shigella sonnei, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumonia, Streptococcus pyogenes* and *Bordetella pertussis* and antiviral activity on Herpes simplex virus 1, Human immuno deficiency virus, Flavi viruses, Pesti viruses and Dengue virus.

CLINICAL TRIALS

Androgapholide administration was reported to cure 91% of acute bacillary dysentery cases and cure rates were found higher than furazolidrne or chloramphenicol. Moreover, the juice of fresh leaves of *Andrographis paniculata*, which generally contains andrographolide, is used as a domestic remedy to treat colic pain, loss of appetite, irregular stool and diarrhea. Phase I dose-escalating clinical trial in HIV positive patients conclude a significant rise in the mean CD4(+) lymphocyte level of HIV subjects occurred after administration of 10 mg/kg andrographolide and this suggest andrographolide may inhibit HIV-induced cell cycle dysregulation (Calabrese *et al.,* 2000). In long term follow up trail of andropholide tablet (30 mg of andrographolide) in 60 rheumatologic patients concludes significant effect in reducing symptoms and serological parameters of the disease. The effect was associated to a reduction of rheumatoid factor, IgA, and CD_4. In addition to this, after one to five years follow up trial in six rheumatologic patients, following three tablets per day concludes well tolerability, safe and efficacious for the symptomatic relief and serological control of underlying inflammation related to their disease activity. In placebo-controlled double blind study in 61 common cold patients, following oral administration of andrographolide tablet (1200mg) resulted to significant reduction in clinical symptoms at day 4 and shortens the course of the disease.

PHARMACOKINETIC STUDIES

Intravenous pharmacokinetic study in rats reveled plasma half-life and volume of distribution as 1.31 h and 0.27 L. Following oral administration of andrographolide (30 mg/kg) in rats, C_{max}, T_{max}, $t_{½}$ and AUC_{0-8} were 115.81 ng/ml, 0.75 h, 2.45 h, 278.44 ng · h/ml reported respectively. Pharmacokinetic study in 16 healthy volunteers (7 male and 9 females), who received four tablets containing 20mg of andrographolide with 200 ml of water shown Tmax (1.6 h), absorption half-life (25 min), Cmax (1.36 h), Vd (55.16) and AUC_{0-8} (1294 µg · h/ml) respectively. The tissue distribution study revealed that the concentration was observed at 1h in liver, lung, kidney, heart, spleen, brain and plasma.

TOXICOLOGICAL STUDY

The LD_{50} of andrographolide in male mice through the intraperitoneal route was 11.46 g/kg. Rats or rabbits

receiving 1g/kg of andrographolide orally showed no changes in body weight, blood count or the functions of the liver, kidney, or other vital organs. Acute toxicity study clearly demonstrated that andrographolide treated animals were devoid of any toxic sign and indicates that it is safe up to the dose of 2000 mg/kg orally. Intravenous administration at 10 mg/kg to rabbits showed no abnormal cardiovascular response and heart, liver, kidney and spleen of these rabbits were found to be normal.

It is reported that in chromosome aberration and micronucleus tests, it did not induce clastogenicity and has no impact on fertility in female mice. However anaphylactic shock and anaphylactic reactions have been reported to the World Health Organization (WHO) collaborating center for International Drug Monitoring as of June 2003. Moreover, dizziness and palpitations after intake of *Andrographis paniculata* extract was observed (Sabnis, 2006) as it is called as "King of bitters" it may cause emesis, gastric instability, loss of appetite and nausea. In addition to this there were no significant effects on the pharmacokinetics/pharmacodynamics of Warfarin and contradindicated in pregnancy

CONCLUSIONS

Andrographis paniculata contain andrographolide as main active principle, grouped as diterpene lactone with a very bitter taste. It had wide range of pharmacological activity including anti-inflammatory, anticancer, antibacterial and antiviral. Pharmacokinetic study suggests that it quickly absorbed into blood and welldistributed including brain after oral administration of andrographolide in human.

Evidence from clinical study suggests that it can be used in uncomplicated upper respiratory tract infections, common cold and rheumatoid arthritis. It occasionally causes dizziness, allergic skin reactions and contraindicated in pregnancy. As a bitter in taste, over dose shows gastric discomfort, vomiting and loss of appetite. Looking to fact andrographolide as active ingredient possesses anti-inflammatory, antioxidant and antimicrobial properties.

NP-01

IMPACT OF GI HELMINTHIASIS ON GROWTH, ANTIOXIDANT, IMMUNE AND METABOLIC STATUS IN KIDS AND ITS AMELIORATION THROUGH SUPPLEMENTATION OF CONDENSED TANNIN

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The present experiment was conducted to assess the impact of GI helminthiasis on production and health status in kids along with its amelioration through supplementation of condensed tannin (CT). Twenty-one Surti kids (4-5 month; 13.04±1.12 kg BW) were divided into three homogenous groups CON (dewormed), PAR (naturally parasitized) and PAR-TAN (naturally parasitized with supplementation of CT @1.5%). Fresh *Ficus bengalensis* leaves were used as source of CT. Blood was collected at 0, 35 and 70 day to assess the metabolic and antioxidant status. Humoral immune status was verified against chicken erythrocyte after one month of experimental period. Fortnightly parasitological status (as faecal egg count; FEC), body weight and voluntary

feed intake were also measured. The *F. bengalensis* leaves contained appreciable amount of tannin particularly CT (10.98%). Hematological data revealed a depression in haemoglobin and hematocrit values, with higher level of leucocyte and granulocyte count in PAR group. Metabolic profile displayed a higher serum glucose (70th day) and lowering trend for alanine aminotransferase in PAR-TAN group. CT supplementation improved the antioxidant status of kids by increasing the erythrocytic reduced glutathione and decreasing the lipid peroxidation. Kids of PAR-TAN group have better immune status as indicated by higher antisera against specified antigen. The anthelmintic effect of tannin feeding was evident from 4th fortnight, where PAR-TAN has displayed comparable FEC with that of control one (dewormed). In spite of comparable body weights, PAR group displayed deprived growth rate. Due to the similar voluntary feed intake with moderate alteration in growth rate, feed conversion ratio did not differ among groups. GI helminthiasis adversely affects the growth and important indices of health in kids, which can be ameliorated through supplementation of CT from *F. bengalensis* leaves. CT fabricates its impact through optimistic alterations in metabolic profile, antioxidant, immune and parasitological indices of kids.

ISVPT-2016

POSTER SESSION – I

P-EP-01

HEMATO- BIOCHEMICAL AND HISTOPATHOLOGICAL ALTERATIONS FOLLOWING ORAL ADMINISTRATION OF AQUEOUS EXTRACTS OF *MURRAYA KOENIGII* LEAVES ON CARBON TETRACHLORIDE-INDUCED HEPATOTOXIC RATS

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The present study was conducted to evaluate hemato-biochemical and histopathological alterations following repeated oral administration of aqueous extracts of *Murraya koenigii* leaves in carbon tetrachloride induced hepatotoxic rats. The study was conducted on thirty six (36) male albino Wistar rats dividing them in various groups having six rats in each group. Group I served as vehicle control and received the normal saline solution. Group II was served as hepatotoxic control and group III served as standard treatment control (received standard drug silymarin @ 50 mg/kg of body weight) and rats of treatment group IV, V and VI received 50 % carbon tetrachloride in olive oil @ 1 ml/kg body weight, intraperitoneally twice in a week throughout the study period for induction of hepatotoxicity. Group IV, V and VI received aqueous extracts of *M. koenigii* @ 100, 200 and 400 mg/kg (P.O.) respectively, daily once for 28 days. Upon acute oral toxicity testing, aqueous extracts of *M. koenigii* were found safe. Phytochemical analysis by GC-MS revealed presence of many compounds in *M. koenigii* aqueous extracts. On 29th day of study, animals were subjected to blood collection; blood and serum sample were analyzed for haematological and serum biochemical parameters, respectively. The result showed significant alterations in hematological and serum biochemical parameters suggest that carbon tetrachloride is useful substance for successful induction of hepatotoxicity in rats. Daily oral administration of silymarin significantly reduced serum ALT, AST, GGT, ALP, bilirubin, creatinine kinase and creatinine and increased albumin, globulin and total protein level as compared to hepatotoxic control rats. Hepatotoxic rats receiving aqueous extracts of *M. koenigii* @ 100, 200 and 400 mg/kg body weight also showed the same changes as compared to the rats of hepatotoxic control group in dose dependent manner except *M. koenigii* (100 mg/kg). Gross pathological examination of liver from rats of hepatotoxic control group showed paleness and diffused necrotic foci and microscopically liver sections showed sinusoidal dilatation, cellular vacuolization, necrosis, distortion of the central venules and ballooning of hepatocytes, kidney sections showed congestion with degeneration, necrosis of renal tubular epithelium and cloudy swelling of tubular cells, spleen sections showed mild congestion and haemorrhage with multifocal area of necrosis and mild lymphoid depletion and heart sections reveled severe congestion. Treatment of hepatotoxic rats with aqueous extracts of *M. koenigii* preserved normal histoarchitecture in dose dependent manner and standard treatment silymarin almost preserved normal histoarchitecture of all the organs as compared to rats of hepatotoxic control group.

P-EP-02

IN VITRO ANTIBACTERIAL ACTIVITY OF ACETONE EXTRACTS OF *CRATAEVA NURVALA*, *CAREYA ARBOREA* AND *OROXYLUM INDICUM* BARK AND *BIXA ORELLANA* AND *ENSETE VENTRICOSUM* SEEDS

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The aim of present study was to evaluate *in-vitro* antibacterial activity of acetone extracts of *Crataeva nurvala*, *Careya arborea* and *Oroxylum indicum* bark as well as *Bixa orellana* and *Ensete ventricosum* seeds. Bark and seeds of these medicinal plants were collected, shade dried, powdered and finally extracts were made using various solvents like hexane, chloroform, acetone, ethanol and water based on their increasing polarity. Acetone from the crude extracts were evaporated and measured to make serial dilutions in 10% DMSO. Antibacterial efficacy was evaluated of these extracts using micro-broth dilution technique and viability of organism was checked by tetrazolium chloride dye keeping gentamicin and enrofloxacin as positive control. All the dilutions were made in triplicate. MIC values of *Crataeva nurvala* against *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes* and *Proteus mirabilis* were observed 2.56 mg/ml, 1.28 mg/ml, 5.12 mg/ml and 1.28 mg/ml, respectively. MICs of *Careya arborea* bark acetone extract against gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* were observed 2.56 mg/ml each; against gram negative bacteria *Salmonella typhimurium* and *Proteus mirabilis* were found 5.12 mg/ml each. MIC values of *Oroxylum indicum* bark against *Staphylococcus aureus, Bacillus subtilis, Salmonella typhimurium* and *Proteus mirabilis* were observed 1.28 mg/ml, 2.56 mg/ml, 5.12 mg/ml and 5.12 mg/ml, respectively. MICs of *Bixa orellana* seeds against various organisms like *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes* were found 2.56 mg/ml, 2.56 mg/ml and 5.12 mg/ml, respectively and against *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Proteus mirabilis* were observed 5.12 mg/ml, 5.12 mg/ml, 5.12 mg/ml and 2.56 mg/ml, respectively. MIC values of *Ensete ventricosum* seeds against *Staphylococcus aureus, Bacillus subtilis, Salmonella typhimurium* and *Proteus mirabilis* were observed 1.28 mg/ml, 2.56 mg/ml, 5.12 mg/ml and 5.12 mg/ml, respectively. These results suggest that acetone extracts of above medicinal plants have potential antibacterial activity.

P-EP-03

IN-VITRO ANTIBACTERIAL ACTIVITY OF CHLOROFORM EXTRACTS OF *ANDROGRAPHIS PANICULATA*, *HELICTERES ISORA* AND *BIXA ORELLANA* LEAVES

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The present study was carried out to evaluate *in-vitro* antibacterial activity of chloroform extracts of *Andrographis paniculata*, *Helicteres isora* and *Bixa orellana* leaves. Plant leaves were collected, shade dried, powdered and further serial cold extractions were carried out using various solvents like hexane, chloroform, acetone, ethanol and water based on their increasing polarity. The chloroform extracts were concentrated using rotatory vacuum evaporator. Reduced extracts were weighed and serial dilutions were made in 10% DMSO to evaluate their antibacterial activities using micro-broth dilution technique in which tetrazolium chloride dye was used to check viability of bacteria in microtiter plate. All the dilutions were made in triplicate. Gentamicin and enrofloxacin were used as positive control. MIC values of *Andrographis paniculata* leaves against various gram positive organisms viz. *Bacillus subtilis, Streptococcus pyogenes* and *Staphylococcus aureus* were observed 1.28 mg/ml each. MIC values of *Helicteres isora* leaves against *Salmonella typhimurium, Pseudomonas aeruginosa* and *Streptococcus pyogenes* were observed 5.12 mg/ml, 5.12 mg/ml and 0.64 mg/ml, respectively. MIC values of *Bixa orellana* leaves against *Bacillus subtilis, Streptococcus pyogenes, Staphylococcus aureus* and *Proteus mirabilis* were observed 2.56 mg/ml, 1.28 mg/ml, 2.56 mg/ml and 1.28 mg/ml, respectively. In conclusion, chloroform extracts of *Andrographis paniculata*, *Helicteres isora* and *Bixa orellana* leaves were found to possess antibacterial efficacy against various gram positive and gram negative bacteria.

P-EP-04

IN VITRO ANTIOXIDANT PROPERTIES OF ETHANOL EXTRACTS OF *DRYPETES ROXBURGHII* LEAVES, *CAREYA ARBOREA* AND *SCHLEICHERA OLEOSA* BARK AND SEEDS OF *ENSETE ENTRICOSUM*

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Present study was planned to examine *in-vitro* free radical scavenging and antioxidant properties of ethanol extracts of *Drypetes roxburghii* leaves, *Careya arborea* and *Schleichera oleosa* bark and seeds of *Ensete ventricosum*. Plant material was collected, shed dried and powdered to evaluate its antioxidant activity using 2,2-diphenyl 1-picrylhydrazyl (DPPH) and 2,2-azinobis (3-ethylbenzothiazoline 6-sulfonic acid) (ABTS) radical scavenging methods. Extracts were made serial extraction method using various solvents like hexane,

chloroform, acetone, ethanol and water based on their increasing polarity. Ethanol was evaporated by negative pressure from the crude extracts and resultants were measured to make serial dilutions in 10% DMSO. Half maximal inhibitory concentrations (IC_{50}) were calculated for each extracts. Trolox was taken as standard antioxidant and radical scavenging agent. Each extracts were verified in triplicate. IC_{50} of trolox in DPPH assay was observed 0.074 mg/ml and in ABTS assay 0.022 mg/ml. Compared to trolox, IC_{50} of ethanol extracts of *Drypetes roxburghii* leaves, *Careya arborea* bark, *Schleichera oleosa* bark and *Ensete ventricosum* seed for DPPH assay were observed 0.13 mg/ml, 0.14 mg/ml, 0.12 mg/ml and 0.12 mg/ml, respectively. Half maximal inhibitory concentrations of ethanol extracts of *Drypetes roxburghii* leaves, *Careya arborea* bark, *Schleichera oleosa* bark and *Ensete ventricosum* seed for DPPH assay were observed 0.05 mg/ml, 0.04 mg/ml, 0.06 mg/ml and 0.05 mg/ml, respectively. Looking to IC_{50} values of ethanol extracts of *Drypetes roxburghii* leaves, *Careya arborea* and *Schleichera oleosa* bark and seeds of *Ensete ventricosum* it can be concluded that ethanol extracts of plants under investigation have good antioxidant and free radical scavenging activity.

P-EP-05

STUDIES ON COMPARATIVE EFFICACY OF ANTI-MICROBIAL ACTIVITY OF *TINOSPORA CORDIFOLIA*, *AZADIRACHTA INDICA* AND *ANDROGRAPHIS PANICULATA* PLANT EXTRACTS AGAINST GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

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The present study was designed to evaluate the phytochemical screening and antimicrobial activity of *Tinospora cordifolia* (stem), *Azadirachta indica* (leaves) and *Andrographis paniculata* (aerial parts). Samples of *Tinospora cordifolia* (stem), *Azadirachta indica* (leaves) and *Andrographis paniculata* (aerial parts) were obtained by Soxhlet's extraction in mixture of 50% methanol and 50% water. Then each extract was initially subjected to phytochemical analysis. The antibacterial activities were assessed by measuring the diameter of the inhibition zones, MIC and MBC values. Antimicrobial activity of these plant extracts against bacterial strains like *Salmonella gallinarum*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli* were assessed. The antimicrobial activity was seen by Disc diffusion method. The results revealed that the TCE and AIE possessed antibacterial activity only against *Salmonella gallinarum* at high concentration and APE showed significant antibacterial activity against *Pseudomonas aeruginosa* and *E. coli*, while it did not show any antibacterial activity against *Staphylococcus aureus* even at high concentrations. The antibacterial activity observed with TCE, AIE and APE might be due to the presence of many potent compounds such as flavonoids, terpenes, phenolics and alkaloids etc.

P-EP-06

EFFECT OF *EMBLICA OFFICINALIS* ON BIOCHEMICAL PROFILE IN MONOCROTOPHOS TOXICITY IN BROILER POULTRY BIRDS

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Present study was undertaken to evaluate the effect of *Emblica officinalis* on biochemical profile in Monocrotophos toxicity in broiler poultry birds. Monocrotophos was given orally to the birds as 1/5th of LD_{50}. After one day of monocrotophos treatment there was a significant increase in activities of ALT, AST and ALP in the birds which were treated with monocrotophos only. *Emblica officinalis* fruit, leaf and bark extracts reduced the activities of ALT and AST significantly in monocrotophos treated birds, whereas, the leaf and bark extracts of the plant could significantly bring the level of ALP to near the normal value after one day. The serum globulin level was increased significantly by *Emblica officinalis* leaf and bark extracts as compare to control and monocrotophos treated birds. The albumin level and Albumin:Globulin ratio were found to be significantly lower in the birds treated with higher dose of fruit and leaf extracts. After seven days of monocrotophos treatment the activities of ALT and LDH were increased along with creatinine level in serum of monocrotophos treated birds as compared to control birds. *Emblica officinalis* fruit, leaf and bark extracts reduced the values of creatinine and level of ALT significantly in monocrotphos treated birds. The values of total proteins and ALP were significantly decreased by *Emblica officinalis* in comparison to control and monocrotophos treated birds. No effect on LDH was recorded. The results of present study confirmed ayurvedic claim using *Emblica officinalis* fruit as hepatoprotective and cardiogenic agent. The antioxidants and flavanoids present in *Emblica officinalis* fruit possibly protected the birds from monocrotophos induced toxicity.

P-EP-07

SURVEY OF ETHNO VETERINARY PRACTICES IN SALEM DISTRICT OF TAMILNADU

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India is one of the richest repositories of traditional information on the medicinal uses of plants. During the last few decades there has been an increasing attention in the investigation of medicinal plants locality and their traditional use in various parts of world. For the same objective a medicinal plants survey was carried out in Salem district of Tamil Nadu which is one of the most botanized areas of South India. This survey was undertaken in Arunoothmalai area of Salem district where the tribal peoples traditionally practicing the ethnoveterinary medicine. Ethnomedicinal information on the plant species was collected by interviewing the

local communities like herbal medicine practitioners and vaidyas. The present survey noted the recipes which were available for treating Rhinitis, Snake bite, Fever and bloat, through questionnaire. They have informed the recipe for Rhinitis (Combination of *Allium sativum*, *Allium ceba*, *Curcuma longa* and Chilli) Snake bite (Combination of *Achyranthes aspera, Cassia obtuse*, *Azadirachta indica*, *Corallocarpus epigaeus*, White Aruku, *Aristolochia indica)* Bloat (*Piper nigrum, Cuminum cyminum, Ferula asafetida, Allium sativum* and Karupatti) and fever *(Piper betle, Piper nigrum and Calotropis gigantea).* The existing scientific information's pertaining to the above mentioned recipes were collected to validate the practices. For examples in case of rhinitis, as per the literatures, *Allium ceba* have shown antiallergic effect due to its antihistaminic, anti-inflammatory and antioxidant activities. And the oral administration of *Piperine* which is present in *Piper betle* have reduced the infiltration of eosinophil, hyperresponsiveness and the Capsaicin present in *Piper nigrum* was used in the non-allergic, non-infectious, perennial rhinitis and *Allium sativum possess a*ntimicrobial property which might be useful in treating the rhinitis condition. A detailed i*n vivo* study of above mentioned recipe may further validate the ethno veterinary practices.

P-EP-08

IN VITRO EFFICACY OF ALCOHOLIC EXTRACTS OF INDIGENOUS MEDICINAL PLANTS AGAINST *RHIPICEPHALUS* (*BOOPHILUS*) *MICROPLUS*

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The acaricidal activity of methanol and ethanol extracts of leaf of *Azadirachta indica*, *Ocimum tenuiflorum*, *Aegle marmelos*, *Eucalyptus alba* and *Saraca indica* were tested against the bovine tick, *Rhipicephalus* (*Boophilus*) *microplus* under *in vitro* condition. The evaporated alcoholic extracts of the leaves were dissolved in 0.1% Tween 20 in the distilled water and thereafter tested for their acaricidal activity using standard adult immersion test (AIT) by measuring mortality after 24 hours. Mortality of the selected tick was merely 20-50% in 100 ppm cypermethrin solution after 24 hours of AIT, indicative of high level of resistance. The mortality recorded in 2.5% methanolic/30% ethanolic extract of *A. indica* and *O. tenuiflorum* as well as 10% methanol extract of *A. marmelos* was 0-30%. The 30% methanol extract of *E. alba* recorded moderate level of mortality (40-50%) while same plant leaf extract in same strength lack mortality in ethanol. Surprisingly, 30% extract of *S. indica* in methanol yielded a high level mortality of 70%. The mortality recorded in the dual combinations of methanolic leaf extracts of *A. indica*: *O. tenuiflorum/ A. indica*: *A. marmelos/ O. tenuiflorum*: *A. marmelos* was 10-20% while it was 30% in triple combinations of *A. indica*: *O. tenuiflorum*: *A. marmelos*. Based on the above experimental results, it is confirmed that the selected plant materials especially methanolic extract of *S. indica* followed by *E. alba* possess considerable degree of acaricidal activity against the targeted hard tick. Plant extracts in combinations are more effective than single. It is opined that further purification studies and in vivo trials are needed to ascertain the active ingredients responsible for the acaricidal properties of the extracts.

P-EP-09

IN VITRO ANTIOXIDANT AND ANTHELMINTIC PROPERTIES OF RHIZOME EXTRACTS OF *HEDYCHIUM SPICATUM*

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The present study was designed to evaluate different extracts of *Hedychium spicatum*, commonly known as *kapur kachri,* for *in vitro* antioxidant property by ABTS, DPPH and Nitric oxide free radical scavenging assay. The methanolic extracts showed IC_{50} minimum as compared to other extracts. Total flavonoid and phenolic contents were determined and methanolic extracts showed the maximum contents of flavonoids 38.70 ±36.63 mg of rutin equivalent/g of extract and phenolic 60.01±1.46 mg of gallic acid equivalent/g of extracts, respectively. The *in vitro* anthelmintic efficacy of methanolic extract @ 2%, 4%, 6% and 8% concentrations against the *Hemonchus contortus* was found maximum as compared to other extracts and standard drug thiabendazole. The time of paralysis and time of death of the parasite were recorded and was found minimum for methanolic extract against *Hemonchus contortus* than the other extracts. It is concluded from this study that methanolic extract of *Hedychium spicatum* contained antioxidant and anthelmintic activities which could further be used for therapeutic applications for the treatment of diseases of man and animals.

P-EP-10

EVALUATION OF ANTICANCER ACTIVITY OF *CHENOPODIUM ALBUM* LINN. EXTRACTS IN HELA CELLS

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Chenopodium album Linn., an edible Indian herb is known o possess many pharmacological properties. The objective of the study was to investigate the anticancer activity of different extracts of the plant in cancer cell line HeLa cell. Cell proliferation assays are widely used for anticancer drug screening. Hence, different solvent extracts of the plant (methanolic, ethanolic, aqueous, 50% hydromethanolic and 50% hydroethanolic) were assessed for their cytotoxicity using MTT [3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium] and neutral red bioassays. These cells were cultured in MEM (minimum essential medium) medium and incubated with the dilution series of extracts (7.81- 1000 ?g/ml) and incubated at 37 °C, 5% CO_2 for 48 h in CO_2 incubator. Dose dependent cytotoxicity to HeLa cells was observed after treatment with various extracts. Among various extracts of *C. album*, hydroethanolic was found to have minimum IC_{50} value with MTT (99.14 ± 4.03 µg/ml) and Neutral red (80.87 ± 4.40 µg/ml) assays. Morphological changes in HeLa cells were observed through phase contrast microscope after 48 h of treatment with the plant extracts at a dose of 250 µg/ml along with control.

The cells reflected highest morphological changes in hydroethanolic extract followed by hydromethanolic, aqueous, ethanolic and least in methanolic extract. The cells were found to lose their morphological characteristics and also proliferation of cells were inhibited by these extracts. There was induction of apoptosis resulting in cell shrinkage, eventually leading to the formation of apoptotic bodies. Because of cytopathic effect, the cells also got detached from the surface showing flagging off the cells and also they formed clumps after detachment from the surface. In hydroethanolic extracts, there were formations of apoptotic bodies also.Caspase-3, a cysteine proteases that mediate apoptosis was assessed after treatment with different extracts. Caspase-3 induction after treatment of HeLa cells with different extracts yielded maximum caspase -3 activity of 16.13 ± 0.81 fold change for hydroethanolic extract followed by ethanolic (15.47 ± 0.69), hydromethanolic (14.43 ± 0.72), methanolic (12.80 ±0.74) and least in aqueous (8.17 ± 0.73) extract.Hence, based on cell culture anticancer studies, hydroethanolic extract was found to have maximum activity. These findings were considered as an indication of the anticancer potential of *C. Album*.

P-EP-11

ANTIPROLIFERATIVE, CYTOTOXIC AND ANTICANCER ACTIVITY OF *MELIA AZEDARACH* EXTRACTS IN HELA CELLS

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Melia azedarach (M. azedarach) belonging to the family Meliaceae is a known plant for its anthelmintic, antibacterial, anti-inflammatory & anti proliferative properties. Present study was conducted to evaluate the *in vitro* antiproliferative, apoptotic and cytotoxic properties of various extracts of *M. azedarach* in human cervical cancer cell line HeLa, grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum. For the study, aqueous, 60% hydro ethanolic, 60% hydro methanolic, ethanolic and methanolic extracts were prepared using dried and powered *M. azedarach* leaves by cold extraction. Cyto toxicity of these extracts at different concentrations ranging from 0.00781- 1mg/ml were studied in HeLa cell line using MTT cytotoxicity assay. Also morphological changes of HeLa cells in the presence of IC_{50} concentrations of extracts were observed through phase contrast microscope after 48 h of treatment, along with proper control. Activity of Caspase 3, a cysteine proteases which cleave the target proteins in the cell, causing morphological and functional changes to cells undergoing apoptosis was also estimated in HeLa cells treated at IC_{50} concentrations of extracts, using colorimetric assay. The extracts of *M. azedarach* showed cytotoxicity in HeLa cell line in a dose dependent manner with IC_{50} concentrations of 123.52, 91.93, 90.68, 64.66 and 66.07 µg/ml for aqueous, hydro ethanolic, hydro methanolic, ethanolic and methanolic extracts respectively. Morphological changes indicating apoptosis like cell shrinkage, cell detachment and clump formation were more evident in presence of ethanolic and hydroethanolic extracts which can be regarded as an indication of their antiproliferative and cytotoxic activity. The caspase 3 activity (units) was found to be high in ethanolic (11.13), methanolic (9.55) and 60% hydro ethanolic extracts (8.25) lowest (4.29) in aqueous extract. The highest anticancer property was

observed in ethanolic extract of *M. azedarach.* All these results suggests that ethanolic, methanolic and hydroethanolic extracts of *M. azedarach* promote the induction of cell cycle arrest and mitochondria mediated apoptosis in HeLa cells.

P-EP-12

MODULATION OF APOPTOTIC PATHWAYS BY HYDROETHANOLIC EXTRACT OF *CUMINUM CYMINUM* IN 7, 12-DIMETHYLBENZ[A] ANTHRACENE INDUCED MAMMARY TUMOURS IN WITAR RATS

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Cancer is one of the most dreaded and life threatening disease of human as well as animals, especially pet animals, despite the significant progress made in its early diagnosis recent years. Among various features of cancerous cells, failure in apoptosis, which can lead to abnormalities in tumour cell death is considered as the main mechanism for tumourogenesis. The present study was conducted to evaluate the role of *C. cyminum* (Cumin) extracts in modulating the apoptotic pathways involved in 7, 12-dimethylbenz[a]anthracene (DMBA) induced mammary tumours. The tumours were induced in Wistar rats by administering DMBA orally in two divided doses of 50 and 30 mg/kg at one week interval. The tumour induction started from 143 day onwards and 24 tumour bearing animals were divided into four groups of 6 animals each. The study was conducted for 30 days by oral feeding of 60% hydroethanolic extracts of cumin at two doses of 150mg/kg and 300mg/kg body weight in 7,12-dimethylbenz[a]anthracene induced mammary tumour bearing Wistar rats. Normal control and cancer control were also included in the study. Various parameters evaluated were general clinical signs, tumour evaluation, mRNA expression studies of apoptosis related genes like Caspase 3, Bcl 2, flow cytometry for detecting percentage of apoptotic cells, ROS generation, mitochondrial transmembrane potential and histopathology of tumour tissues. The tumour regression was found to be highest in cumin extract treatment at a dose of 300mg/kg. Also the results of mRNA expression study showed upregulation of proapoptotic gene caspase 3, and downregulation of antiapoptotic gene Bcl2 in cumin treated groups. Flow cytometric evaluation for detecting percentage of apoptotic cells, mitochondrial transmembrane potential and ROS generation also revealed that cumin extract can modulate apoptosis and prevent cancer progression. These findings were also substantiated by histopathological evaluation of tumour tissues, which revealed considerable loss of cancerous cells in rats treated with high dose (300mg/kg) of cumin extracts.

P-EP-13

PUNARNAVA: THE PLANT OF HOPE IN CHLORPYRIFOS TOXICITY

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Since the insecticides are being used in agriculture, animal husbandry practices and health operations, it is essential to undertake the issue of study and monitoring of remaining amount present after their application especially in animals as they are used for food. However, a very little information is available over this. Further the presence of insecticide residue in animals is not only of food concern and health of the community but it is also of importance to deal with the issue of animal welfare as these have adverse effect on their health and physiology. The residues of organophosphate insecticides like chlorpyrifos which is being commonly used in agriculture and other domestic use, pose adverse health effects as neuro-disorder, liver damage, reproductive dysfunction, endocrine disruption, tissue degeneration, etc. in human beings and animals. Therefore safer alternatives may be of herbal origin are needed to deal with the problems due to prolong exposure of individuals to insecticides. As the plant Punarnava (*Boerhavia diffsusa*) is known for its medicinal value in Ayurveda and reported for therapeutic properties such as anti-oxidant, hepatoprotective, aphrodisiac, anti-inflammatory, analgesic, anti-convulsant, anti-proliferative, adaptogenic, diuretic, antidiabetic, anticancer, etc. in modern studies. The plant is also reported well in modern literature for its pharmacologically important compounds like flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, glycoprotein's, β-sitosterol, etc. As per our study the punarnava extract found effective in the improvement of reduced reproductive performance of male domestic fowl due to prolong low dose administration of chlorpyrifos. Thus the medicinal substances obtained from it may be used as nutritional additive or remedy to prevent the losses due to reproductive insufficiency in animals due to toxic effects of residues of insecticides.

P-EP-14

EXPLORATION OF THE IMMUNOMODULATORY ACTIVITY OF *KEDROSTIS FOETIDISSIMA* (JACQ.) COGN PLANT OF TWO DIFFERENT GEOGRAPHICAL AREAS AND COMPARING ITS BIOLOGICAL CONSISTENCY IN IMMUNOSUPPRESSED BROILERS

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In poultry, because of undesirable effects associated with the commercial immunomodulatory chemicals, awareness on organic poultry production and belief on herbs as they are safe, more promising therapeutic potentials and cheaper has increased the feeding of medicinal herbs in recent years. Study on exploration of herbal immunomodulation and the influence of geographical factors with its biological potentials in broilers is

limited. Hence this experiment was conducted to explore the immunomodulatroy activity of *Kedrostis foetidissima* (Jacq.) Cogn herb of two different places in immunosuppressed broilers. This study design contains five treatment groups. Each group comprised of eight birds and a total number of 40 birds were used. T1, T2, and T3 groups were fixed as normal, positive (Levamisole @ 30 mg/kg BW) and negative (Cyclophosphamide) controls respectively. T4 and T5 groups received 1% of whole plant crude powder of *Kedrostis foetidissima* collected from Namakkal and Coimbatore Districts of Tamilnadu respectively and challenged with an immunosuppressive drug (Cyclophosphamide @ 150mg/Kg BW) on 21st day. The humoral mediated immunity was assessed weekly by Haemagglutination Inhibition (HI) titre against NDV LaSota antigen. In this study, the herb *Kedrostis foetidissima* collected from Coimbatore District (T5) has showed significant difference on HI titre value ($P < 0.05$) with the same herb collected from Namakkal District (T4), T3 and T1 groups. There is no significant difference noticed between T5 and T2 groups. The results proved that the herb from Coimbatore was having significant immunomodulatory activity than herb from Namakkal. Since the herb was proved to possess immunomodulatory property, it may become an alternative source of immune boosting drugs for broilers with further studies. This experiment has also revealed the influence of environmental factors on plant biological activity and strongly recommends the importance of quality assurance of such herbs / herbal products before its usage in animals and poultry.

P-EP-15

RADIOGRARHIC, SCANNING ELECTRON MICROSCOPY AND CYTOKINE PROFILES EVALUATION AND COMPARATIVE STUDY OF QUERCETIN AND IBUPROFEN IN COMPLETE FREUND'S ADJUVANT INDUCED RHEUMATOID ARTHRITIS IN RATS

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Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disease characterized by joint inflammation and progressive destruction of the joints, leading to decreased functional capacity .Thirty male *Wistar* rats aged about 60 days were randomly assigned into five groups comprising of six rats in each and treated as follows: Group 1 was kept as normal control throughout the experimental period. Remaining 4 groups were induced rheumatoid arthritis (RA) by sub plantar injection of CFA. After 72 h, all those induced rats were diagnosed for RA, and were included in the study. Treatment protocols were initiated from day 0 post-confirmation of RA and continued for 21 days. Group 1: Non-RA control; group 2: CFA (0.1 mL @ 10% single dose, sub plantar route) - induced RA control; group 3: Quercetin (160 mg/kg b.wt mixed with tween 80 given orally by gavage on alternate days from 0^{th} day of induction); group 4: Ibuprofen (53 mg/kg b.wt *P.O* from 0^{th} day of induction) treatment in RA rats; group 5: Ibuprofen and quercetin in RA rats. In the present study, the TNF-? and IL-1? levels in serum revealed a significant rise in group 2 as compared to all other groups. The IL-10 levels in serum revealed a significant fall in group 2 as compared to all other groups. Radiograph of RA control group showed tissue swelling, bone erosion and osteophyte formation, quercetin-treated RA rats showed moderate tissue swelling, and Ibuprofen-treated RA rats showed mild tissue swelling, mild bone erosion and mild

osteophyte formation. The radiograph of quercetin + ibuprofen-treated group showed no tissue swelling and no osteophyte formation. SEM of RA control group showed round chondrocytes with abnormal microvilli on their surface and wrap around the collagen fibrils, creating a dense network. From this study, it is concluded that, alterations associated with cytokine-architecture of joints, were significantly alleviated by using quercetin, ibuprofen and their combination.

P-EP-16

ANTIDIABETIC EFFECT OF METHANOL EXTRACT OF *CASSIA AURICULATA* ON EXPERIMENTAL DIABETES IN WISTAR RATS

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The present study was carried out to investigate the effect of *Cassia auriculata* bark extract on alloxan induced diabetes in Wistar rats. The apparently healthy 40 Wistar rats of either sex were randomly selected and were divided into 4 groups consisting of 10 rats. After overnight fasting, hyperglycaemia was induced by administering a single dose of alloxan monohydrate (120 mg/kg b.wt. I/p), prepared in sterile saline in group B, group C and group D. After 5 days of alloxan administration, the animals having blood glucose levels more than 250 mg/dl were selected for studies. Group A rats served as normal control. Alloxan treated Group B rats served as a diabetic control and given distilled water orally. Group C rats were given methanolic bark extract of *Cassia auriculata* @ 500 mg/kg b.wt. for 30 days and served as curative. Group D rats were injected with insulin @ 6 IU/kg b.wt. S/C for 30 days. Rats of diabetic control Group B showed significant reduction in body weight, Lymphocytes, TEC and Hb and significant increase in TLC, Neutrophils, AST, ALT, BUN, serum Triglyceride, Total Cholesterol, serum Creatinine and Glucose values. The Group C rats treated with curative regimen of *C. auriculata* methanolic extract and insulin treated Group D showed significant improvement in body weight, Lymphocytes counts, TEC, Hb levels, TLC, Neutrophils counts and serum levels of AST, ALT, BUN, serum Triglyceride, Total Cholesterol, serum Creatinine and blood Glucose values.

P-EP-17

ANTI-DIARRHEAL ACTIVITY OF STEM BARK OF *FICUS RELIGIOSA* IN WISTAR RATS

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The present investigation was aimed to study the anti-diarrheal potential of ethyl alcohol extract of *Ficus religiosa* stem bark in wistar rats. The stem bark of locally collected *F. religiosa* was subjected to ethanolic extract and preliminary phytochemical analysis was carried out. The anti-diarrheal activity of extract at 200 and 400 mg/kg body weight dose was evaluated using castor oil induced diarrhea in wistar rats. Along with castor oil induced diarrhea, the activity was assessed in Castor oil induced enteropooling and Gastrointestinal

motility test (Charcoal meal test).The preliminary phytochemical analysis revealed the presence of alkaloids, sterols, tannin, flavonoids, saponin and glycosides in the ethanolic extract. The extract at 200 and 400 mg/kg significantly inhibited the castor oil induced diarrheal feces by 64.17% and 67.01%, respectively. In castor oil induced enteropooling test the inhibition observed in weight of intestinal contents was 62.20% and 69.23%; and volume of intestinal content was 60.23% and 70.60%. Similarly the extract also reduced the gastrointestinal motility by 47.36% and 63.21% in charcoal meal test. All the results were found statistically significant ($P<0.01$).The extract at both the dose rates significantly inhibited the castor oil induced diarrhea in terms of reduced the frequency of defecation, fecal volume and gastrointestinal motility. The results obtained were dose dependant.

P-EP-18

IN SILICO AND *IN VITRO* ANTI-BIOFILM ACTIVITY OF SELECTED PHYTOCHEMICALS ON THE BIOFILM-PRODUCING *STAPHYLOCOCCUS AUREUS*

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A biofilm is any group of microorganisms in which cells stick to each other and adhering to a surface using a matrix of extracellular polymeric substance composed of extracellular DNA, proteins, and polysaccharides. *S. aureus,* a panzootic pathogen demonstrates a distinctive ability to produce biofilm which prevent the access of antibacterial agents to the bacterial cells, making it resistant to antibiotics. In the present investigation selected phytochemicals were used for its anti-biofilm formation activity by inhibition of staphylococcal accessory regulator (SarA), a major protein involved in biofilm formation in *S aureus,* using *in silico* and *in vitro* techniques. *S. aureus* isolates were isolated from clinical cases of mastitis and gangrenous dermatitis in poultry. Provisionally confirmed isolates of *S. aureus* were characterized with species specific oligonucleotide primers *Staur* 4 and *Staur* 6 using polymerase chain reaction (PCR). The *S. aureus* isolates were further tested for the production of biofilm phenotypically on Congo Red Agar medium and genotypically by detection of intercellular adhesion D (*icaD*) gene by PCR. The ability of *S. aureus* isolates to produce bio?lm in vitro was determined by microtitre plate assay (MTP). 19 phytochemicals were *in silico* docked with the biofilm producing protein Sar A using Autodock 1.5.6 and the binding energy of the best possible conformations were calculated. Two compounds among them with lowest binding energy, namely piperine and ajmalicine were selected for *in vitro* validation using the isolated biofilm producing *S aureus*. The antibacterial activity of piperine and ajmalicine were tested on Muller Hinton agar (MHA) medium. Antibiofilm activity of the same compounds was tested on Congo Red Agar (CRA) medium. The isolate which is confirmed positive for *S aureus* and positive for biofilm production as confirmed by the presence of *icaD,* produced rough black colour colonies on CRA and also positive for the presence of *icaD* gene. The MTP method showed significant absorbance at 590nm as indicated for strong biofilm producer. Piperine and ajmalicine did not produce any antibacterial

activity on MHA whereas on CRA they showed antibiofilm activity. Hence it can be concluded that the use of piperine and ajmalicine along with antibiotics can significantly reduce the biofilm formation by the bacteria and thus making them susceptible to antibiotic.

P-TOX-01

PRESENCE OF HEAVY METALS IN MILK OF DAIRY ANIMALS IN GANDHINAGAR DISTRICT OF GUJARAT STATE: AN EMPIRICAL EVALUATION

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Possibility of health hazards in human and animal due to heavy metals cannot be ignored at present because of rise in heavy metal persistence in natural resources including soil, water, air and vegetation. Man and animal are continuously exposed to contaminated foods, air and water. The present study was undertaken to evaluate the levels of Cd, Co, Cr, Ni, Pb and Cu in fresh milk samples of cattle and buffalo collected from farmers' doorstep of Kalol taluka, Gandhinagar district. A total of 43 milk samples were analyzed for heavy metals using ICP-AES (Inductively Coupled Plasma Atomic Emission Spectroscopy). The samples preparation involved wet digestion method of milk samples with TCA followed by ashing and reconstitution. Among all samples analyzed, Mean ± SE levels of Cd, Co, Cr, Ni, Pb and Cu in the milk were found as 0.057 ± 0.001, 0.021 ± 0.001, 0.098 ± 0.004, 0.061 ± 0.002, 0.071 ± 0.013 and 0.474 ± 0.194 ppm, respectively. The range of concentration of Cd, Co, Cr, Ni, Pb and Cu in the milk samples analysed were 0.056-0.059, 0.020-0.029, 0.070-0.203, 0.067-0.095, 0.056-0.459 and 0.000-1.080, respectively. In conclusion, the level of heavy metals in all the milk samples analysed were not exceeding recommended MRLs.

P-TOX-02

IN VIVO EVALUATION OF ONCOLYTIC EFFECTS OF R_2B MUKTESHWAR VACCINE OF NEWCASTLE DISEASE VIRUS (NDV) ON BREAST CANCER CELL LINE (MDA-MB-436)

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The present work was aimed to evaluate the oncolytic potential of R_2B Mukteshwar strain of NDV against triple negative breast cancer cell line MDA-MB-436 using tumour xenografts in Severe Combined Immunodeficinet (SCID) mice. Cells were implanted in mouse at the dose rate of $1X10^6$ cells and as volume reached to

approximately 200mm^3, NDV was inoculated as therapeutic agent at dose rate of 1X10^7 PFU/ animal via intratumoral (I/T-NDV) and intravenous (I/V-NDV) routes. Mock control groups of mice were kept corresponding to treatment groups. Oncolytic activity was observed only in I/T-NDV group and tumour growth inhibition was recorded as 21.65, 24.28, 26.69 and 24.87 per cent as mean tumour volume of treatment group were found as 78.35, 75.62, 73.31 and 75.13 per cent to that of control group at day 8th, 12th, 14th and 16th, respectively. Oncolytic acitivity of virus went through day 5th to 19th days. No effect could be observed in treatment through I/V route. On histopathological basis, corresponding to gross level, xenografts showed necrosis in 25 % of cells , slight level of apoptosis and immune cell infiltration were observed as major microscopic changes in I/T treatment groups only. In comparison this only isolated patches of necrosis were observed in control groups and I/V treated group. NDV survival was measured via Taqman based real time PCR of NDV and found till 10th day in 2/2 mice of NDV-I/T group and at 21st day, virus became undetectable in all types of xenofrafts.

P-TOX-03

ANTIOXIDANT AND ENDOCRINE STATUS OF BUFFALO CALVES AFTER SUBCHRONIC CARBARYL EXPOSURE IN RELATION TO PESTICIDE'S SERUM LEVELS

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Sub-chronic carbaryl exposure at the dose rate of 1mg/kg/day for consecutive 105 days produced a significant increase in the extent of lipid peroxidation (42.95%) and in the activity of anti-oxidant enzymes viz. glutathione peroxidase (39.2%), glutathione reductase (45.31%), glutathione-s-transferase (29%), superoxide dismutase (65%), and catalase (34.5%). However, carbaryl caused a significant decrease in the blood glutathione level (26%), which is an important intra-cellular component of defense against oxidative damage. Serum samples were collected from 0 to 90th day of experiment at an interval of 15 days and the samples were processed, extracted, cleaned up and quantified using gas liquid chromatography (GLC). There was variation in serum residue level from 3.31 ± 0.28 to 4.37± 0.57 ppm, through day 15 to day 90, with slight fluctuations. Upon sub-chronic exposure to carbaryl, there was an initial hike and a later depression in the levels of thyroxine and triiodo thyronine. The elevation was significantly different for both T_3 and T_4 at the rate of 46.56 and 61% respectively. The variation in TSH was insignificant except on 30th day with an increase of 69.01%. The insulin levels were decreased by 65% upon sub-chronic carbaryl exposure. On inducing the hypothalamus-pituitary-thyroid axis with thyrotropin releasing hormone in buffalo calves with sub-chronic carbaryl exposure, the maximum variation in the increase in the levels of thyroxine and tri iodo thyronine was at a percentage decrease of 32.1% and 58.4% respectively. There was no significant difference in the variation in the levels of TSH upon induction with TRH.

P-TOX-04

IMMUNOTOXIC EFFECTS OF ACETAMIPRID FOLLOWING SUBACUTE AND SUBCHRONIC EXPOSURE IN SWISS ALBINO MICE

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Acetamiprid, a neonicotinoid insecticide has been used worldwide for several years in agriculture and veterinary medicine. The objective of the present study was to evaluate immunotoxic effects of acetamiprid in male Swiss albino mice. Acetamiprid was administered i.p. daily at 4.6 and 2.3 mg/kg/day for 30, 60 and 90 days. Specific parameters of humoral and cellular immune response including hemagglutinating antibody (HA) titer to sheep red blood cells (SRBC; T-dependent antigen) and delayed type hypersensitivity (DTH) response to SRBC was evaluated. The result showed that acetamiprid suppressed both humoral and cell mediated immune response as evident from decrease in HA titer and decrease in DTH response. In 60 days treatment group, significant decrease in HA titer was observed at 4.6 mg/kg/day. In 30 and 90 days treatment groups, significant decrease in HA titer was observed at 4.6 and 2.3 mg/kg/day as compared to control group. In 30 and 60 days treatment groups, significant decrease in DTH response was observed at 4.6 mg/kg b.wt. both in 24 and 48 hours later. In 90 days treatment group, significant decrease in DTH response was observed at 4.6 and 2.3 mg/kg/day in 24 and 48 hours later. At these dose levels there were prominent histopathological alterations in spleen. Histopathological analysis of footpad sections of mice revealed dose dependent suppression of DTH response. The results indicated that acetamiprid has immunosuppressive effects at both dose levels which could potentially be attributed to direct cytotoxic effects of acetamiprid against T cells and long term exposure could be detrimental to the immune system.

P-TOX-05

ACUTE TOXICITY STUDY OF METHANOLIC EXTRACT OF *BLUMEA VIRENS* IN SPRAGUE DAWLEY RATS

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Blumea virens, belonging to family Asteraceae, is found abundantly in tropical and subtropical zones of Asia, especially in India. The consumption of whole plant has been reported to produce mortality in animals in Kerala. Thus the present study was undertaken to evaluate the *in vivo* acute oral toxicity of methanolic extract of whole plant of *Blumea virens* in Sprague Dawley rats as per OECD guidelines 425. Acute oral toxicity study was conducted at a dose of 2000 mg/Kg. The animals administered with the plant extract did not produce any related signs of toxicity or mortality during the 14-day observation period. Gross and histopathology was performed at the end of the study. Phytochemical analysis of the plant extract revealed the presence of steroids, alkaloids, flavonoids, tannins, diterpenes, triterpenes and cardiac glycosides.

P-TOX-06

EFFECT OF BETULINIC ACID ON RENAL FIBROSIS IN RAT CHRONIC KIDNEY DISEASE MODEL

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Most chronic kidney diseases (CKDs), regardless of the nature of the initial injury, progress to end stage renal disease characterized by fibrosis with irreversible loss of tissue and function. Thus, improved and more effective therapies are critical. Betulinic acid (BA) has been shown to exhibit various pharmacological activities under *in vitro* and *in vivo* conditions. We established a CKD animal model by feeding adenine at 0.75% in feed in male Wistar rats and sought to evaluate the effect of BA on renal fibrosis. Kidney samples from CKD control rats revealed increased pro-fibrotic protein levels like transforming growth factor-beta (TGF-â), connective tissue growth factor (CTGF), fibronectin, collagen type I and hydroxyproline indicating the presence of fibrotic response which was also reflected in histological findings with wide tubular damage and collagen deposition. However, the above mentioned findings in CKD rats are significantly reversed by BA-treatment revealing its nephroprotective potential especially anti-fibrotic activity. The biochemical mechanism of anti-fibrotic effect of BA in the adenine-induced CKD rats might be mediated by inhibition of pro-fibrotic protein production there by hindering the kidney tissue damage and scarring. Thus, BA could be an adjunct agent for retarding the progression of fibrosis in CKD subjects.

P-TOX-07

EFFECT OF LEVOFLOXACIN ON ASPARTATE AMINOTRANSFERASE HEMATOLOGICAL PARAMETERS FOLLOWING REPEATED ORAL ADMINISTRATION IN DUAL PURPOSE CHICKEN

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The present study was to conduct the safety evaluation of the levofloxacin after repeated oral administration in chickens. The experimental birds (35 day old)were randomly allotted into three groups (n=30),Group I birds served as control (Distilled water),Group II and Group III birds were administered with levofloxacin at the dose rate of 10 mg/kg bw and 20 mg/kg bw respectively for five days for 28 days. The food was withheld for 12 h before oral dosing but not water and water was provided *ad libitum* before the drug administration. The serum samples used for the determination of biochemical parameters on day 0, 7, 14, 21 and 28by using clinical biochemical analyzer. The data were analyzed by using one-way ANOVA. The mean serum AST (U/L) values were 168.40±0.60, 214.24±0.98, 224.30±0.78, 219.58±0.90, 228.86±0.85U/L for Group II and 172.32±0.65, 218.64±0.80, 230.64±0.64 250.65±0.90, 267.80±0.68U/L for Group III and 170.40±0.88, 210.34±0.20, 216.44±0.13, 218.97±0.64, 220.64±0.82U/L for control group on day 0, 7,14, 21 and 28 respectively. There was

a significant increase ($p<0.05$) in AST values in Groups III on day 21 and 28 in the experimental birds as compared to control group. There was no significant increase ($P> 0.05$) in AST values in Groups II on day 0,7,14, 21, 28 and in Groups III on day 0,7, 14 as compared to control group throughout the experiment. The degeneration of hepatocytes and subsequent leakage of enzymes were the reasons attributed for increase in the levels for AST in chickens. So the levofloxacin on long time administration causes the liver toxicity in birds.

P-TOX-08

EFFECT OF LEVOFLOXACIN ON CREATININE HEMATOLOGICAL PARAMETERS FOLLOWING REPEATED ORAL ADMINISTRATION IN DUAL PURPOSE CHICKEN

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The present study was to conduct the safety evaluation of the levofloxacin after repeated oral administration in chickens. The experimental birds (35 day old) were randomly allotted into three groups (n=30), Group I birds served as control (Distilled water), Group II and Group III birds were administered with levofloxacin at the dose rate of 10 mg/kg bw and 20 mg/kg bw respectively for five days directly into the crop using a thin plastic tube attached to a syringe for 28 days. The food was withheld for 12 h before oral dosing but not water and water was provided *ad libitum* before the drug administration. The serum samples used for the determination of biochemical parameters on day 0, 7, 14, 21 and 28 by using clinical biochemical analyzer. Data were analyzed by using one-way ANOVA. The mean serum creatinine values were 0.58±0.09, 0.52±0.04, 0.61±0.16, 0.73±0.08, 0.85±0.13 mg/dl for Group II and 0.60±0.03, 0.56±0.16, 0.70±0.04, 0.92±0.12, 1.04±0.06 mg/dl for group III and 0.57±0.04, 0.48±0.07, 0.54±0.05, 0.70±0.16, 0.72±0.10 mg/dl for the control group on day 0,7,14, 21 and 28 respectively. There was a significant ($P<0.05$) increase in creatinine values in Groups III on day 21 and 28 in the experimental birds as compared to control group. There was no significant ($P>0.05$) increase in creatinine values in Group II on day 0,7,14, 21, 28 and in Group III on day 0 and 7 as compared to the control group throughout the experiment. The elevated levels of creatinine observed in this study indicated the kidney damage caused by high dose of levofloxacin at the dose rate of 20 mg/kg bw which drew support from the histopathological observations like tubular epithelial cell degeneration, desquamation, congestion, hemorrhages, necrosis along with infiltration of inflammatory cells in the interstitium of renal tubular epithelium towards the end of the experiment in kidney. So the levofloxacin on long time administration causes the renal toxicity in birds.

P-TOX-09

TOXICITY OF THE LEVOFLOXACIN AT 10MG/KG BODY WEIGHT IN LIVER TISSUES FOLLOWING REPEATED ORAL ADMINISTRATION IN DUAL PURPOSE CHICKEN

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The present was to conduct the toxicity of the levofloxacin in liver tissue following the repeated administration in chickens to see the adverse effect with respect to gross and histopathological examination. The experimental birds (35 day old)were randomly allotted into two groups (n=30),Group I birds served as control (Distilled water), Group II birds were administered with levofloxacin at the dose rate of 10 mg/kg bw respectively directly into the crop using a thin plastic tube attached to a syringe for 28 days. The six birds from each group were randomly selected and sacrificed at weekly interval on day 0, 7, 14, 21 and 28 during the study period. The birds were subjected to a detailed post mortem examination and gross lesions if any were recorded. The samples of liver collected, washed with normal saline and then collected in 10 percent neutral buffered formalin (NBF). They were processed through routine paraffin embedding technique. All the organs were processed for histopathology by cutting sections of 4µm thickness and stained with Haematoxylin and Eosin. In Group II, levofloxacin administered at 10 mg/kg bw, no gross pathological changes were observed on day 0,7,14 and 21. There was enlargement and congestion of liver on day 28 compared to the control group Histologically the sections of liver in Groups II did not reveal any histopathological changes on day 0, 7, 14 and day throughout the experimental period compared to the control group . The section of liver showed mild dilatation of sinusoids with focal infiltration of mono nuclear inflammatory cells, vacuolar degeneration of hepatocytes and dilatation of sinusoids, congestion of central vein, infiltration of mono nuclear cells in Group II on day 21 and 28 respectively. This study shows that levofloxacin at 10mg/kg body weight causes toxicity in liver tissues after repeated administration for 28 days.

P-TOX-10

HISTOMORPHOLOGICAL AMELIORATION OF *ASTERACANTHA LONGIFOLIA* WHOLE PLANT ON CADMIUM CHLORIDE AND THIO UREA INDUCED TOXCITY IN RATS

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The present study was conducted to investigate the histological alterations caused by cadmium chloride and thio urea in albino Wistar rats and protective effect of Ethanolic *Asteracantha longifolia* whole plant extract. Collected plants were subjected to soxhlet extraction with ethanol as solvent and preliminary phytochemical analysis was carried out. Cadmium chloride (@ 200 ppm in water, orally) was administered for 60 days, during last 30 days, cadmium treated animals received plant extract at the dose of 200 and 400 mg/kg orally.

Thyroprotective activity of *A. longifolia* was evaluated in thio urea (250 mg/kg, in feed) induced hypothyroid rats, Ethanolic extract of A.longifolia was administered at the dose of 200 and 400 mg/kg, orally for a period of 30 days. At the end of experiments, all are animals were sacrificed body weight, organ weight of liver, kidney, testes and accessory sex glands were estimated and also weight of thyroid gland recorded. All the major organs including testes and thyroid glands were subjected to gross and histopathological changes. Phytochemical analysis showed the presence of alkaloids, glycosides, tannins, saponins and terpenoids. In cadmium treated groups, the testicular tissue showed widening of inters tubular spaces wrinkling of basement membrane as classic lesion along with pyknosis and depopulation of spermatogonial cells in seminiferous tubules. The thyroid gland histology in thio urea treated group revealed variation in shape, size and chromatin content of nuclei with hyperplasia, the average individual follicle appears to be smaller with dominant lower columnar epithelium and scanty colloids. The group of rats treated with *A. longifolia* extract revealed the restoration of all the histomorphological structure in both cadmium and thio urea treated rats. Hence, this study proves the traditional use of *A. longifolia* plant as aphrodisiac and thyrotrophic agent.

P-TOX-11

STUDIES ON WOUND HEALING EFFICACY AND SAFETY OF ENHANCED SLOW RELEASE IODINE PREPARATION IN EXPERIMENTAL ANIMAL MODELS

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The present study was undertaken to evaluate the wound healing efficacy and safety of Povidone iodine enhanced with silk protein, sericin. Wound area reduction, wound healing time, total protein, hydroxyproline content and histological evaluation were done in the wound healing study in rats. Safety evaluation was carried out by using dermal irritation, acute dermal toxicity and skin sensitization studies in laboratory animals. In excision wound healing study, Wistar rats were divided into four groups. Group I was treated with the test combination (sericin + povidone iodine); Group II with plain silk protein, Group III with povidone iodine (positive control) and Group IV was the negative control. Group I showed significant ($P<0.001$) decrease in wound area and wound healing time, also there was significant ($P<0.01$) increase in protein and hydroxyproline content of the wound tissue compared to Group IV (NC). Histopathologically, Group I showed enhancing effect compared to Group IV. In dermal irritation study, the sericin + povidone iodine treated New Zealand White rabbits showed no signs of erythema, edema and eschar and the scoring was given as '0' for all time points of observations according to Draize scoring system. In acute dermal toxicity study, the rats treated with sericin + povidone iodine did not show any abnormal clinical signs throughout the observation period of 14 days after the removal of patch and the body weight, biochemical parameters and gross pathological observations were not significantly different from the control group. The skin sensitization study on Guinea pig showed no skin reaction 24 and 48 h after the removal of challenge patch, which was scored '0' based on Magnusson/ Kligman grading scale. In the present study, the test materials were made up of silk proteins which

might have enhanced concentrations of important growth factors, retention of water, proteins, and electrolytes and attachment and growth of fibroblast cells. This might be the reason for enhanced wound contraction in Groups treated with sericin based films. Thus, it was concluded that, the test material (sericin + povidone iodine) was safe and had enhancing effect on wound healing.

P-TOX-12

SAFETY ASSESSMENT OF MOXIFLOXACIN AFTER ITS REPEATED INTRAMUSCULAR ADMINISTRATION IN COW CALVES

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The present study was designed to investigate safety of moxifloxacin (5 $mg.kg^{-1}$ b.wt.) after repeated intramuscular administration at 24 hours interval for 5 days in healthy cow calves. Safety of repeated intramuscular administration of moxifloxacin was assessed by studying the haematological and serum biochemical parameters. Blood samples were collected before administration of the drug which served as control (day 0). After administration of drugs, blood samples were collected at day 1^{st} (24 h), 2^{nd} (48 h), 3^{rd} (72 h), 4^{th} (96 h), 5^{th} (120 h) and 6^{th} (144 h) from jugular vein into vacutainers for haematological and serum biochemical analysis. No significant alterations ($p<0.05$) in haemoglobin level, packed cell volume, total leukocyte count, and differential leukocyte count (neutrophil, lymphocyte, basophil, eosinophil and monocyte) have been observed following daily intramuscular administration of moxifloxacin for 5 days in cow calves. No significant alterations ($p<0.05$) in blood biochemical parameters, viz. values of serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum lactate dehydrogenase (LDH), serum alkaline phosphatase (AKP), serum acid phosphatase (ACP), serum bilirubin, serum creatinine, blood urea nitrogen (BUN), total protein, albumin and blood glucose, have been observed following intramuscular administration of moxifloxacin (5 $mg.kg^{-1}$ b.wt., repeated at 24 h interval) for 5 days in cow calves.

P-TOX-13

THIAMETHOXAM SUBCHRONIC TOXICITY AND AMELIORATIVE POTENTIALS OF QUERCETIN ON HEMATOLOGICAL AND PLASMA ELECTROLYTE PARAMETERS IN MALE WISTAR RATS

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Pesticides are chemical substances intended for preventing, destroying and controlling pests including vectors of animal and human diseases. The present study is designed to evaluate the toxic effect of subchronic toxicity of thiamethoxam and its amelioration by quercetin on some hematological and plasma electrolyte

parameters. Thiamethoxam at two dose levels 2.5% and 5% maximum tolerated dose (MTD = 4200 mg/kg) and quercetin (50 mg/kg) was administered orally daily for 60 and 90 days. A gap of 12 hours was maintained between thiamethoxam and quercetin administration. Blood samples taken from the heart were taken in heparinized vials and centrifuged. The blood and plasma obtained was used for the determination of hematological and plasma electrolytes parameters. Thiamethoxam administered at the dose of 2.5% and 5% maximum tolerated dose (MTD = 4200 mg/kg) significantly decreased hemoglobin concentration (Hb) and red blood cell (RBC) count in 60 and 90 days treatment, while packed cell volume (PCV) was decreased at both dose rates in 60 days schedule but it was observed to be increased at lower dose in 90 days schedule. Red blood cells distribution (RDW) was increased at both doses in 60 days and at higher dose in 90 days schedule as compared to control. Thiamethoxam at lower dose significantly reduced mean corpuscular volume (MCV) in 90 days treatment while mean corpuscular hemoglobin (MCH) & mean corpuscular hemoglobin concentration (MCHC) in 60 and 90 days treatment schedule as compared to control. Sodium and chloride plasma levels decreased significantly in rats treated with both doses of thiamethoxam in 90 days treatment schedule, while phosphorous level decreased significantly at both doses in 60 and 90 days treatment schedule compared to control. Potassium level increased significantly at higher dose of treatment in 60 days and at both doses in 90 days schedule as compared to control. Calcium increased significantly at both doses in 60 days treatment compared to control. Ameliorative effect of quercetin was seen on Hb at 2.5% MTD (TMX)+Qu dose at 60 days and at 2.5% MTD (TMX)+Qu and 5.0% MTD (TMX)+Qu dose in 60 and 90 days schedules, respectively. Amelioration in RDW was observed at higher dose in 60 days schedule. The ameliorative effect on PCV was seen at 2.5% MTD (TMX)+Qu and 5.0% MTD (TMX)+Qu in 60 days and 5.0% MTD (TMX)+Qu in 90 days treatment schedule. Amelioration in MCV was seen at 2.5% MTD (TMX)+Qu in 90 days schedule and in MCHC at 2.5% MTD (TMX)+Qu in 60 days schedule. Amelioration effect of quercetin is seen for MCH at 2.5% MTD (TMX)+Qu dose at 60 and 90 days schedule. Ameliorative effect of quercetin on sodium, chloride and calcium levels was observed in 90 days schedule, whereas, in phosphorus and potassium amelioration was observed at both schedules.

P-TOX-14

MONITORING AND STUDY OF CHLORPYRIFOS RESIDUES IS AN ESSENTIALITY IN EASTERN UTTAR PRADESH

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In India, agriculture and animal farming constitute major occupation as well as the principal resource for food sufficiency and chief sector of employment for its majority of people especially in rural areas. Strategic efforts were made in agricultural and animal farming to increase the production to meet out the food demand for the rapidly growing population. Food insufficiency due to diminished production was mainly because of harmful insect pests and diseases during cropping and storage. Insect pests also spread a number of important diseases

drugs and other insecticides. Because of scanty information about the occurance of chlorpyrifos residue and its adverse implications on animals, the periodic monitoring and study by means of survey and laboratory analysis of its residues in animals and their products along with agricultural produce is essential as it is one of the most commonly used insecticide in agriculture in eastern U.P., veterinary medicine and domestic use as well as in community health operations.

ISVPT-2016

POSTER SESSION – II

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P-PK-01

A NOVEL METHOD FOR QUANTIFICATION OF MARBOFLOXACIN IN SHEEP PLASMA BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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The precision pharmacokinetic study of every single novel drug needs a highly sensitive quantification technique for effective outcome. The microbial, spectrophotometric and HPLC assay are several approaches being employed in the analysis of drugs from blood and other biological matrix. However, now-a-days, the popularity of LC MS/MS as a quantification technique is growing because of high sensitivity, specificity and repeatability. In present study, a simple, time saving and sensitive liquid chromatography tandem mass spectrometric method has been developed and validated for determination of marbofloxacin, a newer veterinary exclusive fluoroquinolone, from sheep plasma. Marbofloxacin was extracted from sheep plasma by liquid-liquid extraction. Detection was performed as transition of marbofloxacin into two product ions i.e., 363.00 —› 320.00 and 363.00 —› 245.00 using mass spectrometry. Chromatographic separation was performed using reverse phase C_{18} column at 0.2 ml/min flow rate. The assay of marbofloxacin was linear over the range of 0.001 µg/ml to 10.00 µg/ml with regression co-efficient (R^2) > 0.9994. The observed value of intra-day and inter-day precision of assay was found in between 5.196 to 10.686 % and 2.343 to 13.768 %, respectively. The mean percent recovery of marbofloxacin from sheep plasma varied from 74 to 78 %. The assay was used successfully for pharmacokinetic and bioavailability studies of marbofloxacin in sheep.

P-PK-02

EFFECT OF TOLFENAMIC ACID ON INTRAMUSCULAR PHARMACOKINETICS OF CEFTIZOXIME IN SHEEP

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Successful treatment of infectious diseases largely depends upon use of compatible combination of antimicrobial and anti-inflammatory drugs. Present study was planned to investigate the effect of NSAID drug tolfenamic acid (4 mg/kg b.wt.) on the intramuscular pharmacokinetic behaviour of ceftizoxime (10 mg/kg b.wt.) in six adult female sheep. Plasma concentration of ceftizoxime was analysed using UHPLC with UV detector. The pharmacokinetic parameters were calculated using PK solver software. Following single dose IM administration of ceftizoxime alone and along with tolfenamic acid, the mean peak plasma levels were found as 30.03 and 16.40 µg/ml, respectively, achieved at 15 min. Plasma ceftizoxime concentrations at all time

points (except 8 and 10 h) were found significantly lower (P<0.01) in sheep given tolfenamic acid along with ceftizoxime as compared to sheep which were given ceftizoxime alone. Plasma levels of ceftizoxime above MIC were detected up to 36 h, as 0.34 ± 0.03 and 0.24 ± 0.01 µg/ml, respectively, when administered alone and along with tolfenamic acid in sheep. Following ceftizoxime administration alone and along with tolfenamic acid, mean values of elimination half-life ($t_{1/2\ â}$), apparent volume of distribution [$Vd_{(area)}$], clearance (Cl_B) and bioavailability were determined as 9.97 and 7.84 h, 0.70 and 0.91 $L.kg^{1}$, 0.07 and 0.10 $ml.min^{1}.kg^{1}$ and 132.12 and 122.67 %, respectively. Mean values of $Vd_{(area)}$ and Cl_B after alone IM administration of ceftizoxime were significantly lower (P<0.01) whereas value of $t_{1/2\alpha}$ was found significantly higher (P < 0.05) than their corresponding values observed after concomitant IM administration of ceftizoxime and tolfenamic acid.

P-PK-03

PHENOTYPIC VARIATION IN MOXIFLOXACIN DISPOSITION IN DOMESTIC RUMINANTS

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Moxifloxacin, an antimicrobial drug of flouroquinolone group, was administered intravenously at a dose of 5 mg/kg to 6 each of male Mahesana goats, female Patanwadi sheep and female Mahesani buffalo calves. All animals were kept in natural environment with access to *ad libitum* feed. Heparinised jugular blood samples were taken at pre-determined time interval. HPLC method was developed for plasma moxifloxacin analysis. Disposition of moxifloxacin; pattern of distribution and elimination was studied and compared in all three ruminants. Initially at 5 min. of drug administration, plasma concentration of moxifloxacin in sheep, goats and buffalo calves were found as 10.25, 5.90 and 4.24 µg/ml, respectively. Level of drug at 24 hour post administration was 0.015, 0.014 and 0.021 µg/ml, respectively. Disposition pattern of moxifloxacin was more or less similar in the three species of ruminants. Data of moxifloxacin disposition from sheep, goats and buffalo calves fit unimodal disposition, suggesting that these animals do not exhibit phenotypic polymorphism of moxifloxacin metabolism. More species of animals need to be assessed for studying variation in moxifloxacin disposition as to use drug rationally in clinical veterinary medicine to combat infectious disorders in domestic ruminants.

P-PK-04

DISPOSITION KINETICS, PK-PD INTEGRATION AND TISSUE RESIDUE PROFILE OF ENROFLOXACIN (CIPROFLOXACIN) IN BROILER CHICKENS

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The present study was carried out with the aim to determine pharmacokinetic feature of enrofloxacin in broiler chickens (Vencob®) after single *per-os* or intravenous administration (10 mg.kg^{-1}). Further, tissue residue profile of enrofloxacin or its metabolite (ciprofloxacin) in tissues (breast muscle, liver and kidney) was analysed following enrofloxacin administration *per-os* at the rate of 10 mg.kg^{-1} × 5 days. Attempts were also made to analyse the suitability of dosage regime of enrofloxacin against common pathogens infecting poultry as per PK-PD integration using current or hypothetical MIC values. The disposition kinetic of enrofloxacin in broiler chickens was best described by two-compartment open model following both routes of administration. The plasma elimination half-life ($t_{½\beta}$), mean resident time (MRT) and bioavailability of enrofloxacin were found to be 6.31±0.60 h, 3.72 ±0.25 h and 65 per cent respectively following single *per-os* administration (10 mg.kg^{-1}) in broiler chicken. The PK-PD integration of the data revealed that enrofloxacin was moderately effective against local isolates (*E. coli* 0119 and *Salmonella typhimurium*) based on the measured MIC value, while it was found to be ineffective against organisms whose hypothetical MIC value < 0.25 μg.ml^{-1}. The tissue residue concentration of enrofloxacin or its metabolite (ciprofloxacin) persisted up to 72 hr in breast muscle, while the residue of ciprofloxacin alone persisted up to 92 hr in liver and kidney. Based on the MRL prescribed by the Export Council of India (ECI) for fresh poultry meat, it is suggested to adapt 4 days withdrawal period in broiler chickens medicated (*per-os*) with therapeutic dosage (10 mg.kg^{-1} × 5 days) of enrofloxacin.

P-PK-05

TOXICOKINETICS OF LAMBDA-CYHALOTHRIN IN SERUM AND DIFFERENT TISSUE SAMPLES FOLLOWING ORAL ADMINISTRATION IN WISTAR ALBINO RATS

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The toxicokinetics study of lambda-cyhalothrin in wistar albino rats was conducted at single oral dose 20 mg kg^{-1}. Forty wistar albino rat (male & female) of body weight 200±10 g selected after acclimatization divided into eight groups (Group-I, II, III, IV, V, VI, VII & VIII) of each five animals and lambda-cyhalothrin was administered

separately in each of animals. The lambda-cyhalothrin was administered in overnight fasting rats by mixing in 0.1% Tween 80 and Serial blood samples were collected at 0, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 and 24.0 h after administration. All the animals of each group were killed by cervical dislocation immediately after blood collection at respective time interval. Different tissue samples (liver, kidney, spleen, adrenal, heart, brain, ovaries and testis) were collected at same time interval of blood collection. The concentration of lambda-cyhalothrin concentrations were determined by HPLC. The plasma and tissue concentration time data for lambd-acyhalothrin were found to fit a one-compartment open model. The elimination half-life and mean residence time for lambda-cyhalothrin in serum were found 5.09±0.16 h and 7.32±0.11 h, respectively. The mean volume of distribution (Vd_{area}) in serum was calculated to be 3.94±0.12 L/kg. The total body clearance (ClB) in serum was observed to be 0.54±0.01 $L.kg^{-1}.h^{-1}$. The maximum serum concentration of lambda-cyhalothrin (Cmax) was noted to be 6.42±0.11 $\mu g.ml^{-1}$ at Tmax of 2.00 ± 0.00 h exhibit that after oral administration, lambda-cyhalothrin was extensively but slowly absorbed. The maximum concentrations Cmax was observed highest in spleen (17.9±0.38), ovaries (9.77±0.81), liver (7.75±0.28), kidney (7.75±0.28), heart (4.90±0.42), adrenal (4.87±0.69), brain (6.63±0.25) and testis (1.46±0.10) $\mu g.ml^{-1}$. The elimination half- life ($t_{½}$ α) were calculated to be 4.58±0.23 (liver), 4.29±0.29 (kidney), 4.04±0.27 (heart), 4.39±0.21 (adrenal), 0.16±0.003 (spleen), 4.46±0.26 (brain), 5.65±0.47 (testis) and 7.48±1.65 (ovaries) h. The highest mean value of volume of distribution (Vd_{area}) was calculated in testis 50.6±2.11 and lowest in spleen 2.69±0.06 $L\ kg^{-1}$. The mean value of total body clearance (Cl_B) highest in testis 6.28±0.22 whereas lowest in ovaries 0.39±0.02 $L.kg^{-1}.h^{-1}$. Lambda-cyhalothrin primarily affects nervous tissue and spleen. Therefore, we advise to use this insecticide at the recommended field application levels away from vegetation to be eaten by animals and to minimize the direct exposure to it as much as possible in order to avoid its toxic effects.

P-PK-06

PHARMACOKINETICS OF 6-MERCAPTOPURINE LOADED CHITOSAN NANOPARTICLES IN WISTAR RATS

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6-Mercaptopurine (6-MP) is metabolized to an active form methylated thioinosinic acid (MeTIMP) by hypoxanthine phosphoribosyltransferase (HPRT) within cell to inhibit *de novo* purine synthesis and later converted to thioguanine, which is a DNA intercalating agent. However due to lower bioavailability (16%), short plasma half-life (0.5-1.5 h), with moderate plasma protein binding (19% to 30%), the chemotherapeutic effects failed to achieve. In order to modify kinetic profile, 6-MPCNPs (6-MP chitosan nanoparticles) were synthesised, evaluated at release media (phosphate buffer pH: 3.4, 6.4 and 7.4) and assessed by double beam UV-visible spectrophotometer. 6-MPCNPs showed an initial burst release of 6-MP (@ 4-6 h) in all incubation

media. The cumulative drug release profiles of 6-MPCNPs at pH 3.4 (62%), 6.4 (72%) and 7.4 (82%) were significantly higher in comparison to 6-MP (46%, 48% and 45%) at 48 hr. The Wistar rats were administered with single oral dose (15 mg/kg bwt) of 6-MPCNPs and 6-MP. The pharmacokinetic profile of 6-MPCNPs shown favourable results against 6-MP (F %- 56.52% vs 37.80%; Cmax-25.25 ng/mL vs 13.48 ng/mL at 4 h; MAT-7.98 h vs 3.85 h; $AUC_{0-\infty}$- 59,712.00 ng/mL vs 37987.00 ng/mL; MRT-8.31 hr vs 4.35 hr; Vd-4.76 vs 3.86 L/kg). So, conclusively results of above discussed pharmacokinetic parameters help in understanding 6-MPCNPs advantageous role as a novel oral drug delivery system in comparison to conventional 6-MP.

P-PK-07

PHARMACOKINETICS AND DOSAGE REGIMEN OF MOXIFLOXACIN FOLLOWING SINGLE INTRAMUSCULAR ADMINISTRATION IN COW CALVES

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The disposition kinetics of moxifloxacin after a single intramuscular administration at dosage level of 5 mg/kg b.wt. was investigated in healthy cow calves and an appropriate dosage regimen was calculated. The blood samples were collected at different time intervals and the concentration of drug in plasma was estimated by microbiological assay technique. Intramuscular injection of moxifloxacin in cow calves resulted into appreciable plasma concentration (0.20 ± 0.01 $\mu g.ml^{-1}$) at 1 min, which gradually increased and the peak plasma concentration (4.01 ± 0.10 $\mu g.ml^{-1}$) was observed at 45 min. The drug was detected in plasma up to 12 h. The mean values of absorption rate constant (Ka) and absorption half-life ($t_{1/2Ka}$) were 1.48 ± 0.54 h^{-1}, 0.470 ± 0.017 h, respectively. The mean values of area under curve ($AUC_{0-\infty}$), apparent volume of distribution (Vd_{area}) and mean residence time (MRT) were 11.27 ± 0.200 $\mu g.ml^{-1}.h$, 1.53 ± 0.021 $L.kg^{-1}$ and 3.57 ± 0.021 h, respectively. The mean values of elimination half-life ($t_{1/2\beta}$) and total body clearance were 2.38 ± 0.025 h and 0.444 ± 0.008 $L.kg^{-1}.h^{-1}$, respectively. The systemic bioavailability (F) following i.m. administration of moxifloxacin in cow calves was 88.14 ± 2.72 per cent. To maintain a minimum therapeutic concentration of moxifloxacin as 0.50 $\mu g.ml^{-1}$, a satisfactory dosage regimen of moxifloxacin should be 7.82 $mg.kg^{-1}$ followed by 7.06 $mg.kg^{-1}$ repeated at 8 h intervals.

P-MNP-01

ARSENIC CAUSES THE AORTIC DYSFUNCTION AND SYSTEMIC HYPERTENSION IN RATS: AUGMENTATION OF ANGIOTENSIN II SIGNALING

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The ground water pollutant arsenic can cause various cardiovascular disorders. Angiotensin II, a potent vasoconstrictor, plays an important role in vascular dysfunction by promoting changes in endothelial function, vascular reactivity, tissue remodeling and oxidative stress. We investigated whether modulation of angiotensin II signaling and redox homeostasis could be a mechanism contributing to arsenic-induced vascular disorder. Rats were exposed to arsenic at 25, 50 and 100 ppm of sodium arsenite through drinking water consecutively for 90 days. Blood pressure was recorded weekly. On the 91st days, the rats were sacrificed for blood collection and isolation of thoracic aorta. Angiotensin converting enzyme (ACE) and angiotensin II level were assed in plasma. Aortic reactivity to angiotensin II was assessed in organ-bath system. Westron blot of AT_1 receptors and G protein ($G\alpha_{q/11}$), ELISA of signal transducers of MAP kinase pathway and ROS generation was assessed in aorta. Arsenic caused concentration-dependent increase in systolic, diastolic and mean arterial blood pressure form 10th, 8th and 7th week onwards, respectively. Arsenic caused concentration-dependent enhancement of the angiotensin II- induced aortic contractile response. Arsenic also caused concentration-dependent increase in the plasma levels of angiotensin II and ACE and the expression of aortic AT_1 receptors and $G\alpha_{q/11}$ proteins. Arsenic increased aortic protein kinase C activity and the concentration of protein tyrosine kinase, extracellular signal-regulated kinase-1/2 and vascular endothelial growth factor. Further, arsenic increased aortic mRNA expression of Nox2, Nox4 and p22phox, NADPH oxidase activity and ROS generation. The results suggest that arsenic-mediated enhancement of angiotensin II signaling could be an important mechanism in the arsenic-induced vascular disorder, where ROS could augment the angiotensin II signaling through activation of MAP kinase pathway.

P-MNP-02

IN-VITRO METABOLISM STUDIES OF LINCOMYCIN USING S9 FRACTION FROM GOAT LIVERS

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The present study was planned to study the *in vitro* drug metabolism and time dependent kinetics of lincomycin using S9 liver fraction of goats. *In-vitro* metabolism and time dependent kinetics of lincomycin were performed at a concentration of 50 $\mu g.ml^{-1}$ in S9 fraction; the concentration of drug taken was based on its

Cmax achieved during in-vivo animal studies. Reaction was terminated at 0, 15, 30, 45 and, 60 min and formation of metabolites/lincomycin concentrations left in reaction mixture with respect to time were estimated using Michalis-Menten Kinetic analysis. The total protein and cytochrome P450 content was 23.74± 0.9 mg/g of wet tissue and 0.56± 0.1 nmol/mg of protein, respectively in goat livers. Exposure of lincomycin with S9 fraction in goats caused a decrease in lincomycin concentration in time dependant manner i.e. at 15, 30, 45 and, 60 min, the lincomycin concentrations were 25.27±2.32, 21.55±2.35, 19±2.9 and, 17.6±2.1 µg.ml-1, respectively. The apparent in-vitro kinetic parameters, K_m and V_{max}, were found to be 27.02 µg and 0.18 nM/min/mg of protein, respectively. The $t_{1/2}$ and CL_{int} of lincomycin in S9 fraction were 46.47±7.23 min and was 5.68±0.91 mL/min/g of tissue, respectively. Cytochrome P450 inhibitory assay indicates that CYP1A2, CYP2D6, CYP2C9 and, CYP3A4 enzymes were not involved in metabolism of lincomycin in goats. There was no significant reduction in the protein content following different number of freeze-thaw cycles. However the activity of cytochrome P450 completely disappeared after two freeze-thaw cycles. At the beginning of cycles, the protein content was23.45±0.73 mg/g of wet tissue, whereas at the end of 10th cycle it was 22.24±0.44 mg/g of wet tissue. At the beginning of freeze-thaw cycles, the cytochrome P450 concentration was 0.59±0.03 nmol/mg protein, and it could not be detected after 2nd freeze-thaw cycle.

P-MNP-03

IN-VITRO ASSESSMENT OF CYP-MEDIATED METABOLISM AND KINETICS OF LINCOMYCIN IN SHEEP USING S9 FRACTION

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Mammalian cytochrome P450 (CYP P450 or CYP) enzymes can oxidise both exogenous and endogenous compounds. They play vital role in detoxification, formation of metabolites and, their clearance from body. The present study is the detailed *in vitro* drug metabolism-kinetics of lincomycin and was conducted using S9 liver fractions of sheep (n=5). S9 fraction of liver was prepared and *in-vitro metabolism*-kinetics of lincomycin was performed by adding lincomycin (50 µg.ml^{-1}) in S9 fraction. The total protein and cytochrome P450 content of the isolated S9 fraction was 20.41± 0.62 mg/g of wet tissue and 0.59±0.17 nmol/mg of protein, respectively. Exposure of licomycin with S9 fraction in sheep caused a decrease in lincomycin concentrations in time dependant manner i.e. at 15, 30, 45 and, 60 min concentrations of lincomycin were 26.6±0.71, 24.17±1.09, 21.43±1.08 and, 18±1.64 µg.ml^{-1}, respectively. *In-vitro* kinetic parameters $t_{1/2}$ and CL_{int} of lincomycin in S9 fraction were found to be 46.92±4.24 min and 4.81±0.53 mL/min/g, respectively. Around 65% of lincomycin was metabolized within 1 hour by the S9 fraction. This drug metabolism inhibition study indicates the partial involvement of cytochromes 1A2, 3A4, 2C9 and 2D6 in lincomycin metabolism but does not show any clear-cut major involvement. Further studies are needed to check the involvement of other cytochrome P450 isoenzymes and cytosolic fractions in metabolism of lincomycin.

was metabolized within 1 hour by the S9 fraction. This drug metabolism inhibition study indicates the partial

P-MNP-04

CYTOTOXIC POTENTIAL OF RHIZOME EXTRACT OF *HEDYCHIUM SPICATUM L*. IN HEPG2 CELL LINE

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The objective of the study was to evaluate the cytotoxic effects of methanolic, ethanolic and aqueous extracts of *Hydechium spicatum* rhizome extract on human liver hepatocellular carcinoma (HepG2) cell line using MTT assays. The crude extract of *Hedychium spicatum* was prepared by cold maceration method and then filtered, concentrated and tested on HepG2 cell line. Dose-dependent cytotoxic activities were exhibited on HepG2 cell line. The cell viability was significantly ($P<0.05$) decreased to 81, 70, 63, 53, 47 and 36 % at the concentrations of 50, 100, 250, 500, 1000 and 2000 µg/ml of methanolic extract, respectively; 87, 70, 60, 50 and 31% at the concentration of 50, 100, 250, 500 and 1000µg/ml of ethanolic extract, respectively, and 91, 85, 70, 57, 52, 44 and 31% at the concentrations of 25, 50, 100, 250, 500, 1000 and 500µg/ml of aqueous extracts, respectively. The cell viability was significantly ($P<0.05$) decreased as the dose of the extract increased. Thus, it was concluded from the study that there was a inverse relation between the cell viability and concentration and the aqueous extract revealed least and methanolic extract revealed maximum cytotoxic potential of the rhizome of *H. spicatum.*

P-MNP-05

IN VITRO ANTI CANCEROUS EFFICACY OF 6-MERCAPTOPURINE LOADED CHITOSAN NANOPARTICLES

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6-Mercaptopurine (6-MP) is a cytotoxic and immunosuppressant drug. The use of 6-MP is restricted due to its poor anti cancerous activity. In order to abolish drawbacks, 6-mercaptopurine-chitosan nanoparticles (6-MPCNPs) were prepared and evaluated for physicochemical properties by using DLS, SEM, XRD and FTIR. The *in vitro* cytotoxicity assays showed that, 6-MP and 6-MPCNPs were able to decrease the viability of HT-1080 (76.2% and 89.7%) and MCF-7 cells (69.8% and 93.41%) respectively at the concentration of 10.0 µM (Estimated loading efficiency of 6-MP in chitosan nanoparticles by LCMS/MS technique was 29.1%). G2/M phase arrest was noticed for 6-MP/6-MPCNPs treatment (@IC_{50}) on HT-1080/MCF-7 cell lines. The respective per cent arrest of 6-MP on HT-1080/MCF-7 cell lines (17.56%/18.45%) matched with 6-MPCNPs

(21.25%/14.49%); which is not seen in untreated cell lines (8.96%/9.22%). HT-1080 cells which are untreated (control) have shown 99.73% viability; whereas in case of 6-MP treatment early and late apoptotic per cent were 68.95% and 1.48% respectively and in case of 6-MPNP treatment were 69.84% and 0.36%. Cell viability % of MCF-7 was 99.50 % (control); in contrary 6-MP and 6-MPNP showed respective early apoptotic (46.09% & 58.27%) and late apoptotic (53.56% & 0.82%) effect. The nano formulations showed enhanced *in vitro* anti-cancer activities (MTT assay, apoptosis assay and cell cycle arrest) on HT-1080 and MCF-7 cells. Thus, the results of the present study revealed that, the prepared 6-MP-CNPs have a significant role in increasing anti-cancer efficacy.

P-MNP-06

PHARMACOLOGICAL STUDIES ON EVALUATION OF BEST CONTRACTILE AGENT FOR VASCULODYNAMIC STUDIES ON UTERINE ARTERY OF BUFFALOES

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In the present study, potency and efficacy of different vasoconstrictor agents was studied on the third branch of uterine artery of buffaloes. Isometric tension in arterial rings, mounted in organ bath containing modified Kreb's Heneseleit Solution continuously aerated with medical gas, was measured using data acquisition system-based physiograph and Lab Chart Pro V7.3.7 software. Pilot studies revealed that 2.0 g of passive tension generated maximum active tension (5.05 ± 0.29 g) following exposure of uterine artery to 80mM K^+ depolarising solution. Respective potency and efficacy of endothelin-1, noradrenaline, adrenaline, serotonin and phenylephrine were found to be 7.80 ± 0.91 and 1.41 ± 0.08 g, 6.01 ± 0.10 and 5.80 ± 0.77 g, 6.44 ± 0.06 and 5.17 ± 0.37 g, 6.31 ± 0.05 and 4.05 ± 0.35 g, 5.59 ± 0.00 and 2.85 ± 0.44 g. Among all contractile agents, endothelin-1 produced consistent and sustained contraction while all other agonists induced contractions decayed after some time. Therefore, based on the present study, it can infer that the optimal passive tension for buffalo uterine artery is 2.0 g and endothelin-1 is the most suitable vasoconstrictor for vascular reactivity and pharmacodynamic studies in buffalo uterine artery.

P-NP-01

EFFECT OF FUNCTIONAL FOOD ON GROWTH PERFORMANCE, METABOLIC PROFILE AND CARCASS CHARACTERISTICS IN BROILER FLOCK

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The yogurt, chicory root powder (CRP) and vinegar are regarded as functional food (FF) and well praised for their health benefit on consumers. The present experiment was undertaken to assess the effect of these FF on growth performance, hematology, metabolic profile and carcass characteristics in broiler birds. Two hundred forty, day old broiler chicks (Vencobb-400) were assigned into five groups with 4 replicates for each. The birds were reared on standard diet without any supplementation (control; CON), supplemented with *Lactobacillus sporogenes* through yogurt as probiotics (PRO; @ 3%); Inulin through CRP as prebiotic (PRE; @ 3%); combination of yogurt and CRP as symbiotic (SYN; @ 1.5% each) and acetic acid through food grade vinegar as acidifier (ACI; @ 3%). Yogurt and vinegar were supplemented with drinking water, while CRP with diet. At end of experiment (6 weeks), birds were sacrificed to evaluate their carcass characteristics and blood was collected to assess hematological and metabolic indices. The vinegar supplemented group (ACI) depicted improvement in body weight and thereby growth rate. Due to higher growth rate accompanied with similar feed intake, feed utilization efficiency was better in ACI. Hematological picture did not display any alteration across the groups. Among metabolic profile, there was a reduction in serum cholesterol in PRO and triglyceride in yogurt and vinegar supplemented groups (PRO, PRE and ACI). Yogurt only supplementation (PRO) enhanced the level of serum calcium. Various indices of carcass characteristic like dressing efficiency and yield of splanchnic or other viscera were comparable among groups. Likewise, yield of wholesale cuts of carcass were also similar. The present findings suggests that supplementation of vinegar in drinking water improves growth rate and feed utilization efficiency, while yogurt optimistically modulates the circulating lipid profile in broiler chicken.

P-NP-02

EFFECT OF VITAMIN E SUPPLEMENTATION ON BLOOD PROFILE AND POST THAW SEMEN CHARACTERISTICS IN CROSSBRED BULLS

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The present investigation was designed to study effect of Vitamin E on post-thaw semen characteristics of vaccinated crossbred KF (HF x Tharparkar) bulls along with blood profile of bulls. Twelve KF breeding bulls were selected from herd of Artificial breeding research complex of ICAR-NDRI, Karnal with uniform body weight and semen profile. Bulls were divided into two groups (Control and Vitamin E supplemented of 6 each). Vitamin E supplementation was started two and half months prior to vaccinated and continued for two months post-

vaccinated to treatment group only @ 4000 IU/bull/day. All bulls were vaccinated using FMD vaccine @ 3.0 ml by SC route. 20 freezings of semen were reported, ten from each group. Pre-freez and post-thaw motility was 74.63±0.01, 40.87±0.01 Vs. 74.92±0.01, 42.17±0.01percent in control and supplemented group respectively. Similarly pre-freez and post-thaw HOST was 69.16±0.02, 37.97±0.01 Vs. 70.32±0.01, 39.67±0.01 percent. Non eosinophilic count was 80.67±0.01, 47.88±0.01 Vs 80.71±0.01, 49.19±0.01perecent in control and treatment group respectively. Intact acrosome was 84.92±0.02, 53.90±0.01 Vs. 85.15±0.01, 55.39±0.01 percent respectively in control and treatment group respectively. Total sperm abnormality was more in control as compared to treated animals (7.87±0.01, 18.72±0.01 Vs. 7.93±0.01, 18.34±0.01) however difference not significant in above all parameters. Post vaccination glucose mg/dL (58.76±0.85 Vs. 57.88±1.22), BUN mg/dL (15.68±0.43 Vs.13.58±0.45), NEFA µM/ml (226.46±13.04 Vs. 213.5±11.91) was not significantly different between control and treatment. Similarly plasma phosphorus mg/dL (3.20±0.18 Vs 3.47±0.19), Calcium mg/dL (6.39±0.37 Vs. 6.50±0.40), Mn ppm (0.49±0.02 Vs. 0.0.53±0.02), Fe ppm (0.69±0.06 Vs. 0.80±0.12), Cu ppm (0.90±0.05 Vs. 0.95±0.05) and Zn ppm (1.37±0.13 Vs. 1.52±0.15) were not significantly different. Vitamin E had not shown its effect significantly in reducing vaccination stress but semen and blood profile improved slightly.

P-NP-03

EFFICACY OF VITAMIN C AND VITAMIN E PLUS SELENIUM AS ANTIOXIDANTS IN SUBCLINICAL MASTITIS IN GOATS

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To compare efficacy of antioxidants, Vitamin C and Vitamin E + Se with Gentamicin for the treatment of subclinical mastitis, three hundred goats from various scattered farms of North Gujarat aged between 2 - 5 years were selected and screened using various indirect tests viz., Modified California Mastitis Test (MCMT), Modified White Side Test (MWST) and Somatic Cell Counting (SCC). Thirty goats found positive were divided into three groups viz. unhealthy group (Gr B, n = 10) provided no treatment and treatment group (Gr C, n=20) which was further subdivided into subgroup 1 (T1, Vitamin E + Se) and subgroup 2 (T2, Vitamin C). Group of 10 healthy goats were kept as healthy control (Gr A). After treatment all the mastitis detection test *viz.,* Modified California Mastitis Test (MCMT), Modified White Side Test (MWST) and Somatic Cell Counting (SCC) were significantly decreased in both treatment subgroups. In the present study significant ($p<0.05$) drop was noticed in plasma levels on LPO and SOD in both treatment subgroup, with higher affectivity noticed in Vit. C treated group. From the study it was concluded that Vitamin C is better antioxidant than Vitamin E + Se for overcoming oxidative stress due to subclinical mastitis in goats.

P-NP-04

EFFECT OF FEEDING OF YEAST (*SACCHAROMYCES CEREVISIAE* CNCM I-1077) DURING HOT-HUMID SEASON IN SURTI BUFFALOES ON RUMEN LIQUOR PARAMETERS AND MILK PRODUCTION

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Animal production can be improved by dietary supplementation of yeast and yeast products depending on stage of lactation, diet characteristics and environmental conditions. They manipulate rumen fermentation by stabilizing pH, reducing acidosis, stimulating microbes and improving its anaerobic environment. The present study was conducted to know the effect of feeding of yeast *Saccharomyces cerevisiae* CNCM I-1077 on milk production and ruminal parameters in lactating Surti buffaloes during hot-humid season. The study was carried out during hot humid season on 18 Surti buffaloes divided equally in two groups i.e. control and treatment. Maximum and minimum THI during the period was 85.96 and 81.84 respectively. Diet of treatment group was supplemented daily with 1 g yeast ($10x10^9$ CFU)/animal for 90 days. Milk production was recorded daily. Rumen liquor was collected and analyzed for protozoal motility, pH, volatile fatty acid, ammonia production, protozoal and bacterial count on 0, 45 and 90 days of supplementation. Daily milk production per animal was higher in treatment group owing to stabilization of rumen pH by yeast competing with bacteria that produce lactate and destroy lactate. Rumen pH and protozoal motility were significantly ($P<0.01$) higher along with significantly ($P<0.05$) high levels of protozoal count and VFA production at 45 days in treatment group. There was lowered ammonia production in treatment group possibly due to incorporation of ammonia in microbial protein. Bacterial count was significantly ($P<0.05$) higher in control group at 90 days. Entidinomorphs were significantly ($P<0.05$) higher in treatment group at 90 days. The metabolites of *Saccharomyces cerevisiae* (soluble factors such as vitamin B complex, amino acids, organic acids fumarate, malate, aspartate) as well as cell membrane components (mannans and β-glucans) could stimulate growth of *Entodinium spp*. It may be concluded that feeding of yeast increases VFA production and pH, thus improving rumen health and milk production.

P-NP-05

EFFECT OF BIOCHANIN A PRETREATMENT ON HEMODYNAMIC FUNCTIONS AND HISTOPATHOLOGICAL CHANGES OF MYOCARDIAL INJURED RATS

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The current study was done to assess the effect of biochanin-A (BCA) in isoprenaline-induced myocardial injury in the rats. Different doses of biochanin A were provided to the myocardial injured rats for seven days before induction of injury. Isoprenaline was injected subcutaneously for two subsequent days to induce myocardial

injury in the rats. Assessment of myocardial injury was done by estimation of different hemodynamic functions such as systolic, diastolic, mean arterial blood pressure, heart rate and histopathological analysis of cardiac injured rats. Isoprenaline administration significantly induced the myocardial injury in rats by decreasing diastolic, mean arterial blood pressure and increasing heart rate. Rats were pre-treated with biochanin A but did not show any significant improvement in systolic, diastolic and mean arterial blood pressure in myocardial injured rats. However, biochanin A showed significant improvement in heart rate in cardiac injured rats. Further, pre-treatment with biochanin A showed significant improvement in histopathological changes in myocardial injured rats. The study suggests that biochanin A partially improved the hemodynamic functions and histopathological changes in the isoprenaline-induced myocardial injury in rats.

P-MISC-01

BODY CONDITION SCORE AS TOOL TO EVALUATE PRODUCTION PERFORMANCE AND BLOOD BIO-CHEMICAL PROFILE IN SURTI BUFFALOES

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Present investigation was conducted to study the influence of body condition score on performance and blood-biochemical profile in Surti buffalo. Twenty five Surti buffaloes were evaluated for body condition score, milk yield, reproductive and some blood-biochemical parameters after calving till 84th day of their lactation at fortnight interval. These animals were divided into two groups based on their BCS at calving-Group I: BCS=3.25 and Group II: BCS>3.25. Body condition score of group II animals were significantly higher than group I animals at all stages during the study period. Further, final loss in BCS was 0.35 and 0.22 for group II and I animals between at calving and on the day of estrus. There was significant effect of BCS at calving on milk yield during first 100 days of lactation. Meanwhile, peak day yield was higher and days to attain peak yield lower for group II animals than the group I animals. The postpartum interval to estrus was significantly lower in group II animals than the group I. Additionally, service period, estrus detector reading was lower and estrus intensity score was higher in group II animals. The mean serum glucose, total protein, albumin, globulin, total cholesterol concentrations were almost significantly higher for group II animals at all stages than group I animals. Other blood metabolites like blood urea, triglyceride, and NEFA showed mixed trend for group I and II animals. Further, we observed a significant negative correlation among BCS at calving and postpartum interval to estrus (0.60) and service period (0.42).

P-MISC-02

PHYSIO-BIOCHEMICAL PARAMETERS: A POTENTIAL TOOL FOR TARGET SELECTIVE TREATMENT OF HAEMONCHOSIS IN THE SMALL RUMINANTS

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This study aims to evaluate the eye lid colour based FAffa MAlan CHArt (FAMACHA) system coupled with body condition score (BCS), haemogram and stressor hormone level estimation, in identifying post-mortem (PM)/ coproscopically proven individuals wanting therapy for economically important gastrointestinal (GI) helminths, *Haemonchus contortus* in the small ruminants. Haemonchosis was significantly ($p<0.05$) higher (60.81%) in the ruminants with FAMACHA score (FS)=3. The animals with FS 2, 3 and 4 counted 23.2±0.37, 62±2.5 and 74±3.2 ($p<0.05$) of *H. contortus* [significant positive correlation; r= 0.841 (goats)/ 0.828 (sheep)]/ 77.5±0.65, 97.2±0.46 and 112±0.71 ($p<0.05$) of *P. epiclitum*/ 25±0, 53±1.8 and 31±0 ($p>0.05$) of other helminths with 2.8±0.15, 2±0.3 and 2±0.16 BCS (negative correlation, $p>0.05$), respectively. The *H. contortus* infected goats/ sheep with FS 2, 3 and 4 measured 8.2±0.0, 7.5±0.23 and 6.7±0.34 ($p=0.01$)/ 9.3±0.8, 8.6±0.5 and 7.6±0.3 ($p=0.05$) g/dl of Hb [r=-0.452 (goats)/ -0.511 (sheep), $p<0.05$] with 21.2, 19.8±1.8 and 17.8±0.2 ($p=0.05$)/ 26.7±1.2, 22.2±0.2 and 20.9±0.6% ($p=0.03$) PCV [r= -0.369 (goats)/ -0.251 (sheep), $p<0.05$], respectively. The FS 2, 3 and 4 infected goats/ sheep measured 6.1±0, 7.9±1.0 and 9.5±0.9 ($p<0.05$) / 5.8±2.3, 6.9±1.2 and 7.8±0.2% ($p<0.05$) mid-granulocyte [(r= 0.928 (goats)/ 0.834 (sheep), $p<0.05$] while measured cortisol level was 15.6, 23±4.5 and 42±2.3 ($p=0.23$)/ 12.1±0, 15.9±1.2 and 24±3.4 ($p=0.29$) µg/dl, respectively. The Hb/ PCV and mid-granulocyte/ cortisol level was significantly lower and higher in positive and negative animals, respectively. Specificity was maximized (100%) when FS=4 was considered anemic, but sensitivity was low (35.29% in goats; 25% in sheep). The false negatives was 5.9 (goat)/ 12.5 (sheep)% when FS=3 were considered anemic. By applying TST on the individual with FS=3, BCS= 2.5, Hb=7.5 (goats)/ 8.6 (sheep) g/dl, PCV=19.8 (goats)/ 22.2 (sheep)% and mid-granulocyte= 7.9 (goats)/ 6.9±1.2 (sheep)%, the phenomenon of anthelmintic resistance development can be minimized while maximizing the economic benefit to the farmers.

P-MISC-03

IMMUNODIAGNOSTIC POTENCY OF HOMOLOGOUS ANTIGENS FOR NATURAL *HAEMONCHUS CONTORTUS* INFECTION IN SMALL RUMINANTS IN PLATE AND PAPER ENZYME LINKED IMMUNOSORBENT ASSAY

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The objective of this study was to characterize purify *Haemonchus contortus* antigen, and to standardize and evaluate indirect plate and dot-ELISA using homologous antigens in the small ruminants. Electrophoretic separation of somatic antigen in reducing condition on 15% polyacrylamide gel resolved into 16 proteins of the molecular weight ranging from 14-100 kDa. Two step ethanolic extraction of the supernatant of *in-vitro* culture of *H. contortus* yielded excretory-secretory (E-S) antigen/ cathepsin L cysteine proteinase of molecular weight 28 kDa. The animals (Goats=103; Sheep=91) were broadly kept into post-mortem (PM) and faecal examined groups and further sub-grouped based on mono or multiply helminths infection. At many occasion the somatic antigen found to cross reacts with other helminths parasites thus minimizing the specificity of the selected tests and antigens. There was no any direct correlation between the parasites load and ELISA reactivity pattern. The prevalence rate of haemonchosis was 55.7 (34/61) in goats/ 47.6 (40/84) % in sheep as per PM examination while it was 45.63 (47/103) in goats/ 41.76% (38/91) in sheep and 36.89 (38/103) in goats/ 35.16% (32/91) in sheep using E-S antigen based plate and dot-ELISA, respectively. With E-S antigen, the overall % sensitivity, specificity, positive and negative predictive values of plate-ELISA was 89.74 (goats)/ 80.95 (sheep), 81.25 (goats)/ 91.84 (sheep), 74.47 (goats)/ 89.47 (sheep), 92.86 (goats)/ 84.91 (sheep), respectively while for dot-ELISA was 66.67 (goats)/ 61.9 (sheep), 81.25 (goats)/ 87.76 (sheep), 68.42 (goats)/ 81.25 (sheep), 80 (goats)/ 72.88 (sheep), respectively, so the tests and E-S antigen can be recommended for the detection haemonchosis in the small ruminants.

P-MISC-04

IMMUNODIAGNOSTIC POTENCY OF HOMOLOGOUS ANTIGENS FOR NATURAL *PARAMPHISTOMUM EPICLITUM* INFECTION IN SMALL RUMINANTS IN PLATE AND PAPER ENZYME LINKED IMMUNOSORBENT ASSAY

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The objective of the present work was to standardize and evaluate indirect plate and dot- enzyme linked immunosorbent assay (ELISA) using purified *Paramphistomum epiclitum* homologous antigens in the small ruminants. Electrophoretic separation of somatic antigen (PeSAg) in reducing condition on 15%

polyacrylamide gel resolved into 16 proteins of the molecular weight ranging from 14 -100 kDa. Two step ethanolic precipitation of supernatant of *in-vitro* culture of the fluke yielded *P. epiclitum* excretory-secretory antigen (PeESAg) of molecular weight 28 kDa. The animals (Goats=123; Sheep=91) were broadly kept into post-mortem and faecal examined groups. At many occasion the PeSAg found to cross reacts with other helminths parasites thus minimizing the specificity of the tests and antigens. There was no any direct correlation between the parasites load and ELISA reactivity pattern. The noted prevalence rate after combining the results of post-mortem examination and PeESAg based ELISA (plate and paper/ dot) was 30.08% (37/123) in goats and 28.57% (26/91) in sheep. While using PeESAg, the calculated overall sensitivity% was 92.86 (goats)/ 100 (sheep) in both plate and dot-ELISA, specificity% was 91.58 (goats)/ 91.55 (sheep) in plate ELISA while 88.42 (goats)/ 92.96 (sheep) in dot-ELISA, positive predictive value% was 76.47 (goats)/ 76.92 (sheep) in plate ELISA while 70.27 (goats)/ 80 (sheep) in dot-ELISA and negative predictive value% was 97.75 (goats)/ 100 (sheep) in plate ELISA while 97.67 (goats)/ 100 (sheep) in dot-ELISA, these values were optimum for the field sera sample so the tests and PeESAg can be recommended for the detection *P. epiclitum* infection in the small ruminants.

P-MISC-05

SENSITIVITY PATTERN OF MAJOR ANTIBIOTIC GROUPS AGAINST GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

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The present study was carried out to analyze the sensitivity pattern of different groups of antibiotics against Gram +ve cocci and Gram ve bacilli from year 2012 to 2014. Various clinical samples *viz.* pus swab, milk, faecal sample, nasal swab, sputum, vaginal swab, urine *etc.* submitted to the department were processed for bacterial isolation. Five groups of antibiotics *viz.* aminoglycosides, β-lactams, fluoroquinolones, sulphonamides and tetracyclines were employed for sensitivity testing against Gram +ve cocci and Gram ve bacilli using standard disc diffusion method. The overall sensitivity against was recorded against Gram +ve cocci as 30.51% (15.11 to 29.06), 29.95% (7.89 to 27.58), 67.15% (69.66 to 77.17), 24.35% (22.38 to 27.38) and 34.61% (34.28 to 34.4) and for Gram-ve bacilli 27.77% (13.58 to 42.10), 22% (17.39 to 22.58), 69.36% (62.85 to 76.41), 23.50% (28.39 to 45.45) and 19.63% (1.96 to 22.22) for aminoglycosides, β-lactams, fluoroquinolones, sulphonamides and tetracyclines, respectively. The most effective group was fluoroquinolone, which showed sensitivity against 67.15% for Gram + ve cocci and 69.36% for Gram ve bacilli. Among all antibiotic groups, predominant resistance showed by Gram + ve cocci against Sulphonamides (63.46%) and by Gram ve bacilli against β -lactums (60%).

P-MISC-06

SEROPREVALENCE OF *LEPTOSPIRA HARDJO* IN CATTLE OF SOUTH GUJARAT, INDIA

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The aim of this study was to determine prevalence of *Leptospira interrogans* serovar hardjo in the define area. To serve the purpose a total 398 serum samples were collected from different age group, breed and sex of cattle. Out of 398, some of them (101) cattle had shown history of abortion, mastitis/agalactia/oligolactia, repeat breeder and fever. These samples were screened by I-ELISA kit which detect antibody directed against *L. hardjo*. The distribution of serovar hardjo was significantly different between districts (Navsari, Tapi, Valsad, Surat) of South Gujarat. The highest seroprevalence of *L. hardjo* was found in Valsad district (21.56 %) followed by Surat (6.89 %), Tapi (3.30 %) and Navsari (3.04 %). However, there was statistically insignificant difference was observed in distribution of serovar hardjo between different breeds, age and sex of cattle.

P-MISC-07

ISOLATION OF *STAPHYLOCOCCUS AUREUS* FROM RAW CATTLE MILK AND THEIR DRUG RESISTANCE PATTERN

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Staphylococcus aureus is one of the important human pathogens involved in food related diseases and common community associated infections. This organism proliferates in food and causes food-borne illnesses. Milk serves as an ideal medium for growth of many microorganisms including *Staphylococcus aureus*. A total of 118 raw cattle milk samples were collected under aseptic precautions from different places of Navsari district of South Gujarat, processed under standard bacteriological techniques. The Baird Parker Agar was sued as selective medium for isolation. The presumptive isolates were identified on the basis of their morphological, cultural and biochemical characteristics. The sensitivity pattern of *S. aureus* with different antimicrobial agents was evaluated by disk diffusion method. Analysis of result revealed 12 isolates (10.16 %) of *S. aureus* from 118 milk samples. The *S. aureus* isolates showed cent percent sensitivity towards Amikacin and Gentamicin, followed in reducing levels by Gatifloxacin (91.66 %), Ciprofloxacin (91.66 %), 75 % each, of Streptomycin and Kanamycin; Ampicillin (66.66 %) and Cephalexin (41.66 %). The pattern clearly indicated overall high percent resistance to Cephalexin (58.33%), followed by Ampicillin (33.33 %), Methicillin (25.00%), Kanamycin (16.66 %) and Gatifloxacin (8.33 %), Ciprofloxacin (8.33 %). The isolates showed intermediate sensitivity towards Streptomycin (25.00 %) and Kanamycin (8.33 %). Findings of the study suggested judicious use of antimicrobial therapy in milking animals.

P-MISC-08

EFFECT OF CORPUS LUTEUM ON OVARIAN WEIGHT, FOLLICULAR COUNT AND OOCYTE RECOVERY RATE IN INDIAN BUFFALO

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Ovaries collected from slaughterhouse for *in vitro* maturation and fertilization studies involve oocyte recovery irrespective of stage of the estrous cycle. Present experiment was conducted to assess the effect of presence or absence of corpus luteum on ovarian weight, follicular count, oocyte retrieval and recovery rates from buffalo ovaries obtained from slaughterhouse. Significant (P<0.01) difference was found between the weight (g) of ovaries with corpus luteum (4.84 ± 0.14) than without corpus luteum (3.05 ± 0.07) on ovaries. The mean ovarian weight of pooled ovaries was 3.72 ± 0.09. Significant (P<0.01) difference was observed between the visible follicles on ovaries with corpus luteum (3.81 ± 0.11) than without corpus luteum (4.98 ± 0.19); and overall mean ovarian follicles per ovary were 4.55 ± 0.13. The oocyte retrieval rate was significantly (P<0.01) higher from ovaries without corpus luteum (57.66%) than with corpus luteum (48.25%). The oocyte recovery rate from ovaries without corpus luteum (2.86 ± 0.26) was also significantly (P<0.01) higher than ovaries with corpus luteum (1.87 ± 0.22). The mean oocyte recovery rate of pooled ovaries was 2.36 ± 0.19. Based on the findings, it was concluded that the presence of the corpus luteum on ovaries reflected to the higher ovarian weight as compared to the weight of ovaries without corpus luteum. However, the space occupied by the corpus luteum on ovaries resulted in to the lower follicular count, lower oocyte retrieval as well as lower oocyte recovery rate in buffalo.

P-MISC-09

OOCYTE COLLECTION METHOD: A CRUCIAL FACTOR INFLUENCING QUALITY OF OOCYTES IN BUFFALO

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The method used for oocyte collection is one of the factors affecting quality of *in vitro* collected oocytes. An appropriate oocyte collection method has to be developed to recover the highest number of good quality oocytes from a given ovary. Present investigation was designed to study the effect of oocyte collection

techniques *viz.,* slicing and aspiration on quality of oocytes collected in buffalo. The oocytes recovered from buffalo ovaries were graded based on their morphological criteria in three categories *viz.,* grade I/good oocytes; grade II/fair oocytes and grade III/denuded oocytes. In aspiration technique, highest percentage of grade I oocytes (50.00%) was recorded followed by grade II (31.58%) and grade III (18.42%) oocytes. While; in slicing technique, reverse trend was observed with highest percentage of grade III oocytes (40.89%) followed by grade II (33.55%) and grade I (25.56%) oocytes. The differences between the grades in both the techniques were statistically significant (P<0.01). The mean number of grade I oocytes per ovary in aspiration technique (0.93 $\pm$ 0.03) was non-significantly higher than slicing technique (0.81 $\pm$ 0.06). While, the mean number of grade II oocytes were significantly higher (P<0.01) in slicing technique (1.07 $\pm$ 0.06) than aspiration technique (0.55 $\pm$ 0.04) of oocyte collection. Similarly, the mean number of grade III oocytes were significantly higher (P<0.01) in slicing technique (1.32 $\pm$ 0.05) than aspiration technique (0.34 $\pm$ 0.05) of oocyte collection. It was concluded that in terms of quality of the oocytes, aspiration method of oocyte collection is superior over slicing method in buffalo.

P-MISC-10

COMPARATIVE STUDIES ON DIAPHORETIC POTENTIAL OF DIFFERENT BODY REGIONS IN SURTI BUFFALO

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Heat stress in buffaloes is of great concern. Apart from lactating buffaloes, heifers being potential lactating animal of future should be explored for impact of heat stress. Sweating is a thermoregulatory response which can be used as a criterion to assess thermoregulatory potential. The characteristic hot and humid climate of South Gujarat stimulates diaphoresis and its rate differs in different body regions depending on various factors. Thus to compare the diaphoretic potential between heifer and lactating adults of Surti buffalo, the present study was conducted on heifers and lactating adults (n=6) were exposed to environment having two different temperature humidity index range (THI) i.e. THI_1= 80-85 and THI_2=85-90. The diaphoretic rate of selected body regions was measured. The sweating rate (gm/m^2.hr) in neck dorsum, neck ventral, neck lateral, shoulder, brisket, fore flank, back thoracic and upper hind limb at THI_1 and THI_2 in heifers was (25.63±1.28, 32.48±1.86), (30.06±2.30, 36.82±2.75), (24.71±1.06, 29.86±1.01), (27.95±2.09, 28.87±2.10), (27.50±.69, 33.42±0.32), (24.42±.76, 29.90±0.37), (21.05±.96, 22.43±1.36), (24.40 ±.86,25.66±0.93)and in lactating adults was (27.85±1.30, 32.04±1.28), (31.95±2.36, 33.99±2.34), (26.72±1.16, 30.72±1.06), (31.33±1.89, 32.95±2.10), (27.59±0.68, 31.91±0.69), (26.26±0.77, 30.53±0.77), (26.12±0.87, 27.05±0.97), (28.69±1.01, 29.61±0.87) respectively. The mean sweating rate at THI_1 and THI_2 in heifers was (25.72±0.98, 29.93±1.59) and in lactating adults was (28.31±0.79, 31.10±0.76) respectively. Diaphoresis increased in all the regions at higher THI in both

the groups of animals. In heifers, increase was significant (P<0.05) in neck dorsum and highly significant (P<0.01) in neck lateral, brisket and fore flank. Similarly in lactating adults increase was significant (P<0.05) in Neck dorsum as well as Neck lateral and highly significant (P<0.01) in Brisket and Fore flank. Hence it was concluded that in Surti buffalo, body regions of neck dorsum, neck lateral, brisket and fore flank have higher diaphoretic potential and can be preferred for further diaphoretic studies and subsequent ameliorative strategies.

P-MISC-11

BLOOD PROFILE OF VITAMIN A AND β-CAROTENE IN POST-PARTUM SURTI GOATS

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Surti goat, a dual purpose breed, known for its good production and reproduction potential is acclimatized to hot-humid climate of its native tract Gujarat. Eventhough it is rearing is advantageous but possibilities of its extinction and declaring it as endangered breed has raised concerns over fewer studies on it especially during stressful periods of post-partum and lactation during which losses of vitamin A becomes critical. Vitamin A and β-carotene owing to antioxidant properties affects immune system and if lowered leads to incidences of mastitis and other disorders. Thus considering inadequate availability of information on them in goats, present study was undertaken to investigate changes in vitamin A and β-carotene in blood of post-partum Surti goats. The study was conducted on 40 Surti goats out of which 20 goats who had undergone recent parturition acted as treatment group and 20 non-pregnant animals comprised control group. Blood samples were collected from treatment group on 0, 7, 14, 21, 30 and 45 day post-kidding and once from control group and analyzed for Vitamin A and β-carotene. Vitamin A and β-carotene were significantly low on 0 day owing to increased mammary growth and colostrum production. A significant difference (P<0.05) in vitamin A as well as â-carotene (P<0.01) was observed between 0 to 21st day post-partum followed by non-significant difference in vitamin A after 21 days. However, β-carotene values at 21st day significantly differed from those at 30th and 45th day. Non-significant difference between control and treatment group from 7th day onward was observed in vitamin A. β-carotene values significantly differed between control and treatment group from 0 to 21st day postpartum and were at normal levels from 30th day onwards. It was concluded that on the day of kidding there is decrease in circulatory levels of vitamin A and β-carotene and this decrease can be used as an indicator of stress.

P-MISC-12

ADVANCES IN ANATOMICAL TECHNIQUES FOR MODERN LABORATORY PRACTICES

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Preparation of anatomical specimens with alternative methods imparts the basic conceptual information of veterinary anatomy. The traditional cadaveric exposure minimized and not necessarily to replace the object but model simulations eventually introduced to the students educate the principles of anatomy under practical demonstrations. It may partially achieved by many different technical advancement in teaching tools like plastinates, vascular corrosion casting, taxidermy and freeze drying in combination with appropriate imaging technologies. The view of three dimensional (3D) animations contributes a lot in the applied aspects of veterinary anatomy. The technical advancement has potential role in genetically manipulated animals and supporting drug discovery through a description of vascular defects in various diseases. It reduces student anxiety as well as trauma induced to animals during subsequent learning attempts in live animal demonstrations. The alternative teaching technologies improve the teaching/learning process rather than ethically sourced animals at large. The technology is within reach and uniquely suited to modern laboratory practices in veterinary sciences. It is one of the viable sources of teaching aids or technical tools in the laboratory. The new phase of teaching-learning situation replaced with conventional technologies.

P-MISC-13

BUFFALO CALF REARING WELFARE PRACTICES AT PERI URBAN BUFFALO FARMS OF SURAT CITY OF GUJARAT

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A field survey was conducted purposively in Peri urban areas of Surat city of Gujarat to ascertain the buffalo calf rearing welfare practices followed by buffalo owners during April, 2014 to December, 2015. Data were collected from randomly selected 50 buffalo owners through personal interview with the help of pre-tested structured schedule. The present study revealed that majority (98%) of the respondents attended calving and cleaned the calves soon after parturition. Majority (96%) of respondents did not practice ligation, cutting and disinfection of the naval cord and it was left to fall off itself naturally. Only 42% of the respondents fed colostrum to new born calf within one hour of birth. None of the respondents followed weaning practices and 42% of the respondents allowed calves to suckle their dams till lactation ceased. Majority (60%) of the respondents provided green fodders from two months of age and none of the respondents provided calf starter to the calves. None of respondents practiced castration of male calves and 34% of the respondents gave anthelmetics to the calves regularly. Only 14% of the respondents provided jacketing as well as bedding in

order to protect their calves from cold during winter season. It can be concluded that buffalo calf rearing welfare practices were quite satisfactory and need to improve the more adoption of welfare practices among the farmers of study areas by organizing awareness camps and demonstrations.

P-MISC-14

HUMANE ALTERNATIVE ANIMAL MODELS: ARE WE RESPONSIBLE ENOUGH TO EXTRAPOLATE RESEARCH TO PERSONALIZED VETERINARY MEDICINE?

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Rodents and non-human primates are used for different research and toxicological studies such as acute, sub-chronic, chronic toxicity, reproductive, developmental, genetic testing and eco-toxicology as well as analysis of xenobiotic residues. Each field raises specific ethical, welfare and scientific issues. Tests with laboratory animals are integral part of hazard identification and risk assessment of chemicals, pharmaceuticals, biocides, feed additives, effluents and plant protection products. These preclinical tests raise ethical and economic concerns and are considered as inappropriate for assessing all of the substances and effluents that require regulatory testing. Primary aim should be the replacement of animal experiments with humane alternatives. Besides the rules and procedures laid down by the CPCSEA, Govt. of India, the Indian National Science Academy (INSA), New Delhi has brought out 'guidelines for care and use of animals in scientific research'. These guidelines are widely read and followed by Indian researchers who use animals for experiments. Impediments in research and ethical issues has made researchers/scientists to rely on alternative animal models like genetically engineered cell line cultures, stem cells/regenerative medicine, isolated tissue/organ studies and computer aided data bases. Inspite of advanced technology, standardized alternative model is approved for only acute fish toxicity testing! Lack of successful validation of alternative approaches to replace animal tests, limited access to animal test data hinders the development of alternatives, thus mechanistic approaches should be developed and implemented in regulatory testing along with integrated testing strategies combine experimental and non-testing alternatives. The 4th R of Research implies addition of 'responsibility' to the original 3R's (replacement, reduction and refinement) of Russell and Burch. It has grown into a new era of performance-based outcomes, which reflects integrity, honesty, and scientific correctness in appropriate and reasonable use of laboratory animals. Thus being health care professionals it depends on us, how best we extrapolate laboratory research findings into the field of need based individual cases/personalized veterinary medicine.

P-MISC-15

MULTI-DRUG RESISTANCE PROFILE OF METHICILLIN- RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) ISOLATES FROM BOVINE MILK

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A total of 400 composite and pooled milk samples of bovines were screened for the Methicillin- resistant *Staphylococcus aureus* (MRSA) by two step enrichment method. The methicillin resistance in the 57 presumptive MRSA isolates were confirmed by the presence of *mecA* gene while the β-lactamase resistance was confirmed by the presence of *blaZ* gene. All the isolates (100%) were genotypically positive for both the resistance. The multi-drug resistance profile of the 57 MRSA isolates were studied against the harmonized panel of 12 different categories of anti-microbial agents by disk diffusion assay. The vancomycin resistance was studied additionally using the Ezy MIC method. All the 57 isolates were susceptible to chloramphenicol (phenicols), mupirocin, linezolid (oxazolidinones) and vancomycin (glycopeptides) while a total of 45 (78.95%), 36 (63.16%), 34 (59.65%) number of isolates were resistant to gentamicin, co-trimoxazole and ciprofloxacin respectively. A high level of resistance (15.79% or 9 isolates) was observed against pristinomycin. Similar to the MSSA isolates 9 (15.79%) isolates were resistant to erythromycin while nearly half of the isolates 28 (49.12%) had a resistance categorized as intermediate. The two isolates showed inducible macrolide-lincosamide-streptogramin ($iMLS_B$) resistance by being positive to D test. The presence of multidrug resistant *Staphylococcus aureus* in bovine milk pose a threat to both animal welfare and public health.

AUTHOR INDEX

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