ISVPT – 2019

XIX Annual Conference of

Indian Society of Veterinary Pharmacology and Toxicology

and

National Symposium on

"Pharmacogenomics in the development and validation of indigenous drugs"

December 18-20, 2019

COMPENDIUM OF INVITED PAPERS AND ABSTRACTS

Edited by:

Dr. Usha A. P.

Dr. Ally K.

Dr. Shynu M.

Dr. G. Radhika

Dr. Raji K.

Dr. Beena V.

Dr. Naicy Thomas

Dr. Gleeja V. L.

Dr. Hiron M. Harshan

Dr. Aswathy P.U.

Organised by

Department of Veterinary Pharmacology and Toxicology

College of Veterinary and Animal Sciences, Mannuthy

Kerala Veterinary and Animal Sciences University

Mannuthy, Kerala - 680651

Title: Compendium of invited papers and abstracts of **XIX Annual Conference of ISVPT and National Symposium on "Pharmacogenomics in the development and validation of indigenous drugs"**

Editors: Dr.Usha A. P., Dr.Ally K., Dr. Shynu M., Dr. G. Radhika, Dr. Raji K., Dr. Beena V., Dr. Naicy Thomas, Dr. Gleeja V. L., Dr. Hiron M. Harshan and Dr. Aswathy P.U.

Publication Year: 2019

Copies: 200

Published by:
Department of Veterinary Pharmacology and Toxicology
College of Veterinary and Animal Sciences, Mannuthy
Kerala Veterinary and Animal Sciences University
Mannuthy, Kerala - 680651

Printed by: Phoenix Printers, Thrissur

ARIF MOHAMMED KHAN
GOVERNOR OF KERALA

RAJ BHAVAN
KERALA

10 December 2019

MESSAGE

I am happy to know that the Indian Society of Veterinary Pharmacology and Toxicology along with Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University is organizing the 19th Annual Conference of **Indian Society of Veterinary Pharmacology and Toxicology** and National Symposium on **Pharmacogenomics in the Development and Validation of Indigenous Drugs [ISVPT-2019]** from 18th to 20th December, 2019.

It is commendable that a compendium of the Proceedings of the conference and the symposium will be published shortly.

I wish the Conference, the national Symposium and the Publication all success.

[Arif Mohammed Khan]

Tel. : 0471-2721100 Fax : 0471-2720266

കെ. രാജു
വനം-മൃഗസംരക്ഷണം-ക്ഷീരവികസനം
മൃഗശാല വകുപ്പുമന്ത്രി
കേരള സർക്കാർ

തിരുവനന്തപുരം
തീയതി 10.12.2019

MESSAGE

It is heartening to note that Indian Society of Veterinary Pharmacology and Toxicology along with Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Kerala Veterinary and Animal Sciences University have taken up "Pharmacogenomics in the development and validation of indigenous drugs" as the major theme for deliberation this year.

Pharmacogenomics is the study of how an individual's genetic inheritance affects the body's response to drugs. The biodiversity of indigenous/ traditional medicines of India is amazing. Most of the developed countries implemented pharmacogenomics testing in clinical treatment. Science and research progress through deliberations between various people like, academicians, scientists, researchers etc. Although the use of pharmacogenomics in veterinary field is less, there is a great potential in the application of this new branch in veterinary field, as the whole genome sequences of most of the domestic animals are currently available.

In this context, the theme of this year's symposium is very relevant. I earnestly wish that the society will come up with valuable recommendations for improvements in the new field of pharmacogenomics. I wish the event all success.

Office of the Minister for Forest, Animal Husbandry, Dairy Development and Zoos

Adv. K. Raju

ഓഫീസ് : അഞ്ചാം നില, സെക്രട്ടേറിയറ്റ് അനക്സ്-II, തിരുവനന്തപുരം-695 001
ഫോൺ-ഓഫീസ് : 0471-2335366 ഫാക്സ് : 0471-2332665 മൊബൈൽ : 9447875500, 9447864486
ഇ-മെയിൽ : kraju@kerala.gov.in, rajuminforest@gmail.com

KERALA VETERINARY & ANIMAL SCIENCES UNIVERSITY
Pookode, Lakkidi. P.O., Wayanad, Kerala - 673 576

Prof.(Dr.) M.R. SASEENDRANATH
M.V.Sc., Ph.D., F.N.A.V.Sc., F.I.S.V.M.
VICE - CHANCELLOR

Phone No : 04936 - 209209, 210
Mobile No : +91 9447236514
+91 9400129182

MESSAGE

It is learnt that the Indian Society of Veterinary Pharmacology and Toxicology (ISVPT) is organising a National Symposium on "Pharmacogenomics in the development and validation of indigenous drugs" in connection with the XIX Annual Conference of the Society.

The use of medicinal plants has been a part of people's life and traditions worldwide, especially in India. The motto of Pharmacogenomics is the Right Drug to the Right Person. The concept of pharmacogenomics in the development and validation of indigenous drugs is admirable in that its thrust is on contributing to earth's natural resources for the betterment of the society.

The need of the hour is to reinforce indigenous resources through scientific deliberations and interactions between various researchers. It is my sincere hope that the Symposium would provide an environment for such fruitful interactions.

I sincerely hope that the deliberations taking place during the symposium shall pave way for an alternate development model in pharmacogenomics. I appreciate the organisers for their dedication and wish the event a grand success.

29.11.19

(Prof. (Dr.) M.R. Saseendranath
Vice-Chancellor.

Vice Chancellor's Secretariat Pookode, Lakkidi. P.O, Wayanad, Kerala - 673 576, E Mail : vc@kvasu.ac.in

Dr. A. M.Thaker

President, Indian Society of Veterinary Pharmacology and Toxicology

Message

I am extremely delighted to learn that Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Kerala is organizing XIX Annual Conference of "Indian Society of Veterinary Pharmacology & Toxicology (ISVPT)" and National Symposium on "Pharmacogenomics in the development and validation of indigenous drugs" during 18-20December, 2019.Besides the conference and symposium, a pre-conference workshop on "*In silico* and cell culture techniques as an alternative to the animal use"is also being organized on 17 December, 2019. As a President of the society, it gives me immense pleasure to welcome all delegates at Mannuthy, Thrissur.

Since its inception in 2000, the society has been providing a common platform to researchers, academicians, students, pharmaceutical industry personnel and users to discuss and review the latest global and national advances in Veterinary Pharmacology & Toxicology and to identify the important research areas of national priority.The ISVPT has sizable number of life members, representing different subjects of Veterinary and Animal Sciences besides biomedical, other clinical and para-clinical sciences. The society has been organizing its annual meetings along with national conferences and has been promoting activities to fulfil its mandate. The society has so far organized 18 conferences and the proceedings of these conferences have been published and widely circulated. The society recognizes and honours high quality need-based research by recognizing the scientists and students with several awards.

India is a varietal emporium of medicinal plants and is one of the richest countries in the world as regards genetic resource of medicinal plants. It is need of the hour to explore medicinal plant through pharmacogenomic tools in the development of precise medicine for cure of animal ailments. The present symposium will help the participants in acquainting the area of pharmacogenomics in characterisation and validation of indigenous drugs.

I congratulate Dr. Usha P.T.A. and her team of Veterinary Pharmacology and Toxicology Department for untiring efforts to make the conference scientifically and socially fruitful.

I extend my warm greetings and best wishes for the grand success of the conference.

(A.M. Thaker)
President, ISVPT

KERALA VETERINARY AND ANIMAL SCIENCES UNIVERSITY
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY – 680 651, THRISSUR, KERALA, INDIA

Prof. (Dr.) C. LATHA, M.V.Sc., Ph.D.
Faculty Dean

Phone No : 0487 2370451, 2370344 (O)
Mobile : 9446445100, 9495047649
E. mail : deanvetmty@kvasu.ac.in

Message

It is a matter of immense pleasure to note that the Indian Society of Veterinary Pharmacology and Toxicology is organising a Symposium on "Pharmacogenomics in the development and validation of indigenous drugs" in connection with the XIX Annual Conference of the Society on 18-20 December, 2019 at Mannuthy, Thrissur.

The topic selected for the symposium is very relevant as India has a rich repertoire of traditional medicinal plants and a large population still depends on traditional medicines for treatment and cure. I wish that the symposium succeeds in putting forth concrete suggestions in scientific use of our valuable indigenous drugs.

Mannuthy

11.12.2019

11/12/19.

DEAN

COLLEGE OF VETERINARY & ANIMAL SCIENCES
MANNUTHY
THRISSUR
Date.....
Kerala Veterinary & Animal Sciences University

DEAN
College of Veterinary & Animal Sciences
Kerala Veterinary & Animal Sciences University
Mannuthy, Thrissur-680 651

FROM THE DESK OF ORGANISING SECRETARY

The Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy is honoured to conduct the XIX Annual Conference of Indian Society of Veterinary Pharmacology and Toxicology. It is my great privilege to welcome the distinguished delegates and members of the society to Mannuthy campus and conference.

Pharmacogenomics in Veterinary Medicine, though in the infancy stage, offers immense potential in the target oriented drug development. In the current era of ubiquitous personalised therapy amongst humans, the clients in the veterinary sector also anticipate an individualised strategy based on genetic configuration for the successful therapeutic implementation and recognition of the adverse drug reaction potential. Indigenous drugs are widely used in therapy of various animal diseases in developing countries while scientific validation is cumbersome. Moreover, the precise mechanism of action and pharmacokinetic parameters of these medicines still remain unexplored. This conference provides a national platform for delegates to get acquainted with the technique of pharmacogenomics and its use in the validation of indigenous drugs.

The organising committee has taken laudable effort in arranging the technical programme of the conference which includes invited lectures, oral and poster presentations. I express my sincere gratitude to Indian Council for Agricultural Research, National Bank for Agriculture and Rural Development, Zoetis Pharma and other sponsors for funding this conference. I also take this opportunity to thank all my colleagues, supporting staffs and students for the sincere efforts in making this event a memorable one.

I convey my heartfelt greetings to all the participants and wish them cherishable moments at this campus.

Dr Usha P. T. A.
Organizing Secretary

ABOUT THE UNIVERSITY

Kerala Veterinary and Animal Sciences University (KVASU) came into existence on 14th June, 2010 as per Ordinance No. 44/2010 and later Act 3/2011 of the Government of Kerala by the trifurcation of Kerala Agricultural University. All the Administrative, Academic and Financial matters were initiated under the new University from 01-04-2011. The University headquarters is located Pookode, Wayanad. The University encompasses three faculties- the Faculty of Veterinary and Animal Sciences and the Faculty of Dairy Science and Faculty of Poultry Science; and eight constituent colleges spread across the State.

In addition it has a network of instructional and research farms spread throughout the state which include- University Livestock Farm and Fodder Research and Development Scheme, Mannuthy, Instructional farms attached to College of Veterinary and Animal Sciences, Pookode, Livestock Research Station, Thiruvazhamkunnu, Palakkad, University Poultry and Duck Farm, Mannuthy, Centre for Pig Production and Research, Mannuthy, Goat and Sheep Farm, Mannuthy, Cattle Breeding Farm, Thumburmuzhy and Base Farm, Kolahalamedu.

KVASU hosts the All India Co-ordinated Research Project on Poultry, Centre for Advanced Studies in Poultry Science and Centre for Advanced Studies in Animal Breeding and Genetics at Mannuthy. Also the University offers its services through Teaching Veterinary Clinical Complex at College of Veterinary and Animal Sciences, Pookode, Peripheral Veterinary Clinic and Entrepreneurship Centre, Meenangadi, Veterinary Hospital at Kokkalai, Thrissur and Teaching Veterinary Clinical Complex at Mannuthy. Products from University Meat Plant and Dairy Plant at Mannuthy offers quality assured livestock products to public.

Other than graduate, post graduate and doctoral programmes in Veterinary and Animal sciences, the University offers diploma, post-graduate and doctoral programmes in a number of allied subjects. Also, University successfully runs half a dozen courses in the technology enabled mode. The multi-disciplinary Centres and Schools established under the University undertake research activities transcending boundaries. The University brings out a biannual journal - Journal of Veterinary and Animal Sciences which publishes research articles in various aspects of veterinary and Animal Sciences.

The University received acclaim from across the society for its active participation in rebuilding the state in general and livestock sector in particular after the devastating floods that lashed the State in 2018 and 2019. Kerala Veterinary and Animal Science University is the proud recipient of the Chancellor's Award for Best Young Emerging University instituted by the Governor, consecutively for two years in 2017 and 2018.

The keen interest and commitment of the Officers, dedicated service by the faculty and non-teaching staff, brilliant and hard working students, fuel the University in its march to world class status.

ORGANISING COMMITTEE OF ISVPT-2019

XIX Annual Conference of "Indian Society of Veterinary Pharmacology and Toxicology" and National Symposium on "Pharmacogenomics in the development and validation of indigenous drugs" December 18-20, 2019

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Stage and Venue

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Co-chairman	: Dr.V. Ramnath, Professor & Head, Dept. of Veterinary Physiology
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Members	: Dr. Justin Davis, Assistant Professor, Dept. of LPM
	: Dr. Abhilash R S, Assistant Professor
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Convener	: Dr. Shynu M, Assistant Professor
Members	: Dr. G. Radhika, Assistant Professor

: Dr. Raji K., Assistant Professor

: Dr. Beena V., Assistant Professor

: Dr. Naicy Thomas, Assistant Professor

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: Dr. Hiron M. Harshan, Assistant Professor

: Dr. Aswathy, P.U., Ph.D Scholar

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Members	: Dr. C. Sethulekshmi, Assistant Professor
	: Dr. Bincy Mathew, Assistant Professor
	: Dr. Jamuna Valsalan, Assistant Professor

PROGRAMME

DAY 01 (18/12/2019)	
7.30-9.30 a.m.	Breakfast and Registration
9.30-10.45 a.m. Seminar Hall	Inauguration
10.45-11.00 a.m.	High Tea
11.00a.m. Seminar Hall	**Chellapa Memorial Oration** Chairperson : Dr. J.K.Malik Co-Chairperson : Dr. Pawan Kumar Verma Rapporteur :Dr. Ratn Deep Singh
12.00noon Seminar Hall	**Dr. M. Sabir Oration** Chairperson :Dr. C. Varshneya Co-Chairperson : Dr. P. Sankar Rapporteur :Dr. Patel Harshad Kumar
1.00-2.00p.m.	Lunch
2.00 p.m. Silver Jubilee Hall	**Technical Session I** **Pharmacogenetics/ Pharmacogenomics** Chairperson : Dr. K. Adilaxmamma Co-Chairperson : Dr. A. Manimaran Rapporteur : Dr. Harshad B Patel
2.00 p.m. Seminar Hall	**Technical Session II** **Ethnopharmacology** Chairperson : Dr. Suresh Kumar Sharma Co-Chairperson: Dr. Tapas Kumar Sar Rapporteur : Dr. Archana Lohiya
3.15- 3.30 p.m.	Tea
3.30 p.m. Silver Jubilee Hall	**Technical Session III** **Pharmacokinetics/ Toxicokinetics** Chairperson : Dr. Satya Pal Singh Co-Chairperson : Dr. Atul Prakash Rapporteur : Dr. Preeti Bagri
6.30 p.m.	**Cultural evening,** Auditorium, CVAS, Mannuthy
8.00 p.m.	Dinner

DAY 02 (19/12/2019)	
7.30-9.00a.m.	Breakfast
9.00 a.m. Seminar Hall	**Technical Session IV** **Antimicrobials and Antimicrobial Resistance** Chairperson : Dr. A.M.Chandrasekharan Nair Co-Chairperson : Dr. M.R. Srinivasan Rapporteur : Dr. R Rashmi
9.30 a.m.	**Poster Session** Teaching Veterinary Clinical Complex (Ground Floor)
9.00 a.m. Silver Jubilee Hall	**Technical Session V** **Industry Academia Interactions** Chairperson : Dr. Sanis Juliet Co-Chairperson : Dr. M.J. Raja Rapporteur : Dr. Ramesh Kumar Nirala
10.30 a.m. Seminar hall Dept.of Pharmacology	**Technical Session VI** **Animal Welfare and Alternate Animal Use** Chairperson : Dr. P.Sriram Co-Chairperson : Dr. Rajdeep Kaur Rapporteur : Dr. Jayanthi. M.
11.15- 11.30 a.m.	Tea
10.00 a.m. Silver Jubilee Hall	**Technical Session VII** **Clinical and Regulatory Pharmacology/Toxicology** Chairperson : Dr. Urvesh Kumar D. Patel Co-Chairperson: Dr. P.Mekala Rapporteur : Dr. P. Vikrama Chakravarthy
1.00-2.00 p.m.	Lunch
2.00 p.m. Seminar Hall	**Dr. V.V. Ranade Young Scientist Award** Chairperson : Dr. Prakash Nadoor Co-Chairperson : Dr. Pallavi Bhardwaj Rapporteur : Dr. Preethy John
3.00 p.m. Seminar Hall	**Dr. A.M. Thaker Young Scientist Award for Women** Chairperson : Dr. K.P. Mini Co-Chairperson : Dr. Sujith.S. Rapporteur : Dr. P. Senthil Kumar
3.45- 4.00 p.m.	Tea
4.00p.m. Seminar Hall	**Dr. R. Natarajan Award** Chairperson : Dr. S. Ramesh Co-Chairperson : Dr. Nisha A. R. Rapporteur : Dr. Priyanka Rajput
5.00p.m. Seminar Hall	**Dr. Jayvir Anjaria Award** Chairperson : Dr. A.H. Ahmad Co-Chairperson : Dr. S.P. Preetha Rapporteur : Dr.V. Ramakrishnan

7.30p.m. Seminar Hall	Annual General Body Meeting, ISVPT
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CHELLAPA MEMORIAL ORATION

Chairperson : Dr. J.K.Malik

Co-Chairperson : Dr. Pawan Kumar Verma

Rapporteur :Dr. Ratn Deep Singh

College of Veterinary and Animal Sciences, Mannuthy

Pharmacogenomics in the development and validation of indigenous drugs
XIX ISVPT
CVAS MANNUTHY

CHELLAPA MEMORIAL ORATION

EMERGING ZOONOSIS: REMINDERS OF "ONE HEALTH"

Dr. Jayakrishnan Thayyil

Professor, Department of Community Medicine, Government Medical College, Kozhikode, Kerala

An emerging zoonosis is defined as "a zoonosis that is newly recognized or newly evolved, or that has occurred previously but shows an increase in incidence or expansion in geographical, host or vector range". They have potentially serious human health and economic impacts and their current upwards trends are likely to continue. During the last 3 decades over 30 new infectious agents have been detected and 60 per cent of these are of zoonotic origin and they account for 26 per cent of annual deaths. Since the beginning of 21st century emerging zoonosis like SARS, Avian Influenza, H1N1, MERS-Cov, Nipah attacked human kind in earth.

Many factors lead to the emergence of zoonotic diseases. Environmental changes, human and animal demography, pathogen changes and changes in farming practice are a few of them. Social and cultural factors such as food habits and religious beliefs play a role too. For their control we not only need to know the biology of Virus but history, economics, and politics, social and cultural factors too.In the current globalized world travel, trade and disease become international, all are swimming in the same sea of microbes so any out brake in a remote area- Yesterday can become national public health problem -to day and become international problem -Tomorrow. Emerging infections will continue to challenge public health infrastructure, test credibility of health services, and threaten to devastate health and economic development. So a strategic vision, an effective plan of action such as rational use of drugs and pesticides; Climatic change and environmental impact assessment are the cornerstones of disease prevention and control. Since highly fatal Nipah virus out break had been recently reported in south India for the first time in history; as an emerging zoonotic disease for priority action this paper will look the details of Nipah in detail.

Nipah virus (NiV) is a paramyxovirus (genus Henipavirus) was first identified in 1999 in Malaysia, where it caused an outbreak of respiratory and neurological disease in pigs and encephalitis in humans. Three years later, a genetically distinct NiV independently emerged in India as well as in Bangladesh, where human NiV outbreak events have been reported nearly every year since. A putative NiV also caused an outbreak of disease in horses and people in the Philippines in 2014. To date, there is no reported evidence of NiV outbreaks in humans emerging in any other country than Malaysia, Singapore, Bangladesh, India and Philippines. NiV was first isolated by Chua et al. in 1999 in Malaysia. Currently available sequences

obtained from Malaysia and Cambodia were designated genotype M, while sequences obtained from Bangladesh and India were designated genotype B.

Transmission: Introduction of NiV infection into the human population occurs by two mechanisms of spill over from bats which are the natural symptomless carriers: transmission via an intermediate animal host, which precipitated the outbreak in Malaysia; and bat-to-human transmission, which has occurred in Bangladesh and India, followed by human to human transmission. Infected bats shed the virus in their excretion and secretions such as saliva, urine, semen and excreta. In Bangladesh, a most common risk factor for human NiV infection is drinking contaminated date palm sap or its fermented product. Date palm sap is harvested from December through to March by cutting into the tree trunk and allowing the sap to flow overnight into an open clay pot .The breeding season of bats were from December to May. Corresponds to this all out breaks were reported between December and May.

The other common risk factor for human infection is contact with a patient with Nipah encephalitis. The presence of NiV in respiratory secretions and urine of patients was demonstrated and this posed a danger for nosocomial transmission. Human-to-human transmission is particularly notable in the outbreaks in India and Bangladesh (NiV B) .Study of human NiV infections between 2001 and 2007 in Bangladesh attributed 51% of all cases of transmission from an infected person, although only 7% of people were identified as having transmitted their infection. So far, the basic reproductive number (R0) of the strains of NiV that have spilled over in Bangladesh has remained very low, averaging 0.48 (Luby 2013).

Nipah is one of the Biosafety level 4 agents by WHO. Sera from infected patients contain measurable NiV-specific IgM antibodies as early as four days after exposure, persisting for at least 3 months. Specific IgG can also be detected by day 25 following infection and were shown to persist for several years. The diagnosis of Nipah virus infection can be established by enzyme-linked immunoassay (ELISA). Nipah IgM capture ELISA and an indirect IgG ELISA has high specificity for the diagnosis. Specimens include CSF and serum. RT PCRs can be used for detection of viral sequences in CSF, throat swab or urine specimens.

In the majority of cases, the incubation period of Nipah has been reported to be 5 days to 2 weeks. The majority of patients initially develop influenza-like signs and symptoms, including fever, headache and myalgia and vomiting. In general, the more severe clinical features manifest as either an acute encephalitic syndrome or less frequently a pulmonary syndrome.

Nipah Out Break In Kerala: During May 2 – 29, 2018, 23 human cases of nipah were identified including the primary case; 18 were lab-confirmed and 4 probable cases. On May 17, 2018, a 28 year old male presented to a private facility in Kozhikode district, Kerala State, India with encephalitis (Index case).

His father and aunt developed fever, body ache, and vomiting on the same day. His brother had died following a similar illness 12 days earlier (Primary Case). The family cluster of encephalitis cases among adults prompted the laboratory to test for NiV in addition to common causes of encephalitis.

Sequence analysis of the current outbreak NiV revealed 97% similarity to the NiV-B lineage. Median age of cases was 45; 15 (65%) were male. The median incubation period was 9.5 days (6 – 14 days). Of the 23 cases, 20 (87%) had respiratory symptoms. The case fatality rate (CFR) was 91%; two cases survived. Risk factors for infection included close proximity (touching, feeding or nursing a NiV infected person) enabling exposure to droplet infection. The public health response included isolation of cases, contact tracing, and enforcing hospital infection control practices.Empirically Ribavirin was used in 10 patients with initial dose of 2 gram followed by 1 gram 6^{th} hourly for 4 days and 500 mg 6th hourly for 6 days out of which 2 survived.

Environmental samples: The primary case was supposed to acquire infection from the *Pteropus* bats was confirmed and established by NIV/ICMR, with 19.2% (10/52) of the bats collected from the area testing positive for NiV RNA. Of the 60 environmental samples, including partially eaten mangoes, guava and areca nuts with bite marks of bats, collected from the surroundings of the residence and potential work places of the index case, none had evidence of NiV RNA by real-time RT PCR. His pet rabbits and ducks tested negative for NiV.

Transmission of NiV occurred in three hospital settings: Taluk Headquarters hospital, Perambra, Kozhikode (H-1); Government Medical College, Kozhikode (H-2); and the Community Health Centre, Balussery (H-3). Of the 22 additional NVD cases identified, nine secondary cases contracted the infection from the primary case while he was at H-1. An additional 10 secondary cases were infected while the primary case was in H-2.

The public health response by Kerala Health Services was launched on 18 May with the isolation of cases, contact tracing, enforcing hospital infection control practices and risk communication. The national team of experts deputed by the Ministry of Health and Family Welfare, Government of India guided the response in close collaboration with the Kerala state health services. A total of 2642 contacts were identified and kept under surveillance. The antiviral Ribavirin was imported by the Department of Health & Family Welfare, Government of Kerala. Fifty doses of an experimental monoclonal antibody (M102.4) were provided by the Queensland Department of Health, Australia on the request of the Indian Council of Medical Research, New Delhi for compassionate use and stored at Government Medical College, Kozhikode. Since

30 May, no new cases were reported. Following this out break an International consultation on research to combat Nipah virus was conducted in August6-8 at New Delhi jointly by WHO and ICMR.

First time without requiring sample transport outside of India, Nipah was confirmed with a turn around time of only 12 hours, then paved the way for state and central governments to respond to Nipah more quickly than in previous outbreaks in India, likely limiting the severity of the outbreak and potentially reducing costs in terms of loss of life and commerce (given the role of international fruit and vegetable trade in Kerala farmers' livelihoods). It also may have helped to limit Napa's spread beyond the geographic area initially affected.

One health

Because of their wide distribution and flying range that can cover huge areas of human habitat, pteropid bats are highly effective in NiV dissemination. Moreover, the changing climate can be expected to affect the distribution of this virus reservoir in the future. It has been speculated that migratory fruit bats were forced away from their natural habitats in 1998 because of forest fires prevalent at that time in the region and attracted by the fruit trees in pig farms .As the flying fox habitat is destroyed by human activity the bats become stressed, their immune system weakens, their viral load increases and more virus is shed in the urine and saliva. Similar fluctuations of virus shedding may be associated with stressful physiological conditions or seasons. Increased shedding of virus by the bats was also associated the breeding season of the bats. Habitat destruction also physically brings bats into closer contact with humans. Since Nipah virus encephalitis is a major zoonosis and outbreaks may be associated with multiple factors such as animal reservoirs, socio-cultural practices, food habits and possible human-to-human transmission, a multidisciplinary team is needed and preparation should be done for pre-outbreak, outbreak and post-outbreak phases. One Health approach should be followed within every sector involved with Nipah prevention, control and management.

Therapeutic interventions: Gap Analysis

NiV is identified as one of the 11 diseases in the Blueprint's list of "priority pathogens" by WHO that are likely to cause severe outbreaks in the near future calls for the development of R&D roadmaps for diagnostic assays, novel therapeutics, and effective vaccines. No specific drug has been yet approved for the treatment of this important disease. Limited work has been done to develop therapeutics against NiV infection.

I. Antiviral drugs

1. Ribavirin is a guanosine analogue and broad spectrum nucleoside antimetabolite antiviral drug which features on the WHO Essential medicines list. An inhalation solution of ribavirin is also indicated for the treatment of paramyxovirus. During the NiV outbreak in Malaysia in 1998/99, ribavirin was given empirically to treat 140 patients. The trial was not randomized. Patients who were managed prior to the availability of ribavirin or refused ribavirin were taken as controls. There were 32%deaths in the treated group and 54% in the controls. On Cox regression analysis, younger age and use of ribavirin were independently associated with better survival ($p = 0.011$ and 0.013, respectively). In subsequent animal studies, ribavirin was found to only delay NiV disease and death.

2. Ribavirin was also tested in combination with the antimalarial drug chloroquine. Chloroquine was indeed shown early on to block the critical proteolytic processing needed for the maturation and function of the HeV F glycoprotein), and later shown to inhibit NiV infection in cell culture. However, in vivo trials Chloroquine did not protect hamsters when administered either individually or in combination with ribavirin.

3. A 36 amino acid **HR2-based fusion inhibitor** (NiV-Fc2), analogous to the approved HIV-specific therapeutic peptide enfuvirtide, has been proposed as a specific therapy against henipaviruses

4. In 2017 - 4'-Azidocytidine (R1479), an antiviral developed for hepatitis C therapy, against a range of paramyxoviruses and reported similar levels of in vitro activity against henipaviruses.

5. In Syrian hamster model administration of favipiravir twice daily orally or once daily subcutaneous for 14 days, protected the animals challenged with lethal dose of Nipah virus.

6. Other candidate drugs evaluated in vitro for their activity against henipavirus infections include cationic compounds and calcium influx inhibitors.

Biologicals: In case of severe disease, when no treatment with a proven record of safety and efficacy is available, they may appear as the only available therapeutic option. However, convalescent plasma has not been investigated clinically during outbreaks of NiV infections.

Monoclonal antibodies

Monoclonal antibodies targeting the surface glycoproteins of HeV have shown efficacy against both HeV and NiV as pre- and post-exposure prophylaxis in animal models. The m102.4 mAb has exceptionally potent neutralizing and cross-neutralizing activity against both NiV and HeV viruses and its epitope maps to the ephrin receptor binding site. Testing of m102.4 has confirmed its neutralization activity against several isolates including NiV-M and NiV-B. Effective post-exposure efficacy with m102.4 has been demonstrated in both ferrets and NHPs (AGMs) infected with either HeV or NiV. Over the past years, eleven human

subjects have been reported to receive high-dose m102.4 therapy on an emergency use basis because of high-risk exposure to HeV or NiV, and all have remained well (Broder 2013). A phase 1 clinical trial in human has been completed; include safety/tolerability, pharmacokinetics and immunogenicity parameters. There is evidence of effectiveness in non-human primates (AGM) with low therapeutic window in NiV B strains. Following high-risk NiV exposure or cases of infection, there has been an interest in compassionate use of the m102.4 antibody.

Immunomodulators: A derivative synthetic polyinosinic: polycytidylic acid (poly-IC12U or Rintatolimod), an analogue of double-stranded RNA which strongly activates IFN production, has been shown effective in limiting disease and increasing survival of NiV-infected hamsters. When administered at 3 mg/kg of body weight daily from the day of infection to 10 days post-infection, prevented mortality in 5 of 6 infected animals.

Without any other currently available therapeutic options, ribavirin is still considered today as an option for treatment of NiV infections in emergency settings, but its impact on disease progression is highly questionable. Overall, the development of therapies against NiV infections remains at a very early stage. Research efforts in this area should be encouraged, especially when considering the high case fatality rate associated to NiV infections and the risk of a large outbreak occurring in the future

II.Vaccines

All R&D activities for NiV vaccines are in the pre-clinical stage. Many vaccine candidates including different live recombinant vaccine and subunit vaccines have been produced and tested in various animal models including hamsters, pigs, cats, ferrets and African green monkeys (AGMs). These vaccines are based on the F and/or G glycoproteins, and essentially target the induction of NiV neutralizing antibodies, as done with other human paramyxovirus vaccines such as mumps or measles vaccines.

Vesicular stomatistis virus based vaccine (VSV) showed protection in animal models when the platform was used in Ebola outbreak with single dose one day prior to challenge. Vaccines consisting Measles virus strain as a vector has also shown protection in animal models.Venezuelan Equine Encephalitis Virus (VEE) replicon which has got positive phase 1 trial when tried in animal models has shown no protection in Nipah. A subunit vaccine tried in animal models also gives promising results. Extensive research involving preclinical studies in a number of animals and nonhuman

Primates have identified multiple vaccine candidates, including vectored and subunit vaccines, offering protective immunity. The pharmaceutical companies are hesitant to invest in research on development of vaccines for diseases like Nipah, which are rare occurrences, despite the high fatality.

Conclusion:

Finally, an essential strategy for controlling Nipah should focus on preventing virus transmission from bats to humans. Controlling the virus in its wild reservoir does not seem a feasible approach. However, establishing or reinforcing surveillance systems is of utmost importance to ensure that NiV outbreaks can be detected quickly and appropriate control measures promptly initiated. At present no vaccines or antiviral drugs are available for NiV disease and the treatment is just supportive.

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DR. M. SABIR ORATION

AYURVEDA- A HAND IN HAND APPROACH IN CONCEPTS OF VETERINARY SCIENCE

Dr.Gopikrishna S

Assistant Professor, Department of Agadatantra, V.P.S.V. Ayurveda College, Kottakkal.

Indian sciences have always been one of the most explored and sought after among the scientific communities all over the world. May it be the fields of mathematics, architecture, arts or medical science, our contribution has always stood apart. They have been invaluable and led to many path breaking inventions of today. Among them, the imprints we have put in the medical science is unarguably significant. Ayurveda is one among those medical contributions of India to the world. It believes in "***Swasthasya swaasthya rakshanam aaturasya vikaaraprasamanam cha***" which means the basic need of a medical science is prevention of health and cure from disease. The principle has a wider perspective when we see the evolution of *Ayurveda*. Being a science of five thousand years of heritage, the life and its sustenance, as well as health care were entirely different in those days than we see today. It was from there, *Ayurveda* has evolved and developed. During those days when there was no knowledge about the modern anatomy, physiology and pathology, we had described about all those illnesses and their remedies with precision. The proof is that we still follow the same principles even today with good success rate. Many of the principles were on the basis of observations from the nature.

During the pre-historic era itself man had been friends with animals. He had a concomitant relation with the animals either for food or to live with or even to live in fear of being attacked. During the *Vedic* era sages used to rear animals as pets and also worshipped a few like cows and snakes. When this relation with the animals developed, their care also became inevitable. This led to the beginning of a separate medical science. This ***Pashu Ayurveda*** had enjoyed equal status as any field prevalent during that period.

For any medical science to develop, it needs the help and knowledge from the nature. A reference in *CharakaSamhita*, a book of medicine in *Ayurveda* is as follows: "The knowledge of plants and their medicinal properties can be well understood from the shepherds, cowherds and from those people who dwell near the forests". It is a valuable bridge between *Ayurveda* and Veterinary Science. If we go through the above reference, we can understand that the 'informers' have knowledge about both plants and animals. They use this knowledge to treat their animals when needed.

Ayurveda has its root in *Vedas* and *Puranas*. Being an arm of *Atharva Veda* which deals with the greatest number of plants and also their medicinal properties. We come across the reference of techniques of self-treatment by wild boar and mongoose during their respective illness. It also has dealt with animals and

their illnesses with their remedial measures. In *Rigveda* we get a reference "May we get freed from poverty by the cows".

Probably from there a few branches of *Ayurveda* had later developed in the names ***Hasti or Gaja Ayurveda* (Elephants), *Aswa or Haya Ayurveda* (Horses), *Gava Ayurveda* (Cows).** India can hold the head high that it has been the abode for greats like ***Saalihotra*** referred as ***The Father of Veterinary Science*** and ***Paalakapya*** (author of *Gaja Ayurveda*) who were great Veterinary physicians. "***Saalihotra Samhita***" is available in full form in eight divisions and twelve thousand verses. Further in another text book of *Ayurveda*, *Sushrutha Samhita* we get the symptoms of snake bite in animals and birds. The treatment done is similar to that of humans.

If we go through the *Puranas*, *Matsyapurana* explains treatment of aquatic animals. *Agni purana* and *Garuda purana* have a lot of references which mention about the diseases of the cows, buffalos and horses and also their treatments. There were separate chapter under the name ***Gajaswayurveda***. A few of them to name *Krimijaala* (germs), *Vrina* (ulcers), *Vaarisphota* (eruptive skin disorders), *Kushta* (skin diseases), *Upasarga* (epidemics) etc. Horses and elephants had a great value during those days, may be due to their use in wars. Cows were regarded as holy and had to be specially taken care of. Above all these, ethical concerns also can be seen. There used to be specially appointed physicians, care takers and also a chief to care the animals. Their diet was also very specific and seasonal. We also get the features of how an ideal healthy horse and elephant should be like. The drugs used were also very common. *Triphala* (*Terminalia chebula*, *Terminalia bellerica* and *Emblica officinalis*), *Guggulu* (*Commifora wightii*), *Laksha* (Laccifer lacca), *Ardraka*(*Zingiber officinale*) are a few of them. All these are used in humans for various ailments.

Mahabharata is another classic example for Veterinary Science. The books "***Aswa Sastra***" and "***Nakula Samhita***", written by the *Pandava* princes *Nakula* and *Sahadeva* are in records. Also, in *Mahabharata* during the exile period, *Nakula* had to take care of horses and *Sahadeva*had to take care of cows. *Sahadeva* is believed to be the author of "***Gavayurveda***".

If we go through the history, in later period Ashoka had given due importance to rear, treat, protect and worship the animals. He had even constructed separate residing places for different animals. *Kautilya/ Chanakya* in ***Arthashastra*** has described treatment for animals and birds. *Saalihotra Samhita* was translated to Arabian and Tibetan languages. The *Hasti Ayurveda* was published in the year 1849 and *Gaja Ayurveda* in the year 1958. Another book by the name "***Yuktikalpataru"*** written by *Bhoja Raja* is also available. *Aswa Sastra* is also available published during 1952.

By going through the details of diseases and treatments of *Pasu Ayurveda* in common in various *Puranas* we can come to a conclusion that treatment principles in *Ayurveda* can be adopted in animals also. Further, the dose is to be modified. The dose in animals may be more than for the humans. These data are well enough to conclude that there was a super speciality care available for the animals. It is now the responsibility of *Ayurveda* community to work hand in hand with Veterinary department to open a new area through treatment and research works to prove the records and set the records.

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DR. V.V. RANADE YOUNG SCIENTIST AWARD

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VVR-01

APOPTOTIC POTENTIAL OF GERMINATED SEEDS OF *Hordeum vulgare* AGAINST TRIPLE NEGATIVE BREAST CANCER

Akhil G.H., Bibu J.K., Reni J., Sujith S., Nair S.N., Jisha S., Dhanusha S., and Aathira K.K.

Department of Veterinary Pharmacology and Toxicology,

College of Veterinary and Animal Sciences, Mannuthy, Thrissur – 680 651

akhilgh@gmail.com

Triple negative breast cancer (TNBC) is a subtype of breast cancer constituting 15-20% of all the breast cancer cases diagnosed. It lacks the expression of oestrogen, progesterone and HER2 receptors. This subtype of breast cancer is characterised by aggressive clinical outcome, high risk of metastasis, highfatality and poor prognosis. The present study was undertaken to evaluate the anticancer activity of methanol extract of germinated seeds of *Hordeum vulgare*(barley) in MDA-MB-231 triple negative breast cancer cell line. Qualitative phytochemical analysis of the extract detected the presence of glycosides, steroids, saponins, diterpenes and triterpenes.The RP-HPLC analysis showed the presence of hordenine (4.82%) in the extract. Antioxidant activity of the extract by DPPH and superoxide anion radical scavenging assay revealed a significant concentration-dependent activity with IC_{50} values of 112.75±5.74 and 28.90±1.85µg/mLrespectively.

Cytotoxicity of the extract by MTT assay revealed significant concentration dependent cytotoxicity with IC_{50} value of41.28µg/mL. The same concentration was used for further studies. Morphological assessment of MDA-MB-231 cells treated with hordenine showed similar morphological cytotoxic changes as that of extract such as reduction in cell population, cell shrinkage, vacuole formation and apoptotic bodies formation. Acridine orange/ethidium bromide staining exhibited yellow green fluorescence indicative of early apoptosis. Nuclear morphological changes assessed by Hoechst 33258 staining showed nuclear marginalisation and fragmentation. The results of JC-1 staining showed a partial reduction in mitochondrial membrane potential. The results of comet assay were found to be negative indicative of no DNA damage. Western blotting analysis of the extract on MDA-MB-231 cells showed significant upregulation of caspase-8 by 2.52±0.30 fold whereas no change was found in Bcl-2 expression indicative of extrinsic pathway of apoptosis. Thus, the methanol extract of germinated seeds of *H. vulgare* proved to possess potent anticancer activity against MDA-MB-231 cells.

VVR-02

CANDESARTAN AMELIORATES ARSENIC-INDUCED CARDIAC TOXICITY BY REGULARIZING ANGIOTENSIN II MEDIATED SIGNALING IN RATS

Kesavan, M., Parida, S., Karikalan, M, Pavithra, S., Madhu, C.L., Panigrahi, M., Alam, J., Telang, A. G., Kumar, D.

Division of Pharmacology and Toxicology, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, UP

Arsenic is a groundwater pollutant and can cause various cardiovascular disorders in the exposed population. While candesartan is an antihypertensive drug and an antagonist of angiotensin receptor type-I (AT_1R), prescribed globally for the cardiac patients. The aim of the present study was to assess the arsenic-mediated cardiac toxicity and its modulation with candesartan. Rats were exposed to arsenic at 50 ppm as sodium arsenite through drinking water for 90 days and treated with candesartan (1 mg/kg) by oral gavage during the last 30 days of exposure. Next day, the rats were sacrificed for further studies. The effects of arsenic on heart tissues were evaluated by qPCR, western blotting and histopathology. Real-Time PCR analysis showed up-regulation of AT_{1a} receptor gene and no change in the *anf* gene expression in the heart. Arsenic increased AT_1 receptor and ERK1/2 and phospho ERK1/2 levels in the heart. Arsenic also increased the activity of MMP-9 in the arsenic exposed animals. Candesartan reversed all these effects except ANF gene expression. It up-regulated the ANF gene in candesartan treated groups. Arsenic increased the expression of of transforming growth factor receptor II (TβRII) along with phosphorylated-Smad3 and Smad4. Candesartan significantly reduced the TβRII, pSmad3 and Smad4 protein expression to the level of control. Arsenic caused mild to moderate degeneration of myocardial fibres as evidenced by loss of striations with eosinophilic cytoplasm. In Masson's trichrome staining in arsenic-exposed heart tissues showed the increased proliferation connective tissue in the areas degenerative changes. But candesartan could attenuate the proliferation of collagen in the myocardial layer as well as the degenerative changes. Overall, these findings reveal that the sub-chronic exposure to arsenic through drinking water can cause cardiac dysfunction in rats and candesartan has the cardio-protective potentials.

VVR-03

HEPATORENAL AND INTESTINAL PROTECTIVE EFFECTS OF QUERCETIN AND CURCUMIN AGAINST CADMIUM INDUCED TOXICITY

Shreesha Rao S., Patel U.D., Makwana C. N., Ladumor V. C., Modi C. M., Patel H. B.

Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and AH, Junagadh Agricultural University, Junagadh-362001, Gujarat, India

The study was performed to evaluate the hepatorenal and intestinal protective effect of quercetin (50 mg/kg, P.O. for 28 days) and curcumin (100 mg/kg, P.O. for 28 days) alone as well as in combination against cadmium-induced toxicity (100 ppm $CdCl_2$ in drinking water for 28 days) in rats. Noticeable signs of toxicity were not observed in rats of any groups except hair fall and diarrhea in toxicity controls. Cadmium exposure did not produce significant effect on hematological parameters. However, in rats treated with quercetin + curcumin significantly lower values of ALT, AST and ALP were observed and total bilirubin level was significantly lowered in all treatment groups compared to toxicity control. Treatment with quercetin and quercetin + curcumin were able to lower the blood glucose level as compared to that of toxicity group. In liver, cadmium exposure caused significant increase in MDA level, higher SOD and reduced catalase activity. The quercetin treatment was found to reduce the increased MDA level and SOD activity in liver; simultaneously it increased the catalase activity and GSH level. In curcumin treatment group, significantly decreased SOD activity, MDA level and significantly increased GSH level were observed. Quercetin + curcumin resulted significant lower MDA level and SOD activity. Cadmium exposure in kidney resulted increased MDA level, SOD activity and significant decrease in catalase activity. Quercetin and curcumin alone as well as in combination caused non-significant lower SOD activity and improved catalase activity with stimulation to GSH content which resulted in reduction in lipid peroxidation. In intestine, MDA level and SOD enzyme activity were significantly ($P<0.05$) increased upon cadmium exposure but catalase activity and GSH level were lowered and which were reversed upon treatment of quercetin and curcumin alone as well as in combination. Quercetin and curcumin in combination significantly prevented the histopathological changes caused by cadmium. Quercetin along with curcumin showed comparatively more ameliorating effect against cadmium-induced alterations in liver, kidney and intestine.

VVR-04

COMPARATIVE PHARMACOKINETICS OF SPARFLOXACIN IN GLIBENCLAMIDE TREATED AND UNTREATED DIABETIC RATS

Suraj S.V. [1], Nair S. N. [1],Nisha A.R. [1], Anoop Raj[2], Muhasin A. [3], Sanjay B.M. [1], Saranya C. [1], Megha M. [1], Mery, S. J. [1], Ranjith D. [1] and Juliet S.[1]

[1]Department of Veterinary Pharmacology and Toxicology, [2]Department of Veterinary Pathology, [3] Department of Animal Genetics and Breeding,College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala- 673 576.

Diabetes mellitus (DM) is an endocrinerelated metabolic diseases that result in hyperglycaemia. In diabetes, patients are pronefor multiplediseases. Hence to control these complications diabetic patients use medications like antihypertensive drugs, antiarrhythmic, antibiotics, and antiplatelet aggregant drugs along with the regular anti-diabetic drugs. Diabetes can cause alteration in pharmacokinetics and pharmacodynamics due to alteration in drug transporters like P-glycoprotein, along with this multidrug usage may result in drug interactions. Hence the effect of alteration in pharmacokinetics of drug and also the interaction kinetics has to be foundout in diabetes condition to ensure safe and effective use for the wellbeing of animals.Sparfloxacin is a third generation fluoroquinolone antimicrobial agent which is having activity against wide range of organisms.There are very limited studies related to pharmacokinetics of drugs in diabetes condition. The current study was undertaken to investigate the pharmacokinetic parameters of sparfloxacin in diabetic rats after oral administration and also to study alteration in pharmacokineticparameters of sparfloxacin in glibenclamide treated diabetic rats when compared to control rats. The analysis was carried out using RP- HPLC with the mobile phase of 1% aqueous acetic acid and acetonitrile (20:80) on a phenomenexluna 5µC_{18} column at the flow rate of 0.7ml/min and column temperature of 30^0C. The photodiode array detector (PDA) set to 280 nm was used for detection.The method was validated with respect to accuracy, precision, specificity and linearity. The retention time obtained for sparfloxacin was 8.5 min.The recovery was found to be 87±1.547 % in spiked plasma sample. The intraday precision with the co-efficient of variation ranged from 2.474to 8.218%. And interday mean error ranged from 0.0035 to 0.0512.Control rats, HFD rats, diabetic rats and glibenclamide treated rats were given sparfloxacin at the dose rate of 200 mg/kg and blood was collected at different time intervals and was analysed using RP- HPLC. Pharmacokinetics of orally administered sparfloxacin in control, HFD and diabetic rats were fitted into two compartment model where as in glibenclamide treated diabetic rats the pharmacokinetics of sparfloxacin were fitted to non-compartment model. In HFD, diabetic and

glibenclamide treated diabetic animals, the maximum plasma concentration achieved by sparfloxacin gradually decreased in the respective order.The rate absorption of sparfloxacin from the intestine was somehow decreased when glibenclamidewas co-administered.Probable reasons may be the pre-enterocytic interaction of glibenclamide with sparfloxacin as indicated by increased T_{max}, decreased slope of absorption, decreased$AUC_{(0-t)}$ and $AUC_{(0-\infty)}$. The maximumplasma concentration achieved by the drug is less in HFD rats group, diabetic rats group and glibenclamide treated diabetic rats group dueto higher apparent volume of distribution. Rate of elimination in diabetic rats is faster, due to polyuria hence half-life of sparfloxacin is less in diabetic rat. However, in glibenclamide treated diabetic rats the half-life is increased due to slower rate of absorption of drug and correction of polyuria. In both diabetic rats and glibenclamide treated diabetic rats group, maximum plasma concentration achieved by sparfloxacin is decreased when compared to control rats. Sparfloxacin being an antibiotic, depend on its attained plasma concentration for its pharmacodynamic activity. From this it can be concluded that dose adjustment of sparfloxacinis required in diabetes and in glibenclamide treatment, to attain effective therapeutic concentration.

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College of Veterinary and Animal Sciences, Mannuthy

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AMT-01

REVERSAL OF $PGF_{2\alpha}$ INDUCED CHANGES BY QUERCETIN IN MYOMETRIUM OF MICE WITHEXPERIMENTAL DYSMENORRHOEA

Afroz Jahan[1], Gissa M Jose[1], Vani Geeta[3], Sanweer Khatoon[2], M Muralidhar[1], M K Arunasree[3], P Anand Kumar[1], M. Lakshman[4], K. Bharavi[1], G. SrinivasaRao[1]

[1]NTR CVSc, Gannavaram, [2]CVSc, Udaipur,[3]School of Life Sciences,Hyderabad, [4]CVSc, Telangana

afrozvet@gmail.com

Primary Dysmenorrhea(PD) is one of the most common complaints among adolescent and young adult women and is the leading cause of absenteeism because of possible colic like pain due to contraction of myometrium induced by prostaglandins. Till date, the cause of dysmenorrhea remains unclear. Because of limitations of conventional treatments like NSAIDs and OCP's, herbal medicines are considered as feasible alternatives for the treatment of PD. Keeping all these facts in view the present study was carried out to screen the relaxant activity of quercetin on isolated mice uterus that is pre-contracted with $PGF_{2\alpha}$ and to compare relaxant activity of quercetin with ritodrine, a beta adrenergic agonist and role of COX inhibitor in mice with experimental dysmenorrhoea. The experiment was conducted in six groups. Group 1 was control or non dysmenorrhoeic group whereas Group 2 was experimental dysmenorrhoea group without any treatment.Group 3, 4, 5 and 6 received meloxicam (5 mg/kg) and quercetin(20, 40 and 80 mg/kg, PO) for 28 days followed by induction of experimental dysmenorrhoea. The uterine smooth muscle was pre-contracted with $PGF_{2\alpha}$ ($1.34x10^{-7}$M) and recorded dose dependent relaxation with Quercetin ($5x10^{-9}$to $15x10^{-5}$ M) and ritodrine ($5x10^{-12}$to $15x10^{-8}$M). Both quercetin and ritodrineshowed dose-dependent tocolytic action on pre-contractile response of $PGF_{2\alpha}$. The histological and ultra-structural changes observed in uterus and altered hormonal levels of $PGF_{2\alpha}$, PGE_2, PGI_2, TXB_2 and NOin plasma and uterine tissue homogenates of dysmenorrhoea were restored to normal by both meloxicam and quercetin.Both meloxicam and quercetin could restore the histological and ultra-structural changes in myometrium. Quercetin has alone restored COX-2 up-regulation in dysmenorrhea. The present study revealed the potential of quercetin in relieving pain induced by $PGF_2\alpha$ in dysmenorrhoea.

AMT-02

GENERAL ANAESTHESIA DECREASES THE DOSAGE REQUIREMENT OF AMPICILLIN-CLOXACILLIN COMBINATION IN HORSES

K. Kasturi Devi[1]*, Arun Anand[2], B. Srinu[3], J.S. Bedi[3], S.K. Sharma[1] and S.P.S. Saini[1]

Department Veterinary Pharmacology & Toxicology[1],Department Veterinary Surgery & Radiology[2] ,Department of Veterinary Public Health & Epidemiology[3],GADVASU, Ludhiana

kasturi120@gmail.com

Ampicillin-cloxacillin combination is commonly used by equine practitioners as a primary prophylactic measure, owing to its broad spectrum of activity, low toxicity and low cost. However in horses undergoing different anesthesia protocols, there is decrease in renal and portal blood flow due to diminished cardiac output as well as compensatory increase in blood supply to vital tissues like brain. The present study was aimed to determine the influence of various anesthetic protocols on the disposition kinetics of ampicillin-cloxacillin combination, when used as surgical prophylaxis in horses. Animals were categorised into 3 groups (n=6 for each group); group I was unanesthetised horses, group IIcomprised horses undergoing total intravenous anesthesia (TIVA) and group III consisted of horses maintained under inhalant anesthesia. In all the groups, ampicillin-cloxacillin was administered intravenously at the dose rate of 10 $mg.kg^{-1}$ (in group II & III, ampicillin-cloxacillin was administered when the animal had attained proper anesthetic depth *i.e* in plane II of stage 3 of mainteneceanesthesia). Blood samples were collected at different time intervals after antibiotic administration, and plasma concentrations of both the drugs were quantified by simultaneous detection method in HPLC. Disposition curves for both the drugs were best described in biexponential equation (two compartment model). Mean(±SE) pharmacokinetic paramters for ampicillin under TIVA and inhalant anesthesia were: area under plasma drug concentration time curve (AUC) (27±2.84 $\mu g.hr.ml^{-1}$ and 24.819±1.506 $\mu g.hr.ml^{-1}$, respectively), mean residence time (MRT) (1.639±0.09 hr and 1.684±0.07 hr, respectively) which were significantly($P<0.01$) greater and clearance rates (0.3858±0.04 $litre.h^{-1}kg^{-1}$ and 0.412±0.03 $L.h^{-1}kg^{-1}$, respectively) were significantly($P<0.01$) lower when compared to unanesthetised horses. Similarily the pharmacokinetic paramters for cloxacillin under TIVA and inhalant anesthesia were: elimination half-life (1.789 ±0.05 hr and 1.729±0.09 hr, respectively), AUC (42.171±3.86 $\mu g.hr.ml^{-1}$and 44.938±2.83$\mu g.hr.ml^{-1}$, respectively), MRT (1.725±0.05 hr and 1.827±0.05 hr, respectively) which were significantly($P<0.01$) greater and clearance rates (0.2464±0.02 $L.h^{-1}$ kg^{-1} and 0.228±0.01 $L.h^{-1}$ kg^{-1}, respectively) were significantly ($P<0.01$) lower when compared tounanesthetised horses. The MIC and MBC values of ampicillin-cloxacillin against *Escherichia colli* and *Staphylococcus aureus*in mullerhinton broth were determined by broth microdilution method. MIC of ampicillin-cloxacillin against *E.colli* and *S.aureus*was 0.39$\mu g.ml^{-1}$. PK-PD integration indicated that to maintain %T>MICvalue above 50% for bacteria with MIC≤ 1$\mu g.ml^{-1}$, an appropriate dosage regimen for ampicillin-cloxacillin in horses would be 10 $mg.kg^{-1}$i.v. , repeated 6 h intervals during anesthesia and at 4 hour interval in unanesthetised horses.

Since ampicillin and cloxacillin are largely excreted via glomerular filteration/tubular secretion, decrease in renal blood flow during anesthesia significantly alters the plasma concentrations and kinetic parameters during anesthesia. The results of the present study warrant the use of approximately 33% lower

dose in anesthetizeed horses than unanesthetized horses in order to maintain blood concentrations above therapeutic concentrations which is very crucial to prevent surgical site infections.

AMT-03

AN ITK FORMULATION ALLEVIATES DIABETIC-NEPHROPATHY BY DOWN-REGULATING BLOOD GLUCOSE AND SGLT-2 EXPRESSION IN HIGH FAT DIET/STREPTOZOTOCIN TREATED RATS

Rajput Priyanka, PatilChandan, PrakashAtul, ChoudhurySoumen, Mandil Rajesh and GargSatish K.

Department of Veterinary Pharmacology and Toxicology

College of Veterinary Science and Animal Husbandry

U.P. PanditDeenDayalUpadhyayaPashu-ChikitsaVigyanVishwavidyalayaEvam Go AnusandhanSansthan,

(DUVASU), Mathura – 281001

Diabetic nephropathy (DN) is a leading cause of end stage renal failure and death worldwide in about 30-40% cases. DN results from several factors including hyperglycaemia, hyperglycemia-induced oxidative stress and advanced glycation end products. Present study was designed to explore the therapeutic potential of an Indian Traditional Knowledge (ITK) based formulation in high-fat diet fed (obese) streptozotocin-induced type II diabetes and diabetic nephropathy in male Wistar rats. Thirty obese male Wistar rats divided into four groups viz. Normal control, obese, obese-diabetic, obese-diabetic+metformin and obese-diabetic+ITK consisting six animals in each, were experimentally-induced-diabetes with streptozotocin @ 35 mg/kg body weight intraperitoneal. ITK formulation and metformin were given @ 445 mg/kg and 50 mg/kg body weight, respectively by oral gavage continuously for 60 days. Significant increase in fasting blood glucose and percent glycatedhaemoglobin (HbA1C) and renal function biomarkers like serum albumin, globulin, total protein and albumin to globulin ratio were decreased and an increase in the levels of creatinine, BUN, uric acid were recorded in obese diabetic rats. Expression study by Western blot method revealed up-regulation (0.79±0.08 fold) of SGLT-2 protein in kidney tissues of obese diabetic rats compared to normal control (0.65±0.04 fold). Histopathological findings also revealed severe collagen fibre deposition in kidney tubules and glomeruli in obese diabetic group rats. Treatment with ITK formulation and metformin significantly lowered blood glucose and moderately percent HbA1C, however it was observed ITK was superior to metformin in reducing blood glucose. Kidney injury markers and biochemical indices were partially to significantly restored in rats treated with hot aqueous ITK formulation. A significant decrease (0.30±0.09 fold) in SGLT-2 expression and reversed the pathological features of kidney injury and showed less degenerative change was observed in ITK treated groups compared to obese diabetic group revealing ameliorative action against hyperglycemia induced kidney injury. It can be concluded that ITK formulation has better hypoglycemic property.

AMT-04

EVALUATION OF THE VASCULAR PROTECTIVE EFFECT OF L - ARGININE (L-ARG) AND L - CITRULLINE (L-CIT) IN EXPERIMENTAL ATHEROSCLEROSIS AND ASSOCIATED NEPHROPATHY

Rekha, C., Kalakumar, B., Gopala Reddy, A., Lakshman, M. and Rajkumar, U.

cahker@gmail.com

Cardiovascular dysfunction is considered as one of the leading causes of death in human beings around the globe.We evaluated the vascular protective effect of L - arginine (L-arg) and L -citrulline (L-cit) in experimental atherosclerosis and associated nephropathy. Sixty C57BL/6J mice were divided into six groups often animals each. All the treatment groups were fed with high fat diet along with cyclosporine A (first line immunosuppressant in solid organ transplantation) and Group I was kept as normal control with standard diet throughout the study period. Group II was positive control for atherosclerosis and nephropathy. Group III and IV were administered with L-arg and L-cit@ 2.5% in drinking water respectively and Group V with a combination of L-arg and L-cit @ 1.25%each. Group VI were treated with simvastatin @10mg/kg body weight orally. On day 45 and 90 blood samples were collected to estimate the serum biochemistry and nitric oxide (NO) level. Samples of aorta and kidney were collected on day 90 for histopathology and electron microscopy. Our results demonstrated significant alterations in serum biochemistry and NO levels in blood. Moreover, there were marked histopathological changes in aorta and kidney in group II.We found that the effects of L-arg were comparable with L-cit while the combination of both provided better vascular protection.These results suggest that nitric oxide boosting substances including L–arg and L–cit can ameliorate the biochemical changes and overcome the progression of vascular disorders induced by cyclosporine A along with high fat diet.

AMT-05

EFFECT OF RACTOPAMINE HYDROCHLORIDE ON BEHAVIOURAL AND REPRODUTIVE ALTERATIONS IN ZEBRAFISH *(Daniorerio)*

Lonare S.M., Sole S.S., Kambli A.P.,Kamble K.K. and Umap S.A.

Department of Veterinary Pharmacology and Toxicology, Bombay Veterinary College, Mumbai,

Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra

lsnehal47@gmail.com.

Ractopamine (RAC) a β-adrenergic and TAAR1 agonist is used as feed additive in cattle, pigs and turkeys for promoting leanness ideal for meat production. Its widespread use has resulted in its appearance in aquatic environments. In this study, we have evaluated the effect of RAC on endocrine system of male zebrafish by exposing them to different concentrations for the period of 21 days. Adult male zebrafish were

divided into 4 groups [one control group and 3 RAC exposed group (n=8 in each)]. Three different concentrations of RAC were selected (250 ppm, 350 ppm and 450 ppm) on the basis of Maximum Tolerable Dose (MTD). RAC exposed zebrafish were evaluated for behavioral alterations (colliding to walls, hyperventilation, un-coordinated swimming and territorial behavior daily during 21 days of exposure) and reproductive performance (on 7, 14 and 21 days of exposure). On the specified timelines, RAC exposed males zebrafish were allowed to mate with unexposed females and their eggs were evaluated for spawn count, fecundity%, mortality% and abnormal growth count for three consecutive weeks, and at the end of the study period vitellogenin (VTG) levels (indicator of exposure to estrogenic endocrine disruptive chemicals) was evaluated using ELISA. RAC exposed zebrafish showed signs of behavioral alteration (colliding to walls, hyperventilation, un-coordinated swimming and decreased territorial behavior). Regarding reproductive performance, alterations in spawn count, fecundity%, mortality% and abnormal growth count was observed compared to the control ($p<0.05$). Higher vitellogenin levels were found in zebra fish exposed to RAC at 250 ppm. This study highlights the ecotoxicological effect of RAC and highlights importance of screening for RAC in water reservoirs with possibility of RAC contamination and also for random screening for RAC in poultry meat. We need regulation to control RAC use in livestock in India.

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NAT-01

AMELIORATIVE EFFECT OF QUERCETIN ON CADMIUM INDUCED MYOMETRIAL CHANGES IN MICE WITH ESTRUS

Gissa M. Jose[1], K. Bharavi[2], Afroz Jahan[3], P. Anand Kumar[4], G. Srinivasa Rao[5]

[1]Department of Veterinary Pharmacology & Toxicology, RIVER, Puducherry, [2,3,5] Dept. of Veterinary Pharmacology & Toxicology, [4] Dept. of Veterinary Microbiology, NTR CVSc, Gannavaram. gissamary@gmail.com

Uterine abnormalities either hereditary or acquired are the major cause of infertility among reproductive females that leads to recurrent miscarriages, spontaneous abortions, pre-term labour, infertility etc. Cadmium is an important endocrine disruptor and reproductive toxicant causing uterine abnormalities. Quercetin, a powerful antioxidant flavonoid have the ability to scavenge the free radicals and also have heavy metal chelating property, smooth muscle relaxant property and estrogenic effect. The present study evaluated the structural and functional changes caused by cadmium in mice myometrium and also studied the ameliorative effect of quercetin on cadmium induced myometrial changes. The animals were divided into four groups viz control, cadmium, cadmium & quercetin of and quercetin. The study was conducted in both non-estrus and estrus mice. The *exvivo* study revealed a decrease in the mean EC50 value for oxytocin and KCl in cadmium group, suggesting the estrogen mimicking effect of cadmium. The co-administration of quercetin and cadmium increases the mean EC50 value, suggesting the reversal effect of quercetin in cadmium induced changes. Histopathology of cadmium group in both non-estrus and estrus animals revealed degenerative changes in the glandular epithelium, congestion and desquamation of lining epithelium. The co-administration of quercetin and cadmium showed normal structure with lining epithelium and glands. There was significant difference in the TBAR'S, SOD and GSH values of liver and kidney among the treatment groups, suggesting the ameliorative effect of quercetin on cadmium induced oxidative damages. Hence we can conclude that quercetin is having an ameliorative effect on cadmium induced structural and functional changes.

NAT-02

SEPSIS-INDUCED ATTENUATION OF ANGIOTENSIN AND PURINOCEPTOR- MEDIATED AORTIC REACTIVITY: EFFECT ON VASCULAR AT1 AND P2Y6 RECEPTORS

T Jagadeesh, Gari Manju, Choudhury Soumen and Garg Satish K

Department of Pharmacology & Toxicology College of Veterinary Science & A.H.

U.P. Pt. Deen Dayal Upadhyaya Pashu-Chikitsa Vigyan Vishwavidyalaya Evam Go- Anusandhan Sansthan (DUVASU), Mathura (U.P.) - 281 001

Up-regulation of P_2Y_6 purinergic receptors during inflammatory conditions and interplay between P_2Y_6 and angiotensin II type I (AT1R) receptors in hypertension has been reported. But their involvement in modulating vascular response during sepsis is yet to be explained. Thus the present study was conducted to assess the effect of sepsis on P_2Y_6 and AT1R receptors expression and their signal transduction mechanism(s) in mediating vascular hyporeactivity during late phase of sepsis. Sepsis was induced by caecal-ligation and puncture (CLP) in mice. Isometric tension was recorded in isolated aortic rings, harvested during late phase (20 h) of sepsis and mounted in thermostatically controlled (37 ± 1 °C) organ bath using data-acquisition system based polyphysiograph. The mRNA expression of AT1a and P_2Y_6 was investigated using qRT-PCR. The results obtained revealed that UDP and Ang-II-induced vasoconstriction was significantly ($P<0.05$) higher in endothelium-denuded mouse aorta compared to the endothelium-intact aorta, and these responses were mediated through P_2Y_6 and AT1 receptors, respectively. UDP and Ang-II produced higher contractile effect in the presence of L-NAME, the non-specific inhibitor of nitric oxide synthase (NOS), in endothelium-intact mouse aortic rings. But compared to healthy, aortic rings of sepsis-mice showed significant ($p<0.05$) decrease in contractile response following exposure to UDP and Ang-II. Sepsis caused up-regulation of P_2Y_6 receptor mRNA and down-regulation of AT1a receptor mRNA expression in endothelium-intact mouse aorta. Ang-II produced higher contractile effect in the presence of 1400W, the specific inhibitor of iNOS, in aorta of sepsis group. Ang-II-induced contraction was reduced in the presence of MRS-2578, the selective antagonists of P_2Y_6 receptor, in sham operated mouse aorta, but the contractile effect of UDP was not altered in the presence of losartan, the selective antagonists of AT1 receptor. Our findings revealed that NO attenuated the contractile effect of both Ang-II and UDP in endothelium-intact aortic rings of sham operated mice. Sepsis–induced vascular hyporeactivity in endothelial-denuded mouse aorta is possibly due to iNOS-derived NO and down regulation of P_2Y_6 and AT1a receptors. Up-regulation of P_2Y_6 receptor mRNA expression in intact septic mouse aorta was possibly due to change in expression of both vascular smooth muscle and endothelium.

NAT-03

EVALUATION OF DEVELOPMENTAL TOXICITY OF LEAD IN ZEBRAFISH AND AMELIORATION BY GARLIC AQUEOUS EXTRACT

R. Yogeswari, A. Arivuchelvan, S. Murugesan, G.A.Balasubramaniam, P. Selvaraj, A. Jagadeeswaran, A. Raja and Dr. K. Balasundaram

Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal-2

Lead is a non-biodegradable toxic heavy metal in the environment and now, it has become a global issue. Lead is a cumulative toxicant that affects multiple body systems, especially developing nervous system. Garlic, *Allium sativum* L. is a member of the Alliaceae family, has been widely recognized as a valuable spice and a popular remedy for various ailments. Hence in this study the effect of lead acetate on zebrafish embryo/larvae and its amelioration by garlic aqueous extract (GAE) was assessed. Normally dividing 5 hpf (hours post fertilization) embryos were allotted into different groups, in six well plates. Two control groups - one with plain embryo water and the other with garlic aqueous extract. Lead acetate was exposed at three dose levels 0.1, 0.5 and 1 ppm to next three groups. The other three groups were exposed with lead acetate at three levels along with 1 µg garlic aqueous extract.The livability and aberration percentage was determined by examining the embryo/larvae at 5, 24, 48, 72, 96 hpf and 5, 6, 7 and 8 dpf under inverted microscope. Apoptosis was determined by staining the embryo/larvae from each group with Acridine orange dye at 24, 48 and 72 hpf and 5 dpf (days post fertilization). Locomotor behaviour was assessed by Kinovea software at 6 dpfand histopathology was done at 8 dpf. Lead induces significant ($P \leq 0.01$) dose dependenttoxic effect on livability, hatching, morphological and histopathological changes, apoptosis and locomotor behaviour in zebrafish embryo / larvae. GAE has partial protective effect on lead acetate treated embryos. The amelioration by GAE was better in 0.1 ppm lead acetate treated group followed by 0.5 and 1.0 ppm lead acetate treated groups respectively.

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ANJ-01

COMBATING METHICILLIN/OXACILLIN RESISTANCE VIA SYNERGISM AND *mecA* MODULATION BY LEMONGRASS OIL AND CITRAL IN CLINICAL ISOLATES OF *STAPHYLOCOCCUS AUREUS*

Jisha, S.[1], Suja, R.S.[1], Sujith, S. [1], Nair, S.N. [1], Kariyil, B.J. [1], Surya, S.[2], Ambily, R.[2], Akhil, G.H.[1] and Usha, P.T.A.[1]

[1]Department of Veterinary Pharmacology and Toxicology, [2]Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala.

E mail: jisha792@gmail.com

The mounting tide of bovine mastitis by methicillin-resistant *Staphylococcus aureus* (MRSA) infection allied with biofilm formation poses serious threat to efficacy of antibiotic treatment. The gene encoding modified pencillin binding protein, PBP2a, referred to as *mecA* is responsible for methicillin /oxacillin resistance. Combination therapy, particularly combining essential oil with conventional antibiotics represents a vital strategy to ameliorate antimicrobial resistance. Hence, the study was conducted to evaluate antibacterial and antibiofilm activities of lemongrass oil (LGO) and its active principle, citral, individually as well as in combination with methicillin/oxacillin against *mecA* positive *S. aureus* isolates from bovine mastitic milk, which are resistant to methicillin and oxacillin,. The effect of LGO and citral on *mecA* gene modulation was also analyzed. Antibacterial effect of the test substances were evaluated by disc diffusion and microbroth dilution method whereas, combination effect was assayed using checkerboard method. Biofilm formers among the MRSA isolates were identified using congored method while, antibiofilm activity of LGO and citral was ascertained using tissue culture plate based crystal violet assay. Further, realtime PCR was done to elucidate downregulation of *mecA* gene expression by LGO and citral. The results of the study revealed significant antimicrobial activity of LGO and citral as evident from the minimum inhibitory concentration (MIC) and diameter of zone of inhibition values. Besides, LGO and citral produced remarkable inhibition of both preformed biofilm and biofilm formation, manifested as significantly reduced minimum biofilm inhibitory concentration (MBIC) and minimum biofilm eradication concentration (MBEC). Furthermore, the combination therapy of LGO and citral with methicllin/oxacillin showed synergistic interaction in both antimicrobial and antibiofilm assays, establishing the implication of combination strategy in mitigating antibiotic resistance. Therefore, present findings are indicative of the essential role of LGO and citral in alleviation of methicllin/oxacillin resistance partly via downregulation of *mecA* and antibiofilm activities.

ANJ-02

SYNTHESIS, CHARACTERISATION AND PHARMACOLOGICAL ASSESSMENT OF NANOPARTICLES OF *TINOSPORA CORDIFOLIA* FOR ITS CYTOTOXIC ACTIVITY

Sakthi Priya, M*., Sarathchandra,G[b]., Jagadeeswaran, A[c]., Preetha, S.P[d] and S.Partiban[e]

[b]Dean, Faculty of Basic Sciences, TANUVAS, MVC Campus, Chennai.

[c]Professor and Head, Department of Veterinary Pharmacology and Toxicology, VCRI, Namakkal.

[d]Assistant Professor, Department of Veterinary Pharmacology and Toxicology, MVC, Chennai.

[e]Professor, Department of Animal Biotechnology, MVC, Chennai.

* Assistant Professor, Department of Veterinary Pharmacology and Toxicology, VCRI, Namakkal

The present study was taken up to evaluate the use of *Tinospora cordifolia* stem extract as a reducing agent for silver nanoparticle formation and characterization of the synthesized nanoparticles and assessment of its cytotoxic effect on the Hep2 G cancer cell line. Silver nanoparticles were synthesized and characterized by visualizing the colour change, observing for the concentric rings under an optical microscope, analyzing the particle size and measuring the zeta potential. The extract was also phytochemically characterized and subjected to MTT assay to evaluate its cytotoxic activity at three different doses of 200 µg/ml, 400 µg/ml and 600 µg/ml against a negative control and a positive control 5-Flurouracil 5 µg/ml. The synthesized nanoparticles were confirmed by visualizing the colour change from colourless to slight reddish brown colour, observing concentric rings under optical microscope, determining the particle size in the range of 0.4 nm and zeta potential was measured to be -13.4mv with peak area of 100 percentage intensity. Phytochemical analysis revealed the presence of saponin, terpenoids, flavanoids, hydrolysable tannin, glycosides and cardiac glycosides in the aqueous extract. The cytotoxicity assay revealed a dose dependent significant decrease in the percentage inhibition of growth of cells with the highest level of inhibition noticed at the highest dose similar to positive control group. Thus the cytotoxic effect of the extract could be attributed to the presence of the secondary metabolites in them which could get converted into nanoparticles with silver and establishes its potential as a chemotherapeutic option for cancer.

ANJ-03

ANTIDIABETIC POTENTIAL OF AN ITK FORMULATION AND *TRIBULUS TERRESTRIS* IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

Rajput Priyanka, Patil Chandan, Prakash Atul, Mandil Rajesh and Garg Satish K.

Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry U.P. Pandit Deen Dayal Upadhyaya Pashu-Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, (DUVASU), Mathura – 281001

drpriyanka12apr@gmail.com

The objective of the present study was to explore the therapeutic potential of hot aqueous extract of ITK formulation and 70% hot ethanolic extract of *Tribulus terrestris*. Extracts have potent *in vitro* antioxidant (2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and 2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging assay), antidiabetic (α-amylase and α-glucosidase inhibition assay) and were observed to possess active principles having antidiabetic, antioxidant, nephroprotective, cardioprotective and anti-inflammatory properties. Further, 42 obese male Wistar rats were divided into seven groups viz. Normal control, Obese control, Obese diabetic, Obese diabetic+Metformin, Obese diabetic+*Tribulus terrestris*, Obese diabetic+ITK and Obese diabetic+*Tribulus terrestris*+ITK consisting six animals in each, were induced diabetes with streptozotocin @ 35 mg/kg body weight, i.p. *Tribulus terrestris* extract, ITK formulation and metformin were given @ 200 mg/kg, 445 mg/kg and 50 mg/kg body weight by oral gavage for 60 days. Increase in feed and water intake, decrease in percent weight gain and anthropometric parameters. Increased triglycerides, total cholesterol, LDL, ALP, GGT, ALT, AST (liver injury markers) and decrease in HDL were evident in obese diabetic rats which were partially and significantly restored in rats treated with hot aqueous ITK formulation and metformin, however ITK was not observed to be very effective in regulating dyslipidemia induced by experimental T2DM as compared to metformin treated group. Obese diabetic rats also revealed significant increase in MDA and decrease in GSH level, decreased activity of CAT, SOD, GST and GP_X in liver, kidney, brain, spleen, testes and heart whereas, significant improvement was observed in antioxidant system and enzymes and reduction in lipid peroxidation in ITK formulation treatment group irrespective of tissues than the metformin treatment group. Thus, it can be concluded that, hot aqueous extract of ITK formulation possess good antioxidant potential to combat free-radical mediated derangements in the body and reversal of hyperglycemia-induced oxidative insults in tissues in experimental streptozotocin-induced type-2 diabetic rats.

ANJ-04

ASSESSMENT OF *IN-VIVO* ANTI-INFLAMMATORY EFFECT AND SAFETY OF CLOVE OIL IN WISTAR RATS

Humbal, B.R., Sadariya, K. A., Prajapati, J.A., Bhavsar, S.K. and Thaker, A. M.

Department of Veterinary Pharmacology & Toxicology College of Veterinary Science and Animal Husbandry AAU, Anand - 388 001, Gujarat, INDIA. humbalbrijesh@gmail.com

The present study was planned to evaluate *in vivo* anti-inflammatory and safety of *Syzygium aromaticum* (clove) oil in male and female wistar rats. The *in-vivo* anti-inflammatory assay of clove oil was carried out using carrageenan induced paw edema model in rats. All rats were injected subcutaneously with 0.1 ml of a 10% w/v carrageenan suspension in the sub- planter region of the left hind limb after 30 minutes subsequent to oral administration of clove oil. Indomethacin was administered @ 10 mg/kg in standard drug control rats. Rats of control groups were kept untreated and other groups were treated with clove oil @ 100, 250 and 500 mg/kg b.wt., respectively. Volume of edematous paw will be measured by using plethysmometer at 0, 1, 2, 4, 6, 12 and 24 h after treatments. Increase in paw thickness and per cent inhibition was calculated. The anti-inflammatory effect of clove oil was highest at 3h in male and female rats 35.77% and 35.46%, respectively at the dose of 500 mg/kg. It was lower than anti-inflammatory effect of indomethacin at 3h in male and female rats 41.75% and 42.99%, respectively. Safety evaluation of clove oil was carried out following repeated oral administration at dose of 50, 100 and 200 mg/kg once daily for 28 days. Body weight and feed consumption of rats were monitored at weekly interval. At the end of experiment, blood sample were collected for the hematological and serum biochemical investigations as well as organs were collected for histopathological examination. No significant differences were observed in body weight and feed consumption of clove oil treated rats. No significant changes have been observed in Hb, RBCs, PCV, TLCs, MCV, MCH and MCHC in clove oil treated rats as compared to control rats. No significant change have been observed in serum creatinine, BUN, total bilirubin, AST, ALT, total cholesterol, total protein and albumin in clove oil treated as compared to control rats at the end of experiment. Organs like kidney, liver, spleen and heart from control and clove oil treated rats did not revealed any marked gross or histopathological changes. Results of the present study suggest that clove oil has dose dependent anti-inflammatory activity and is safe following repeated oral administration @ 50, 100 and 200 mg/kg for 28 days in wistar rats.

ANJ-05

EVALUATION OF *IN VITRO* AND *IN VIVO* ANTITUMOUR PROPERTY OF *THESPESIA POPULNEA* ('POOVARASU')

Anu G[1], Usha PTA, Bibu John Kariyil, Suja Rani, Ajith Jacob George and Reni K John

Veterinary surgeon, Animal husbandry department Kerala

dranugvet@gmail.com

Increased level of toxicity of chemotherapeutic agents and development of resistance to anticancer drugs are the major concerns in cancer chemotherapy. Based on traditional knowledge and previous researches,*Thespesiapopulnea* ('Poovarasu') bark was tested for itsantitumour propertyin MCF-7 human breast carcinoma cell line and in DMBA induced mammary tumours in rats. *In vitro* cytotoxic effect of methanolic extract and its fractions were assessed using MTT assay and based on IC_{50} value chloroform soluble fraction (CSF)was selected for the studies. *In vivo* effect of CSF was tested at dose rates 50 mg/kg and 100 mg/kg bodyweight in mammary tumourinduced rats for 14 days.*In vitro* apoptotic studies were conducted using phase contrast microscopy, DNA ladder assay, TUNEL assay and acridine orange/ethidium bromide (AO/EB) staining. *Invitro* and *invivo* antioxidantassays and expression study of antiapoptotic gene *bcl2*were performed.Histopathologicalexamination of tumour masses was doneusing H&E and AO/EB staining.On phase contrast microscopy and AO/EB staining, CSF treated cellsexhibited marked apoptotic changes.Typical ladder pattern characteristic of apoptosis was not observed in DNA ladder assay whereas, an increase in positive reactions noticed in TUNEL assay. CSF exhibited an*in vitro* antioxidant effect in a dose dependent manner. In AO/EB staining of tumour masses an increased apoptotic and necrotic cell density was noted in CSF treated groups. On histopathological examination oftumour masses a progressive reduction in cellularity and apoptotic changes were noticed.*Bcl2* expression was downregulated both in *in vitro* and *in vivo* studies. Hence in the present study, CSF of methanolic extract of *T. populnea* bark exhibited a dose dependent cytotoxic and antitumour activity. The plant fraction was effective in inducing apoptotic cell death and may be considered as a potent source for isolating therapeutic molecules for cancer treatment.

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TECHNICAL SESSION

PHARMACOGENETICS/ PHARMACOGENOMICS

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Pharmacogenomics in the development and validation of indigenous drugs
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LP-PG-01

POLYMORPHISMS: THE BASIC CONCEPT OF PHARMACOGENOMICS

S.P. Singh and Disha Pant

Department of Pharmacology & Toxicology,

College of Veterinary and Animal Sciences

G. B. P.U. A & T, Pantnagar-263145 (U.K.)

Introduction

As of now, the promising benefits of pharmacogenomics in therapeutics are known. To mention a few, individual based drugs and dosage regimen resulting into a more selective and targeted drug therapy, early disease diagnosis and remedial measures, development of more effective DNA and RNA vaccines and rapid drug discovery with minimum toxicity. Responses to drugs and adverse reaction to drug treatment may occur in a sector of animal population, which may be influenced by age, gender, environment, and by an individual's genetic make-up which in the case of animals is breed variation. Genetic variants arise within a species as a result of DNA base pair substitutions resulting in single nucleotide polymorphisms (SNPs) or as a result of either an insertion or deletion in a sequence (Brunton *et al*., 2008).

For clinicians, genetic information obtained from polymorphism-based pharmacogenetic tests is highly crucial for the better prediction ability of drug response and life-threatening toxic reactions due to the narrow therapeutic index of some chemotherapeutic agents viz., anticancer drugs. Pharmacogenotyping utilizes different examination strategies, such as single nucleotide polymorphism analysis, somatic/germline mutation analysis and partial/full genome sequencing. The promising effect of pharmacogenetics on the solving of the individual variability in drug response and toxic reactions is being observed with the accumulation of the information that unravel the human genomic variations from large-scale population and multi-parameter-based pharmacogenetic studies of the postgenomic era. Polymorphisms contribute wide variations in human genome and may define how individuals respond to medications, either by changing the pharmacokinetics and pharmacodynamics of drugs or by altering the cellular response to therapeutic agents. To define the effect of polymorphisms on the targets of chemotherapeutics is necessary for the prediction of altered pharmacokinetics of therapeutic agents.

Genetic polymorphisms

If the genomic DNA sequences of two individuals are compared, substantial sequence variations can be detected at different points of the whole genome. There are many forms of these genetic variations. Polymorphism (poly- multiple; morph- form), is used in genetics to describe multiple forms of a single gene that exist in a population. Polymorphisms are genetic variants and refer to the occurrence of various

phenotypes in a certain population. A polymorphism is a DNA sequence variation and does not classify as mutation. In genetic polymorphisms, there are two or more equally acceptable sequence of a gene and the common allele must have a frequency of 1% or more in the population. If the frequency is lower than 1%, the allele is accepted as a mutation.

Specific single nucleotide polymorphisms (SNPs) are referred by using 'rs' number (reference sequence). It stands for Reference SNP cluster ID. The rs number allows the precise identification of a polymorphic variation in the numerous databases (NCBI, HapMap, SNP500 Cancer, etc.). For instance, a SNP causes a replacement of an amino acid by another amino acid; this can be defined by the name and the position of the replaced amino acid, followed by the name of the novel amino acid. As an example, a common SNP in the DPYD gene is identified as rs 1801160 [V732I or Val732Ile]. SNPs are also identified by the name and position of nucleotide in the reference DNA sequence. The same SNP in the DPYD gene, presented with rs 1801160, is identified as 2194G>A. The letters A, T, C, and G can be used for both nucleotides and amino acids, and this can cause confusion (Robert *et al*., 2014).

Genetic polymorphisms include minor changes on DNA sequence such as substitutions, deletions, insertions, and repeats. These changes influence the three-dimensional structure, expression and activity of the proteins encoded by these genes. The occurrence of single nucleotide polymorphisms (SNPs) in DNA sequence occurs when a single nucleotide (A,T,C,or G) in the genome sequence is altered. This type of alteration generates single nucleotide variants (SNVs). SNPs are relatively common and are considered to occur in approximately 1 in 1,000 base pairs in the human genome, therefore, millions of SNPs must be identified and analyzed to determine their any involvement in drug response. In addition, our limited knowledge of gene linked drug response also complicates the process of drug development. Since many genes are likely to influence responses, obtaining the big picture on the impact of gene variations is highly time-consuming and complicated. SNPs and indels, if located in the coding region of genes, may have significant effects on key protein production within cells. SNPs can affect gene function due to the change of protein but can also occur in noncoding parts of the gene so they would not be seen in the protein product (Strachan *et al.*, 2015). Ethnicity is one of the key factors that can explain the observed variability in both pharmacokinetics (PK) and pharmacodynamics (PD) of therapeutics, resulting in differences in response to drug therapy.

Identification of polymorphism

Polymorphism can be detected by various ways. A number of good polymorphism genotyping technologies such as allele-specific single-base primer extension, allele-specific enzymatic cleavage –

restriction fragment length polymorphism (RFLP), pyrosequencing, mass spectrometry and invader assay are available but only one genotyping method is not ideal for all applications. High-throughput polymorphism genotyping process includes fast and cost-effective identification of polymorphisms in different individuals and lead to the determination of associations between genotype and phenotype. Genotyping starts with the isolation of DNA from patient followed by amplification to increase the sample amount and later polymerase chain reaction (PCR), sequencing or array-based technologies are employed (Kocal *et al.*, 2017). However, main challenges of polymorphism genotyping technologies are slow speed of assays due to the time-consuming protocols, high instrument and consumable costs, and requirements on the performing multiple assays in parallel. Studies on ideal polymorphism genotyping technologies are still in development process (Twyman *et al*., 2005).

ADME-related polymorphisms

DMET™ Microarray Technology for pharmacogenomics-based personalized medicine is a novel concept. Drug metabolizing **e**nzymes and **t**ransporters (DMET) enables highly multiplexed genotyping of known polymorphisms in absorption, distribution, metabolism, and elimination (ADME)-related genes on a single array. The DMET Plus Panel interrogates markers in 225 genes that have documented functional significance in phase I and phase II drug metabolism enzymes as well as drug transporters. The power of the DMET Assay has previously been demonstrated with regard to several different drugs including warfarin and clopidogrel. In a research study using an earlier four-color version of the assay, it was demonstrated that warfarin dosing can be influenced by a cytochrome P450 (CYP) 4F2 variant. Additionally, the assay has been used to demonstrate that CYP2C19 variants with decreased enzyme activity led to lower levels of the active clopidogrel metabolite, resulting in a decreased inhibition of platelets and a higher rate of cardiovascular events when compared to noncarriers of the DNA variant. Thus, highly multiplexed SNP genotyping focused on ADME-related polymorphisms should enable research into development of safer drugs with greater efficacy (Burmester *et al.*, 2010).

Adverse drug reactions (ADRs)

Genetic differences due to the polymorphisms are thought as one of the strongest reasons in adverse drug reactions (ADRs). Genetic polymorphisms are considered as molecular biomarkers in pharmacogenetic-based studies both in clinic and research to predict the ADRs and apply the medications as personal. Pharmacogenetic associations are important in cancer chemotherapy due to the extremely narrow chemotherapeutic index of anticancer drugs given for cancer management. Polymorphisms in both patient's genome and tumor genome affect the regulation of drug transport, retention and efflux of anticancer drugs,

determining the penetration into tumor tissue. The tumor genome possesses most of the polymorphisms that influence the sensitivity or resistance of, hence, treatment efficacy and tumor genome will have a key role as a dose limiting factors in cancer management.

Polymorphisms sometimes do not cause significant alterations on the final product, but may have an effect on the substrate specificity and activity of the product (especially for enzymes) or other characteristics and functions. For example, polymorphisms in cytochrome P450 2D6 (CYP2D6) are one of the cytochrome P450 enzymes of the liver that can influence how humans metabolize cancer drugs, although the enzymes are basically the same sequence and structure. Polymorphisms in CYP2D6 have been seen in the general population about 10% and it has been associated with poor-metabolizer phenotype of enzyme. This is important for codeine-based pain medications due to the activation of codeine to morphine and includes CYP2D6-dependent step.

Clinical importance

In the context of therapeutics, one of the examples of clinical pharmacogenomics involves the genetic polymorphism of thiopurine methyltransferase (TPMT). TPMT catalyzes the S-methylation of the thiopurine agents such as azathioprine, mercaptopurine, and thioguanine. These agents are commonly used for a diverse range of medical indications, including infants leukemia, rheumatoid arthritis, inflammatory bowel disease, dermatologic disorders, and solid organ transplantation. The principal cytotoxic mechanism of these agents is generally considered to be mediated via the incorporation of thioguanine nucleotides (TGN) into DNA. Thus, thiopurines, the inactive prodrugs, require metabolism to TGN to exert cytotoxicity. This activation is catalysed by multiple enzymes, the first of which is hypoxanthine phosphoribosyl transferase. Alternatively, these agents can be inactivated via oxidation by xanthine oxidase or methylation by TPMT. In hematopoietic tissues, xanthine oxidase is negligible, leaving TPMT as the only inactivation pathways (Mc Leod *et al*., 2000).

Pharmacogenomics in Veterinary Science

Domestic animal species also served as experimental models for heritable human disease. The animal model for the heritable condition malignant hyperthermia (MH), a hypermetabolic adverse reaction to succinylcholine and volatile anesthetics , was first identified in 3 Landrace cross pig littermates. The Pietrain breed of pigs was found to have a high likelihood of MH susceptibility. Pigs susceptible to MH also have an increased likelihood of developing porcine stress syndrome (PSS), which affects meat quality and appearance pointing to an issue relating to an excitable cell protein. The genes responsible for MH in pigs was subsequently identified as due to a ryanodine receptor 1 (RYR-1) mutation and RYR-1 gene

polymorphisms have since been identified in a range of pig breeds. Following immediately on from this work in pigs, variation in human RYR-1 gene, was identified as a genetic biomarker for human MH.

Ivermectin toxicity was reported in dogs, particularly Collie and herding breeds by 1983. The drug was authorized for use in dogs in 1987 as a heartworm preventative, specific testing had been carried out to ensure that the dosage chosen was safe in what were termed "sensitive" breeds, specifically Collies. The role of P-glycoprotein as a blood-brain barrier "gatekeeper" was discovered as an "incidental finding" in experimental laboratory animals. When ATP binding cassette subfamily B member 1a (ABCB-1a, then named MDR-1a) knockout mice were treated with ivermectin for mite infestation, nearly all of the group died from neurotoxicity. Ivermectin toxicity in dogs was then subsequently identified as a deletion mutation of the ABCB-1 gene encoding P-glycoprotein. Like the Human Genome Project, with the first full genome sequenced in 2001, the dog and cat draft genomes were published in 2005 and 2007, respectively. Domestic cattle (*Bos taurus*) and horse genome sequences were published in 2009.

Like human medicine, substantial progress has also been made in the study of pharmacokinetic variation in animal species. Much of the current scientific understanding of CYP metabolism has been elucidated in humans and rodents, although in recent decades, exploration has commenced in veterinary species. It is important to note that interspecies differences in drug metabolism mean that information derived from human gene-biomarker: probe substance pairs do not automatically extrapolate to veterinary species. The genetics of canine CYP, extensively reviewed in 2013 showed specific similarities but also significant differences from human CYP. There are species differences in gene sequences, and there are clearly identifiable orthologs, the genes derived from common and sometimes ancient ancestral genes. As an example, CYP2D15 is the canine ortholog of human CYP2D6 . Human CYP2D6, whose polymorphisms were first identified with debrisoquine, is considered be involved in the metabolism of over 25% of currently marketed drugs in humans, including antiarrhythmics, adrenoceptor antagonists, and tricyclic antidepressants. Canine CYP2D15 shares many, although not all therapeutic drug substrates with human CYP2D6 but has a greater relative degree of expression in the normal canine the intestine and liver than CYP2D6 in normal human intestine and liver, which explains why drugs which are CYP2D16 substrates have lower oral bioavailability in dogs than in humans.

In addition, equine ortholog for human CYP2D6, the equine CYP2D50 gene, is polymorphic, and effects on drug metabolism have been identified. 126 exonic SNPs have been identified, of which 31 appeared in more than one horse. A subset of the genotyped horses (23 Thoroughbreds, 1Standard bred) were administered the known human CYP2D6 substrate tramadol by nasogastric tube, and subsequent

plasma levels of tramadol were measured, using jugular samples taken over the following 96 hours. Although the number of animals used was very small, the resulting pharmacokinetic data was suggestive of specific CYP2D50 SNPs being associated with slower drug metabolism in horses.

Canine CYP polymorphisms have been identified is Canine CYP2B11, found primarily in Labrador retrievers, Collies, Uraguayan Cimarrons, Silken Windhound, Scottish Deerhounds, Greyhounds, and Welsh Corgis. Canine CYP2B11 is the canine ortholog of the human clinically actionable highly polymorphic pharmacogene CYP2B6, and is considered to act on a number of important substrates including propofol in both species. Other known substrates of CYP2B11 include a range of drugs used frequently in veterinary practice, including atipamezole, diclofenac, ketamine, medetomidine, midazolam, and temazepam and further evaluation of Canine CYP2B11 polymorphisms combined with hepatic microsomal studies may inform the therapeutic use of these drugs in the veterinary clinic. The gene encoding P-glycoprotein in dogs and cats is also named ABCB-1. Experiments in dogs using the sedative drug and P-glycoprotein substrate acepromazine found that those dogs which were homozygous for the mutation showed increased sedative effects to IV acepromazine while dogs which were heterozygous for the mutation did not differ from genetically normal dogs. Canine breeds with a high risk of ABCB-1 mutation, particularly under treatment with vincristine, vinblastine, doxorubicin, loperamide, or a macrocyclic lactone should have pharmacogenetic testing (Campion *et al*., 2019).

Conclusion

Pharmacogenetics and its backbone studies in terms of polymorphisms present new developments and trends in the field of tailored medications and advancements in the modification of therapeutic choices utilizing genotypic information from polymorphism analysis. Pharmacogenetic biomarker studies have multiple processes from discovery to clinical implementation. The ultimate aim of the biomarker studies is to find a clinically accessible decision-maker biomarker to improve patient outcomes. Biomarker discovery studies should be performed as the screening of genotype-phenotype relations in large cohorts with statistical and bioinformatics tools. The most significant markers obtained from discovery studies are replicated for analytical validation in different cohorts with the evaluation of assay reproducibility and robustness. And finally, analytically and clinically validated biomarker assay is ready for implementation phase that includes regulatory approval and incorporation in clinical practice guidelines, commercialization and coverage by health insurance.

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F-PG-O1

ACRAB-TOLCEFFLUX PUMP INHIBITORY ACTION OF PIPERINE ON *ESCHERICHIA COLI* ISOLATES FROM BOVINE MASTITIS MILK SAMPLES: A NOVEL MEASURE TO OVERCOME ANTIMICROBIAL RESISTANCE.

Vanishree, Nisha A.R., Sanis Juliet, Naicy Thomas, Suja Rani S.

Department of Veterinary Pharmacology and Toxicology,

College of Veterinary and Animal Sciences,

Pookode, Kerala

'Antimicrobial resistance' is a global epidemic issue nowadays. In the recent past, role of efflux pumps in development of resistance is intervened so as to overcome bacterial resistance to wide class of chemotherapeutic agents. In the current study an attempt has been made to investigate the ability of 'Piperine', a phytochemical to overcome the efflux pump (AcrAB-TolC) mediated resistance in *E. coli* isolates from bovine mastitis.In the current study antimicrobial activity of Piperine was carried out by antibiotic disc diffusion assay and microdilution method for Enrofloxacin, Ciprofloxacin (fluoroquinolones) andOxytetracycline (Tetracyclines) in the *E. coli* isolates from bovine mastitis. Geneexpression of *acr*A, *acr*B and *tol*C genes were studied by reverse transcription and real time PCR. Biofilm formation was examined in the resistant colonies in the presence of each of the above antibiotics and Piperine by Congo red assay. *In silico* analysis for binding of *acr*Bprotein of efflux pump and Piperine were carried out by using autodocksoftware.In the present study Piperine potentiated the antibacterial activity of the Enrofloxacin, Ciprofloxacin, Tetracycline and Oxytetracycline.The study also revealed Piperine reduced the MIC of Enrofloxacin,Ciprofloxacin and Oxytetracycline against *E.coli* isolates from mastitis and showed synergistic action at a concentration of 100 ppm. Gene expression studies indicated down regulation of expression of *acr*A, *acr*B and *tol C* efflux pump in *E. coli* isolates resistant to Enrofloxacin and Ciprofloxacin inhibited expression of only *acr B*. Relatively Piperine showed more efflux pump inhibitory activity in Ciprofloxacin resistant *E. coli* isolates. *In silico* analysis revealed that Piperine binds with acrB protein with least binding energy. Thus Piperine potentiate the action of Enrofloxacin and Ciprofloxacin by inhibition of AcrAB-TolC efflux pump.

F-PG-O2

AYURGENOMICS – A NOVEL PHARMACOGENETIC APPROACH FOR RESEARCH IN AYURVEDA

Bindya Liz Abraham*

Department of Animal Breeding and Genetics, College of Veterinary and Animal Sciences

Mannuthy, Thrissur, Kerala-680651, *Email: bindya@kvasu.ac.in

The traditional medicine of Ayurveda is nowadays used side by side with contemporary medicine. Ayurveda works on the principles of *Panchbhutha* and *Tridoshas* explaining an individual's *Prakriti* or constitutional type (*Vata, Pitta, Kapha*) determined using physical, psychological, physiological and behavioural traits and supported by biochemical profiles of lipid, liver function, hematocrit and micronutrient levels. *Prakritis* are discreet phenotypes that determine predisposition to diseases and responses to treatment. The science of Ayurgenomics incorporating Genomics into Ayurveda research has become successful in exploring the molecular basis of *Prakriti* through Genome-Wide DNA variations like single nucleotide, insertion-deletion (Indel), block substitution, inversion and copy number or tandem repeats. Single nucleotide polymorphisms (SNP) in genes like *HLA-DRB1*, *CYP2C19* and CD markers for various blood cells have also been associated with *Prakriti* phenotypes. *Prakriti* is also found to be associated with some inflammatory biomarkers like C-reactive protein and macrophage-colony stimulating factor (M-CSF). Genetic markers of an oxygen sensor gene, *EGLN1* and its expression levels have also been found to be correlated with *Pitta Prakriti* natives adapted to complex hypoxic conditions like High Altitude Pulmonary Edema (HAPE). The immunomodulatory Ayurvedic drug, *Indukantha Gritha* working on the principles of *Ojas, Ojabala, Baladosha* and *Vyadhikshamatva* is found to enhance T-cell mediated immunity as revealed through profiling of differentially expressed genes. The Ayurvedic formulation of *Amalaki Rasayana* is found to enhance levels of heterogeneous RNA-binding proteins (*hnRNPs*) while providing neuro-protective effects. Some Ayurvedic drugs are also found to regulate chronic inflammatory conditions (*Shotha*) by modifying epigenetic dysregulations like DNA methylation, histone modification and micro-RNA (*miRNAs*) alterations in target tissues or in immune cells like macrophages. The validation of Ayurvedic formulations in synergy with elucidation of underlying molecular mechanisms using genetics, genomics, cell and molecular biology would be extremely beneficial for the practice of Predictive, Preventive, Personalized and Participatory (P4) stratified medicine

F-PG-O3

PHYLLANTHUS EMBLICA AMELIORATES FATTY LIVER DISEASE IN RATS: MODULATION OF PPAR-α AND NF-κB AS PLAUSIBLE MECHANISMS

Suja R.S.[1], Dhanya, T. [1], Mathew J.[1], Anoop, R.R. [2], Renjith, S.[1], Usha P.T.A.[3] and Sanis, J.[1]

[1]Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala. [2]Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Pookode, Wayanad , Kerala, [3]Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala.

E mail: sujarani@kvasu.ac.in

Fatty liver, the prevalence of which is rising globally in both humans and domestic animals, poses enormous demand for development of potent lipotropic agents, which are safer and easily available. Emerging evidence suggests that liver peroxisome proliferator-activated receptor-alpha (PPARα) is protective against fatty liver disease and directly inhibits inflammatory genes induced by NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells). Hence, the present study was undertaken to evaluate alcoholic, aqueous and hydro-alcoholic extracts of *Phyllanthus emblica* leaves in amelioration of fatty liver in CCl_4 induced fatty liver model and also to explore the effect on modulation of hepatic PPARα and NF-κB gene expressions. The extracts were evaluated at 100 mg/kg b.wt. dose level for a treatment period of 14 days in Wistar albino rats affected with fatty liver. The CCl_4 control rats showed significant increase in serum and hepatic lipids and lipid peroxide (LPO) levels, indicating effective induction of fatty liver, hyperlipidemia and oxidative stress while histopathological examination showed moderate to severe diffused hepatic fatty infiltration. The extracts of *P. emblica,* significantly ameliorated hepatic lipids concentration with a distinct reduction in degree of hepatic steatosis histologically, depicting their lipotropic potential. Furthermore, levels of hepatic LPO and serum cholesterol, ALT, AST and ALP showed a significant attenuation after treatment. The hepatic gene expressions of PPARα and NF-κB in the extracts treated rats were significantly upregulated and downregulated respectively as compared to CCl_4 control rats. Besides, the extracts were found to be safe upto the limit test dose level of 2000 mg/kg body weight in acute oral toxicity study. Therefore, the present findings are indicative of the plausible role of *P. Emblica* leaves in alleviation of fatty liver, acting partly via upregulation of PPARα, a major regulator of hepatic lipid metabolism and downregulation of NF-κB, a key regulator of inflammation.

S-PG-O1

ALTERED INTESTINAL P-GLYCOPROTEIN mRNA EXPRESSION LEVELS IN HIGH FAT DIET, STREPTOZOTOCIN INDUCED DIABETIC RATS AND GLIBENCLAMIDE TREATED DIABETIC RATS

Saranya C[1]., Suraj S.V[1].,Nair S. N[1]., Nisha A.R[1]., Anoop Raj[2]., Muhasin A[3]., Sanjay B.M[1]., Megha M[1]., Mery, S. J[1]., Ranjith D[1]., and Juliet S[1]

[1]Department of Veterinary Pharmacology and Toxicology, [2]Department of Veterinary Pathology, [3] Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala- 673 576.

P-glycoprotein, the most abundantly studied ATP binding cassette (ABC) transporter protein, functioned as biological barrier by pumping out xenobiotic and toxins out of the cell. Rodents havethree P-glycoprotein genes *ABCB1a/mdr1a, ABCB1b/ mdr1b and mdr2. ABCB1a/mdr1a, ABCB1b/ mdr1b* genes functions as drug efflux transporter whereas *mdr2* functions as phospholipid transport protein.The aim of the present study was to find the mRNA expression of P-glycoprotein in high fat diet (HFD) fed, streptozotocin induced type 2 diabetic rats and glibenclamide treated type 2 diabetic rats. Expression study was conducted on twelve adult Sprague Dawley rats of either sex weighing 250-350 grams (g) body weight. These rats were grouped into control, HFD, streptozotocin induced type 2diabetic and glibenclamide treatedstreptozotocin induced type 2 diabetic groups. The animals were sacrificedby anaesthetic overdose on the day 10th and colon part of the intestine was collected, processed as per standard molecular protocols and gene expression study was conducted using real-time quantitative polymerase chain reaction (RT-qPCR).Theresults showed anupregulation of intestinal (colon) *ABCB1a/mdr1a* gene expression by1.13 fold in HFD rats, 6.62 fold in diabetic ratsand1.6 fold in glibenclamide treated diabetic rats when compared to control rats. There was upregulation of intestinal (colon) *ABCB1b/mdr1b* gene expression by 5.55 fold in diabetic rats whereas there was downregulation of *ABCB1b/mdr1b* gene expression by 1.15 fold in HFD rats and1.09 fold down-regulation in glibenclamide treated diabetic rats when compared to control rats. Alterationsof intestinal P-glycoprotein expression under hyperglycaemic condition associated with obesity must be taken into consideration in designing appropriate individual targeted pharmacotherapy.

ISVPT – 2019

TECHNICAL SESSION

PHARMACOKINETICS/ TOXICOKINETICS

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RAPPORTEUR : DR. PREETI BAGRI

College of Veterinary and Animal Sciences, Mannuthy

Pharmacogenomics in the development and validation of indigenous drugs
XIX ISVPT
CVAS MANNUTHY

LP-PK-01

ADVANCES IN TRANSDERMAL DRUG DELIVERY AND PHARMACOKINETICS OF DRUGS

S. K. Bhavsar, J. K. Parmar, B. G. Goswami, K. A. Sadariya, Bhavisa R. Patel and A. M. Thaker

Department of Veterinary Pharmacology and Toxicology,

College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand (Gujarat)

skbhavsar@aau.in

Drug delivery system is used as a medium or carrier for administering a pharmaceutical product to a patient. Limitations of conventional drug delivery system includes requirement of high dose, possibility of first pass metabolism of drugs and risk of over and under-dosing. Novel drug delivery system is a combination of advance technique and new dosage forms which are far better than conventional system. It provides site specific delivery of drug, provide optimum dose at the right time and right location, decreased toxicity, efficient use of expensive drugs and increased efficacy of the drug (Bhagwat & Vaidhya, 2013). The major problem associated with delivery of the drug through the skin is that many of the drugs are not able to cross the skin at the required rate necessary for the therapeutic action. The stratum corneum behaves like a major barrier as it allows only lipophilic and low molecular weight drugs to pass through it and prevent entry of hydrophilic and high molecular weight drugs. It has been reported that only 10–20% of total drug loaded in cream or transdermal patch permeates through the skin. The hypodermic needle can deliver 90–100% of the loaded drug but it is very painful which results in poor patient compliance (Waghule *et al.*,2019). Transdermal drug delivery is becoming increasingly popular in veterinary medicine due to ease of administration, prolonged delivery and avoidance of a first-pass effect. Not all drugs can be applied topically, as size and lipophilicity of a compound determines its potential for transdermal delivery. Furthermore, species and regional (on the body) differences in skin, including thickness of the skin layers, hair type and density, and cutaneous blood flow, suggest that formulations developed for one species (or body region) may have different pharmacokinetics and pharmacodynamics when applied to other species. (Mills, P., 2013)

To overcome limitations of topical cream, transdermal patches, hypodermic needles, the concept of microneedles-based drug delivery was introduced. Microneedles are multiple micron-scale needles attached to a supporting base or patch. It can be used for delivery of vaccines, drugs, biopharmaceuticals, local anesthetics and for collection of blood (Nayak *et al* 2016). Microneedles creates temporary mechanical disruption of the skin and produce micro-channels by piercing into the stratum corneum of skin. The drug is directly placed in the epidermis or upper dermis layer which then goes into the systemic circulation and shows a therapeutic response on reaching the site of action (Henry *et al.*, 1998). Five types of microneedles

have been made like solid, coated, dissolving, hollow and hydrogel forming microneedles. Solid microneedles are arrays of projections that are employed for creating micron scale holes in stratum corneum of skin and the drug delivery occurs through "poke and patch" approach. Coated microneedles are coated with drugs at their tips. And the drug is delivered via "Coat and poke" approach. Dissolving microneedles typically constitute of water-soluble materials which completely dissolve in the skin and thus leave no bio hazardous waste after use. Drug delivery occurs via "poke and release" approach. Hollow microneedles contain a hollow bore in the center of the needle. It's used to inject the drug solutions directly into skin and also to remove the fluid from the body for analysis. Hollow microneedles deliver drugs via "poke and flow" approach. Hydrogel-forming microneedles are made up of a swelling polymeric materials with a drug reservoir attached to the base plate of the array. Upon insertion into the skin, the array absorbs interstitial fluid and swells to form continuous conduits between the dermal microcirculation and patch-type drug reservoir leading to the diffusion of the drug into the skin (Bora and Bansal, 2008; Kumar *et al.*, 2011; Donnelly *et al.*, 2012).

Diphtheria and influenza vaccine given using solid microneedles showed significantly higher antibody titers as compared to vaccination given by subcutaneously in mice (Ding *et al*., 2009). Dissolving microneedle patch produced a strong rabies-specific immune response with no adverse effects following dissolving microneedles patch rabies DNA vaccination in dogs (Arya *et al.*,2016). Lidocaine-coated microneedle can successfully deliver drugs into the skin within seconds and provide rapid onset (~1 min) and long duration of action as compare to conventional creams in swine (Zhang *et al., 2012*)**.** Pharmacokinetics of Ketoprofen was improved following its administration via coated microneedles as compared to transdermal formulations in male rats. (So *et al.*, 2009). Fukushima *et al.* (2010) evaluated pharmacokinetic and pharmacodynamics of insulin dissolving microneedles in dogs. They found dose dependent hypoglycemic effect following administration of two and four patches of insulin dissolving microneedles. Hydrogel-forming microneedle patch treatment produced therapeutic plasma concentration of metformin and the steady-state concentration was achieved within 24 hours as compared to control in rats (Dangol *et al.*, 2017). Microneedle delivery is applicable to a wide variety of protein drugs and it alters absorption kinetics via targeting a tissue bed better perfused with lymphatic and blood vessels than the SC space. Microneedle delivery increase the absorption rate and bioavailability of proteins that have been challenging to deliver at therapeutic levels (Harvey et al., 2011)

Topical application can be extremely useful in veterinary medicine for intractable animals, for large scale applications and to improve owner compliance. Transdermal drug delivery using microneedle is

convenient, painless, biologically non-toxic and less invasive alternative to conventional injection. Significant differences exist between species in the rate and extent of transdermal drug penetration. Microneedles made of metal, stainless steel or silicon have fracture risks and may leave fragments in the skin. Microneedles can cause skin irritation or allergy in some cases. Extensive studies would be required to promote the application of microneedle in the clinical set up in veterinary field.

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F-PK-O1

ORAL PHARMACOKINETICS OF ROXITHROMYCIN IN POULTRY

Singh R.D., Mody S. K., Patel H. B., Sarvaiya V. N. and Patel B. R.

Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry

Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat- 385506

ratn1709@yahoo.com

Roxithromycin is a semi-synthetic, long-acting, orally administered antibacterial drug of macrolide class.The present study was undertaken to study oral pharmacokinetics in eight broiler chickens of Vencobb strain aged from 4 to 6 weeks. Plasma concentrations of roxithromycin were measured by optimized and validated Ultra High Performance Liquid Chromatography (UHPLC) method. The pharmacokinetic parameters were calculated from plasma concentrations*versus*time data by non-compartmental analysis using 'PK Solver 2.0' software.Following oral administration of roxithromycin at the dose rate of 20 mg/kg body weightin broilers, a characteristic short lag phase of 15 minutes in absorption was observed and thereafter mean maximal plasma concentration (C_{max}: 3.60 µg/ml) was achieved at 2 h. The plasma concentration of roxithromycin > 0.5 µg/ml (target MIC for most of common susceptible bacterial infections) was maintained upto 12 h.The mean values of $t_{1/2\beta}$, AUC, MRT, $V_{d(area)}$ and Cl_B were 8.30 h, 25.59 µg·h/ml, 9.57 h and 9.39 L/kgand 0.79 L/h/kg, respectively.

F-PK-O2

ACTIVE IMMUNE SYSTEM AND DRY MATTER INTAKE DURING THE TRANSITION PERIOD ARE ASSOCIATED WITH LACTATION PERFORMANCE IN ZEBU (*BOS INDICUS)* COWS

Pratik Ramesh Wankhade[1], Ayyasamy Manimaran[2*], Arumugam Kumaresan[2], Sakthivel Jeyakumar[2], Kerekoppa P. Ramesha[2], Veerasamy Sejian[3], Duraisamy Rajendran[3], Madiajagan Bagath[3] and Muniandy Sivaram[2]

[1]Research Scholar, Livestock Research Centre (LRC), Southern Regional Station (SRS), ICAR-National Dairy Research Institute (NDRI), Adugodi, Bengaluru-560 030, Karnataka, (India), [2]Scientist, SRS, ICAR-NDRI, Adugodi, Bengaluru-560 030, [3]Scientist, ICAR-National Institute of Animal Nutrition and Physiology (NIANP), Adugodi, Bengaluru - 560 030, Karnataka, (India)

We evaluated the changes in the concentration of innate immune molecules (Haptoglobin: Hp,Serum Amyloid A: SAA, IL-6, TNF-α, IL-1β, and IL-8) and energy indicators [NEFA, Dry Matter Intake (DMI) and Body Condition Scoring (BCS)] during transition period in dual purpose Zebu (Deoni breed) cows in relation to milk yield. Blood collection was done at weekly intervals (-21±2, -14±1, -7±1, d pre-partum, day

0 (date of calving) and 3±1, 7±1, 14±1, 21±2 d postpartum period) for estimation of above plasma variables using commercially available bovine specific ELISA kits. We also recorded DMI and BCS during thecorresponding period. Transition cows were classified based on their milk yield during study period as high (N=6), medium (N=6) and low (N=6) yielding cows and data were analyzed by using Mixed-model repeated measure analysis. We found that high yielding (HY) cows had significantly (P<0.05) higher concentrations of SAA, TNF-α, and IL-6 during pre-partum and early postpartum period than low yielding (LY) cows. DMI was significantly (P< 0.05) higher in HY cows than MY (3^{rd} and 7^{th} d) or LY cows (21^{st} d) while,BCS was significantly (P < 0.05) higher in HY than LY cows during pre-partum period (-7^{th} d). LY cows had significantly (P< 0.05) higher concentration of NEFA during postpartum period (14^{th} and 21^{st}d). It is concluded that active functioning of immune system and more dry matter intake in transition Deoni cows enabled to synthesis more milk during thepostpartum period.

F-PK-O3

DISPOSITION KINETICS OF CEFTRIAXONE IN HEALTHY AND CLINICAL MASTITIC CROSSBRED COWS FOLLOWING SINGLE INTRAVENOUS ADMINISTRATION

Buragohain R., Sar T. K., Mandal T.K., Biswas U. and Samanta I.

Department of Veterinary Pharmacology and Toxicology, West Bengal University of Animal and Fishery Sciences, 37 K. B. Sarani, Kolkata-700037

Mastitis is the most prevalent disease in Indian crossbred cows and its prevalence rate was reported to be more than 90%. Therefore, disposition kinetics of ceftriaxone was conducted in healthy lactating and crossbred cows diagnosed with clinical mastitis following single intravenous administration at 20 mg kg^{-1} body weightin field condition to evaluate the efficacy. The cows were grouped into two groups namely Group I (Healthy) and II (Mastitic) containing 6 animals in each. Blood and milk samples were collected at predetermined time intervals and drug concentration was estimated using HPLC. The pharmacokinetic profile of ceftriaxone followed "One Compartment Model". The mean value of $t_{1/2}\beta$ was significantly increased from0.47±0.002 hrin Group I to1.21±0.04 hrin Group II. The mean cl_{β} values were 0.33±0.005 L kg^{-1} hr^{-1} and 0.16±0.002 L kg^{-1} hr^{-1} in Group I and Group II, respectively. Ceftizoxime, a major active metabolite of ceftriaxone persisted in milk up to 120 hr in Group I (6.08±0.29µg mL^{-1}) and up to 96 hr in Group II (28.32±1.55 µg mL^{-1}). The *staphylococcal* colony count in mastitic cows was 49.33±6.55 × 10^5 c.f.u./mL and lowest colony count was achieved at at 72hr (0.66±0.33× 10^5 c.f.u./mL). No *Staphylococcal* colony in milk was detected after 120 hr of treatment in mastitic cows. So, it may be concluded that single

intravenous injection of ceftriaxone at 20 mg kg^{-1} body weight may be effective in treatment of mastitis in Indian crossbred cows.

S-PK-O1

DISPOSITION KINETICS OF CEFTIZOXIME IN HEALTHY AND CLINICAL MASTITIC CROSSBRED COWS FOLLOWING SINGLE INTRAVENOUS ADMINISTRATION

Buragohain R., Sar T.K., Mandal T.K., Biswas U. andSamanta I.

Department of Veterinary Pharmacology and Toxicology, West Bengal University of Animal and Fishery Sciences, 37 K. B. Sarani, Kolkata-700037

Mastitis is adreadful disease of dairy animals as it adversely affects animal health, milk quality and economics of milk production. The incidence of mastitis has been increased from 20% to 50% in the Indian dairy animals. Therefore, disposition kinetics of ceftizoxime was studied inhealthy lactating and crossbred cows diagnosed with clinical mastitis following single intravenous administrationat20 mg kg^{-1} body weightin field condition to evaluate the efficacy of the drug. Six healthy lactating crossbred cows (Group I) and six clinical mastitic crossbred cows (Group II) were used in the study. Blood and milk samples were collected at predetermined time intervals and drug concentration was estimated using HPLC. Bacterial colony count in milk was performed for efficacy evaluation.The pharmacokinetic profile of ceftizoxime followed "One Compartment Model". The mean value of $t_{1/2}\beta$ was significantly increased from 0.90±0.002hr in Group I to3.09±0.009 hr in Group II. The mean cl_{β} values were 0.36±0.01 $Lkg^{-1}hr^{-1}$ and 0.03±0.001 $Lkg^{-1}hr^{-1}$ in Group I and Group II, respectively. Mean Vd_{area} values were 0.47±0.008 Lkg^{-1} and 0.14±0.001 Lkg^{-1} in Group I and Group II, respectively.Ceftizoxime persisted in milk upto 120 hr in both Group I (9.78±0.46 µg mL^{-1}) and Group II (36.71±0.96 µg mL^{-1}).The *staphylococcal* colony count in Group II was$52.33\pm4.98 \times 10^5$ c.f.u./mL and lowest colony count was achievedat 48 hr($0.67\pm0.66 \times 10^5$ c.f.u./mL). No *staphylococcal* colony in milk was detected after 96hr of ceftizoxime administration in mastitic cows. So, it may be concluded thatsingle intravenous injection of ceftizoxime at 20 mg kg^{-1} body weight may be effective in treatment of mastitis in crossbred cows.

S-PK-O2

PHARMACOKINETICS AND *IN VITRO* PLASMA PROTEIN BINDING OF CEFQUINOME FOLLOWING A SINGLE INTRAMUSCULAR ADMINISTRATION IN BUFFALOES SUFFERING FROM DYSTOCIA

D G Kishor[1], Kaur R, Singh N, Sharma S K, Saini S P S

1. MVSc, Department of Pharmacology and Toxicology, GADVASU, Ludhiana. Mob: 8277307356, Email:dgkishor@gmail.com,

Present affiliation: PhD Scholar, Division of Pharmacology and Toxicology, IVRI, Bareilly, Uttar Pradesh.

Cefquinome is a fourth generation cephalosporin, having broad spectrum of activity against both gram positive and gram negative bacteria, approved for use exclusively in animals throughout the world. Despite the proven efficiency of cefquinome in treating uterine infections post dystocia, pharmacokinetic study of the drug has not been investigated in dystocia so far in buffaloes. The objective of this study was to investigate the disposition kinetics and to compute the dosage regimen of cefquinome in buffaloes suffering from dystocia following its single intramuscular (1 $mg.kg^{-1}$ bodyweight) administration. Blood samples were collected prior to drug administration and up to 24 hr after injection. Plasma concentrations of cefquinome were estimated by HPLC. Pharmacokinetics of cefquinome in buffaloes suffering from dystocia was determined by one-compartment open model, the important pharmacokinetic parameters analyzed were: Cmax 3.06 ± 0.08 $\mu g.m^{-1}$, tmax 1 h, Ka 5.85 ± 0.56 h^{-1}, $t_{½Ka}$ 0.128 ± 0.01 h, AUC 10.19 ± 0.30 $\mu g.ml^{-1}.h$, AUMC 23.90 ± 0.84 $\mu g.ml^{-1}.h^2$, $Vd_{(area)}$ 0.13 ± 0.02 $L.kg^{-1}$, $t_{1/2\beta}$ 1.095 ± 0.011 h, Cl_B 97.12 ± 2.24 $ml.kg^{-1}.h^{-1}$, MRT 2.38 ± 0.01 h and td 5.41 ± 0.05 h and binding of cefquinome to plasma proteins was 15.21 ± 0.63%. The dosage regimen of cefquinome to maintain a MIC above that reported in major uterine pathogens (> 0.125 $\mu g.ml^{-1}$) was optimised to be 2.3 $mg.kg^{-1}$, to be repeated at 8 hourly interval by intramuscular route in buffaloes suffering from dystocia.

ISVPT – 2019

TECHNICAL SESSION

ETHNOPHARMACOLOGY

CHAIRPERSON : DR. SURESH KUMAR SHARMA

CO-CHAIRPERSON: DR. TAPAS KUMAR SAR

RAPPORTEUR : DR. ARCHANA LOHIYA

College of Veterinary and Animal Sciences, Mannuthy

Pharmacogenomics in the development and validation of indigenous drugs
XIX ISVPT
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LP-EP-01

HORMETIC APPROACH TO ETHNOPHARMACOLOGY: LEADS FROM ETHNOVETERINARY HERBAL-CLINICAL EXPERIENCE IN INDIA

N.Punniamurthy PhD FISVPT FNSE

Former Prof & Head, Ethnoveterinary Herbal Res. Centre, TANUVAS

Prof. Emeritus, Trans-Disciplinary University, Bangalore.

Hormesis, is a 'biphasic-dose–response' (of a biological system) to an environmental agent characterized by a 'low- dose-stimulation' (beneficial effect) and a 'high-dose-inhibition' (toxic effect). The enormity and diversity of hormetic responses across the plant, microbe and animal kingdoms are well known. Hormesis, is often produced in response to stimulatory processes and across all forms of life; it suggests that its origins are evolutionary and highly conserved. Responses to hormetic challenges are coordinated across multiple organ systems, involving both cell autonomous molecular mechanisms, and signals transmitted between different tissues.

The central idea of the pharmacologists/researchers working with phytochemical components in health is that, the beneficial effects of phytochemicals are due to their intrinsic antioxidant properties, as oxidative stress plays a key role in most chronic diseases. However to achieve such antioxidant capacity in the blood requires micromolar concentrations of the phytochemicals, requiring fruits/vegetables in greater orders of magnitude than we normally consume. Hence, it is suggested that 'neurohormetic-phytochemicals' by plants have beneficial effects on animals/humans when consumed in moderate amounts.

The familiar example of hormetic response for veterinarians, is 'vitamin A' in low amounts essential for normal development and eye function, but in high amounts can cause anorexia, headaches and other symptoms (hyper vitaminosis- A).

The author's own experience in treating many thousands of livestock across India (through TANUVAS-TDU-NDDB-Glohmsiwa efforts) in the last three years against 14 clinical conditions with over 80 % success under clinical conditions may well augur for the scientific community in general and pharmacologists in particular to work on ethnopharmacology with hormesis as the starting point.

The general belief of the pharmacologists working with phytochemical components in health, is that the beneficial effects of phytochemicals are due to antioxidant properties. However to achieve such antioxidant capacity in the blood requires micromolar concentrations of the phytochemicals, which would require fruits and vegetables in greater orders of magnitude. Alternatively, lower amounts of some

phytochemicals may exert disease preventive and therapeutic effects by activation of adaptive cellular stress responses. Phytochemicals can induce the expression of endogenous antioxidant enzymes and/or redox enzymes.

Plants / animals and their co-evolution

Interactions of plants and animals during their co-evolution, and resulting reciprocal adaptations, have shaped the remarkable characteristics of phytochemicals and their effects on the physiology of animal cells in general, and neurons in particular. Survival advantages were conferred upon plants capable of producing noxious bitter-tasting chemicals, and on animals able to tolerate the phytochemicals and consume the plants as an energy source.

Hormesis is viewed in the light of evolutionary-based adaptive responses, as a measure of performance and resilience of cell proliferation, fecundity, cell and tissue repair, disease resistance, behavioral/cognitive endpoints, aging/longevity and others that are fundamental for survival and thriving in challenging environments. The fact that hormesis is often produced in response to stimulatory processes and across all forms of life strongly suggests that its origins are evolutionary and highly conserved.

The most familiar example of hormetic response for veterinarians is of 'vitamin A' which in low amounts is essential for normal development and eye function, but in high amounts can cause anorexia, headaches (hyper vitaminosis- A).

Although some phytochemicals possess direct free radical-scavenging properties at high concentrations, in lower amounts typical of those obtained in the diet, phytochemicals may activate one or more adaptive cellular stress responses pathways. Activation of such hormetic pathways in neurons results in the production of several types of cytoprotective proteins including neurotrophic factors, protein chaperones, antioxidant and phase II enzymes and anti-apoptotic proteins. One specific pathway that is receiving considerable attention in regards to hormesis in the nervous system involves the transcription factor Nrf2 which binds the ARE, thereby inducing the expression of genes encoding phase II detoxifying enzymes.

Curcumin exert neuroprotective actions in animal and cell culture models

Many phytochemicals such as curcumin have been shown to exert neuroprotective actions in animal and cell culture models of neurological disorders, but in most cases their mechanism of action is unknown a more compelling case can be made for a hormetic mechanism of action of health-promoting phytochemicals.

Hormesis is a holistic response and may get inherited

Hormesis is also characterized by the simultaneous stimulation of many independent cellular functions/endpoints—each with its own set of quantitatively hormetic features (such as enhancements of

DNA repair, antioxidant defenses, autophagy, and others)—whose actions are regulated by multiple interacting receptor/signaling pathways that ultimately produce a metabolically integrated and coherent cellular response. In other words, hormesis is a coordinated response of cells and organisms to an imposed or intrinsically generated challenge that involves multiple integrative signal-transduction processes, each of which is quantitatively hormetic, to coordinate a final holistic response.

Interestingly, a case for extending the resilient phenotype transgenerationally via hormetic mechanisms has been reported using *C. elegans* that hormetic effects induced in the parental generation can be inherited. When the parental *C. elegans* were exposed to a wide range of stressors during developmental stages enhanced resistance to both oxidative stress and proteotoxicity was observed. These adaptations were passed on to subsequent generations via epigenetic mechanisms even when grown in unstressed conditions. These findings reveal a cross-generational communication strategy, which provides the offspring with survival advantages for dealing within a range environmental change.

Reciprocal evolutionary changes during the co-evolution of plants and animals

One type of evolutionary adaptation of plants is physical 'armor' such as hard exposed surfaces and thorns that prevent consumption by animals. A second defense mechanism of plants is the production of noxious phytochemicals that are toxic to animal cells. Animals, in turn, developed novel mechanisms to protect cells against damage by phytochemicals and mechanisms to detoxify the phytochemicals.

Diversification of phytochemical synthesis by plants has driven the evolution of the CYP450 super family of phytochemical-metabolizing enzymes in animals which, in mammals, are concentrated in the liver. More recently evolved plants, produce phytochemicals that when metabolized by CYP450 enzymes generate toxic metabolites. To counteract these phytochemicals, animals have developed the so called "phase 3 transporter proteins" such as the P-glycoprotein or multidrug resistant (MDR) pumps and organic ion transporters.

Ethno Veterinary Medicine (EVM) to rationalize antibiotic usage

EVM is used to manage acute mastitis, sans antibiotics. The EVM treatment for acute mastitis and other conditions are used widely across India as per the ratio and procedure formulated by Punniamurthy (2009). Antimicrobial activity of EVM preparation and probable mode of action for his mastitis formula have also been reported (Punniamurthy et al, 2017a, b).

Mastitis control: a sustainable model for the developing world

The NDDB, India has published booklets on "Ethnoveterinary Formulations for Important Ailments in Bovines" for 14 clinical conditions in 12 languages.(DKP-NDDB 2018) EVM is cost

effective/efficacious and has helped in significantly reducing the use of antibiotics (Punniamurthy2009, Punniamurthy et al 2017a 2017b Nair et al 2017).

A total of 48,469 of acute mastitis cases in 24 milk unions (1500 societies) were treated using EVM alone 78% (38,045) showed complete clinical recovery. When SOPs were followed judiciously, the success rates were above 90% (Rana et al 2019).

Hormetic-approach may well be the starting point in Ethnopharmacology

The author's own experience in treating many thousands of livestock across India (through TANUVAS-TDU-NDDB-Glohmsiwa efforts) in the last three years against 14 clinical conditions with over 80 % success under clinical conditions may well augur for the scientific community in general and pharmacologists in particular to work on ethnopharmacology with hormesis as the starting point.

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LP-EP-02

ANTHELMINTIC ACTIVITY OF PLANTS IN HAEMONCHOSIS

K.P. Mini

Professor and Head Department of Veterinary Pharmacology and Toxicology

Rajiv Gandhi Institute of Veterinary Education and Research, Pondicherry-605 009, INDIA Cell. 919489585699, e-mail: minikpm@gmail.com

INTRODUCTION

Haemonchus is the highly pathogenic nematode parasite of livestock, especially small ruminants capable of causing acute disease and high mortality (Soulsby, 2006;). Haemonchosis is characterized by haemorrhagic anaemia due to the blood sucking activities of the worms in the abomasum. This is one of the most important parasites causing economic loss in livestock production throughout the tropics and in most countries. Over the past five decades, intensive chemo prophylaxis using anthelmintic drugs has lead to the emergence of resistance towards broad spectrum anthelmintics worldwide.

The necessity of anthelmintics with different modes of action has paved the way for screening of medicinal plants for anthelmintic activity. From time immemorial, plant derivatives like *Nicotiana tabaccum* have been used against *Moneizia*, *Ascaridia* and several species of GI nematodes including *Cooperia*, *Haemonchus*, Nematodirus *and Trichostrongylus*. Arecoline and several other alkaloids from the dried ripe seeds of *Areca catechu* were found to be active against tapeworms in dogs and poultry. Plants of the genus Artemisia were used against the nematodes *Ascaris suum* and *Toxocara* spp. as well as cestodes of poultry.

PLANTS WITH ANTHELMINTIC ACTIVITY AGAINST *HAEMONCHUS*

Calotropis:

Anthelmintic property of *Calotropis procera* latex was investigated in experimentally induced *H. contortus* infection in najdi sheep where there was reduction in the number of abomasal parasites. In the same study concentration dependant *in vitro* larvicidal activity was also demonstrated (Al-Qarawi *et al.*, 2001). Iqbal *et al.*(2005) studied the anthelmintic activity of *C.procera* flowers in comparison with levamisole through *in vivo* and *in vitro* methods and observed that, though the *in vitro* methods showed good anthelmintic activity,*in vivo* only the aqueous extract and crude powder were effective which was also not significant compared to that of positive control, levamisole.

Cucurbita:

The anthelmintic effect of *C. maxima* seed powder was evaluated against *H. Contortus* by Aswinikumar (1999) and found that it could cause effective reduction in EPG in sheep. The in vitro

anthelmintic activity of *C.Mexicana* was analysed for its antiparasitic effect against *H. contortus* adult worms by Iqbal *et al.* (2001) in Pakistan and the results showed that *Cucurbita* possessed good wormicidal effect.

Punica:

The alcoholic extract of *Punica granatum* showed anthelmintic activity as revealed by a dose dependant inhibition of transformation of *H. contortus* eggs to larvae (Prakash *et al.*, 1980). Therapeutic efficacy of *P.granatum* against clinical cases of nematodiasis in calves had been documented by Pradhan *et al.*(1992). Aswinikumar (1999) conducted studies on the anthelmintic effect of *P.granatum* fruit rind, stem, root powders against *H. contortus* and found that all the components used were effective in reducing EPG in sheep.Mali and Mehta (2008) also reported on the efficacy of alcoholic extract of stem bark of *P. granatum Linn* in inhibiting transformation of *H. contortus* eggs to larvae and also suggested that the stem bark contains an alkaloid pelletierine.

Fumaria:

Akhtar and Javed (1985) found *Fumaria parviflora* to have anthelmintic activity against *Trichostrongylus,Haemonchus,* and *Trichuris* nematodes in sheep through their studies. Hordegen *et al.* (2003) conducted an *in vivo* study on the efficacy of *F.parviflora* in artificially infected lambs and observed a reduction in FEC. The whole plant extract of *F. parviflora* was subjected to both *in vitro* and *in vivo* tests for anthelmintic activity against nematodes of sheep namely,*H. contortus* and *Strongyloides papillosus* by Al-Shaibani *et al.* (2009) and it was found that the plant extracts had anthelmintic activity.

Artemisia:

Iqbal *et al.* (2004) carried out both *in vitro* and *invivo* studies on the anthelmintic effect of whole plant of *A.brevifolia* against *Haemonchus contortus* and mixed species of GI nematodes in sheep and observed that the plant possessed anthelmintic activity against them.Tariq *et al.* (2009) evaluated the anthelmintic efficacy of aerial parts of *A.absinthium* against GI nematodes of sheep and found that the extracts produced results, which were comparable to albendazole both in *in vitro* and *in vivo* assays.

Tannin Containing Plants:

Alonso-Diaz *et al.*(2008) through their studies on the *in vitro* larval migration and kinetics of exsheathment of *H. contortus* larvae exposed to extracts of four tropical tanniferous plants namely *Acacia*

pennatula, Lysiloma latisiliquum, Piscidiapiscipula and *Leucaena leucocephala* revealed that the larval migration inhibition assay (LMIA) showed a dose-dependent anthelmintic effect for all the three plants except *P.piscipula,*eventhough all plants interfered with the process of L_3exsheathment.

Montellano *et al.* (2010) studied the effect of a tropical tannin-rich plant *L.latisiliquum* on adult populations of *H. contortus* in sheep and suggested that a short term consumption of this legume could modulate directly the biology of adult *H. contortus* affecting the worm size and female faecundity. Alonso-Diaz *et al.*(2011) conducted a study on the tropical tannin rich plant extracts of *A.gaumeri, Brosimumalicastrum, H. albicans,* and *Leucaena leucocephala* against *H.contortus* infective larvae and their results showed that tannin rich plant extracts were more potent inhibitors of the exsheathment *of H. contortus* L_3 larvae than their motility.

Miscellaneous Plants:

Carvalho *et al.* (2011) studied the anthelmintic effects of plant extracts from *Piper tuberculatum, Lippiasidoides, Mentha piperita, Huracrepitans* and *Carapaguianensis* through *in vitro* and *in vivo* methods and suggested that the extracts of *P. tuberculatum*, *L. sidoides* and *M. piperita* were found to be effective *in vitro* against *H.contortus.* The *in vitro* anthelmintic activity of crude extracts of five medicinal plants (*Senna occidentalis, Leonotisocymifolia, Leucas martinicensis, Rumexabyssinicus* and *Albiziaschmperiana*) were tested by Eguale *et al.*(2011) to determine the possible anthelmintic activity against *H. contortus* by *in vitro* means and it was opined that all the plants had potential activity.

The study by Hernandez-Villegas *et al.* (2011) evaluated the leaf extracts derived from *Phytolacca icosandra* against infective L_3 larvae and eggs from *H. contortus* collected from sheep and the results revealed that the ethanolic and dichloromethane extracts possessed clear *in vitro* anthelmintic activity.

The aqueous, ethanol and chloroform extracts of *Aristolochia indica* at 100 mg/ml produced 90, 70 and 64.69% inhibition respectively, in egg hatch assay of *H. Contortus*. The aqueous, ethanolic and chloroform extracts of *Aristolochia bracteolata* at 100 mg/ml produced 80, 69, and 56% inhibition in egg hatch, respectively (Mini, 2012). Aqueous and ethanolic extracts of *A. indica* were most effective, which produced larval development inhibition of 60.20 and 50.83% at 100 mg/ml dose, and was found to be higher than that of fenbendazole at 1µg/ml, however, aqueous extract of *A. bracteolata* was more effective compared to its other extracts (Mini et al., 2015).

Mini (2012) showed that the L_3 paralytic activity on *Haemonchus contortus* was consistently above 90% in aqueous, ethanol and chloroform extracts of *A. indica* at 100mg/ml. On the other hand chloroform

extract of *A. bracteolata* produced maximum larval paralytic activity (96%), whereas, aqueous, acetone and ethanol extracts maintained consistency with 80% efficacy.

Mini et al. (2013,) showed that motility of adult worms was first suppressed by *A. indica* acetone extract followed by its chloroform extract. However, other extracts caused paralysis variably in the range of 120 to 175 min (*A. indica* aqueous and ethanol extracts, *A. bracteolata* chloroform and aqueous extracts)

Mini (2017) has used scanning electron microscopy after subjecting the adult *Haemonchus contortus* worms to 100 mg/ml concentration of the aqueous, ethanolic, acetone and chloroform extracts of *A. indica*, *A. bracteolata* and it was found that the extracts induced cuticular damages similar to the standard drugs.

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F-EP-O1

IMMUNOMODULATORY EFFECT OF *KAEMPFERIA ROTUNDA* AGAINST CYCLOPHOSPHAMIDE INDUCED IMMUNE SUPPRESSION IN SWISS ALBINO MICE

Bibu J. K.[*], Devi, A. R[**], MiniRaj. N.[**]

Department of Veterinary Pharmacology & Toxicology, CVAS, Pookode, KVASU[*]

Department of Plantation crops & Spices, COH, KAU, Vellanikkara[**]

Email id - bibujohn@kvasu.ac.in

Kaempferia rotunda or Indian crocus is a fragrant aromatic herb, distributed throughout India. This plant is considered as an important medicinal plant in the ancient system of traditional medicine in India and Indonesia against abdominal pain, wounds, diarrhoea and colic disorder. The present study was carried out to investigate the immunomodulatory effect of ethanolic extract of the rhizome of *K. rotunda* (Zingiberaceae) in cyclophosphamide induced immune suppression in Swiss albino mice. Immunomodulatory status was assessed by physiological, hematological, biochemical and immunological parameters The animals were randomly divided into two sets comprising 48 animals each. In each set, there were four groups *viz.* vehicle control group, cyclophosphamide alone treated group, ethanolic extract of rhizome of *K. rotunda* alone without cyclophosphamide treated group and ethanolic extract of rhizome of *K. rotunda* with cyclophosphamide treated group. The weight of organs like liver and spleen was recorded at the time of sacrifice. Total leukocyte count was significantly higher in *K. rotunda* alone treated group on 12th day while on 19th day *K. rotunda* treated cyclophosphamide immunosuppressed mice showed significantly higher leukocyte count. In lymphocyte and monocyte count, significantly higher value were observed in both *K. rotunda* alone treated as well as *K. rotunda* treated cyclophosphamide immunosuppressed mice. Haemagglutination test conducted for the evaluation of humoral immune response showed that both *K. rotunda* alone and *K. rotunda* treated immunosuppressed mice showed significant increase in titre value compared with cyclophosphamide control. Bone marrow cellularity test performed for evaluation of cellular immune response showed cyclophosphamide control group with significant lower bone marrow cellularity on 12th and 19th day while *K. rotunda* alone treated and *K. rotunda* treated immunosuppressed mice showed a significant increase in the bone marrow cellularity. The result of histopathology of spleen revealed that *K. rotunda* induced hyperplasia of lymphocyte in white and red pulps while *K. rotunda* treated cyclophosphamide immunosuppressed mice showed proliferation of lymphocytes in white pulp and mild depletion in the marginal zone at 12th day. GCMS profiling of *K. rotunda* extract showed the presence of 18

compounds. The study concluded that the administration of ethanolic extract of *K. rotunda* rhizome enhanced the immune response in *in vivo* experiment in cyclophosphamide immunosuppressed Swiss albino mice.

F-EP-O2

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF CINNAMON OIL IN MALE WISTAR RATS

Prajapati, J.A., Humbal, B.R., Sadariya, K. A., Bhavsar, S.K. and Thaker, A. M.

Department of Veterinary Pharmacology & Toxicology

College of Veterinary Science and Animal Husbandry

AAU, Anand - 388 001, Gujarat, INDIA. aswinthaker@gmail.com

The present study was planned to evaluate *in vivo* anti-inflammatory activity of cinnamon oil (*Cinnamomum zeylanicum*) following single dose oral administration (50, 100 and 200 mg/kg) using carrageenan induced paw edema model in male wistar rats. All rats were injected subcutaneously with 0.1 ml of a 10% w/v carrageenan suspension (0.1 ml of a 1% suspension in 10% saline) in the sub-planter region of the left hind limb as a local acute oedema inducer after 30 minutes subsequent to oral administration of clove oil. Indomethacin was administared @ 10 mg/kg in standard drug control rats. Rats of control groups were kept untreated and other groups were treated with cinnamon oil @ 50, 100 and 200 mg/kg b.wt., respectively. Volume of edematous paw will be measured by using plethysmometer (PLM-01 plus, Orchid) at 0 hr (before treatment), 1, 2, 4, 6, 12 and 24 hours after treatments. Increase in paw thickness and per cent inhibition was calculated. The anti-inflammatory effect of cinnamon oil was highest at 3h (30.58%) at the dose of 200 mg/kg. It was lower than anti-inflammatory effect of standard drug indomethacin at 3h (42.99%). Cinnamon oil showed dose dependent anti-inflammatory activity in wistar rats.

F-EP-O3

HEPATOPROTECTIVE AND ANTIOXIDANT POTENTIAL OF MALABAR TAMARIND (*Garcinia gummi-gutta*) FRUIT RIND EXTRACT IN ACETAMINOPHEN INDUCED TOXICITY IN RATS

Rehna,A[1*] Deepa, A.K.[2], Preethy John[3] and Anu, G.[4]

[1]Veterinary Surgeon, Cochin Municipal Corportion, [2] Assistant Professor, Department of Veterinary Pharmacology &Toxicology, College of Veterinary & Animal Sciences, Pookot, Wayanad, [3]Assistant Professor, Department of Veterinary Pharmacology &Toxicology, College of Veterinary & Animal Sciences, Mannuthy, Thrissur.,

[4]Veterinary Surgeon, Veterinary Dispensary, Thozhannor

Email ID: avrehna@gmail.com

The present study was aimed to evaluate the antioxidant and hepatoprotective effect of ethanolic extract of Malabar tamarind (*G. gummi-gutta*) fruit rind on acetaminophen induced toxicity in rats and to the compare the level of expression of *CYP2E1* gene in liver. Ethanolic extract of *G. gummi-gutta* fruit rind (50,100 and 200 mg/kg orally) were given for 10 days and hepatotoxicity was induced by single oral administration of acetaminophen at a dose rate of 2 g/kg body weight on day 8 of the study. Silymarin (100 mg/kg body weight) was used as reference standard. On the 10^{th} day, animals were sacrificed and the level of liver function markers (ALT, AST, ALP) in blood serum, the level of lipid peroxidation and the activity of hepatic antioxidants (SOD,GSH) in liver homogenate along with histological assessment of hepatic tissue sections were carried out. Furthermore molecular mechanism behind the protective effect was explored by RT-qPCR technique by assessing the expression level of *CYP2E1* gene in liver. Results revealed that fruit rind extract produced significant ($P<0.001$) dose-dependent hepatoprotective effect against acetaminophen-induced hepatic damage by improving the serum biochemical profile and tissue antioxidant activity towards the normal range. Histopathology of the liver tissue showed that pretreatment with fruit rind extract attenuated the toxicant-induced hepatocellular necrosis and enhanced regeneration of hepatic cells. The expression level of *CYP2E1* gene in liver was found to be significantly ($P<0.001$) down regulated in the liver of extract-treated group in a dose dependent manner when compared with that of control group. It could be concluded that the dried fruit rind of *G. gummi-gutta* possess hepatoprotective effect in acetaminophen induced toxicity in rats through modulation of *CYP2E1* gene expression and antioxidant capacity in liver.

F-EP-O4

ALTERATIONS IN BLOOD GLUCOSE, HbA1C AND OXIDATIVE STRESS INDICES IN DIABETIC CARDIOMYOPATHY WISTAR RAT MODEL: COMPARATIVE AMELIORATIVE EFFICACY OF *GYMNEMA SYLVESTRE,* ITK FORMULATION AND METFORMIN

Patil Chandan, Rajput Priyanka, Pathak Abhishek, Prakash Atul, Mandil Rajesh and Garg Satish K.

Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, U.P. Pandit Deen Dayal Upadhyaya Pashu-Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, (DUVASU), Mathura – 281001 email id : dratul2510@rediffmail.com

Type-2 diabetes mellitus (T2DM) is a common metabolic disease in canines and felines and has similarities with diabetes in human. The objective of the study was to evaluate the comparative ameliorative potential of hot methanolic extracts of *Gymnema sylvestre* (GSME) leaves, hot aqueous extract of Indian

traditional knowledge (ITK) based formulation and compare with metformin in streptozotocin-induced type-2 diabetes mellitus obese rat model. Forty two male Wistar rats were divided into seven groups of six animals each (healthy and obese controls, obese-diabetic, obese-diabetic+metformin, obese-diabetic+GSME, obese-diabetic+ ITK and obese-diabetic+GSME+ITK). Experimental type-2 diabetes was induced by streptozotocin (35 mg/kg body weight, intraperitoneal). GSME, ITK and metformin were administered @ 400 mg/kg, 445 mg/kg and 50 mg/kg body weight, respectively by oral gavage continuously for 60 days. Significant increase in fasting blood glucose on 3rd, 15th, 30th, 45th and 60th day and per cent HbA1C were recorded on 60th day in rats of obese-diabetic group. Significant increase in lipid-peroxidation (LPO), decrease in reduced glutathione (GSH) level, decreased activities of catalase (CAT), superoxide dismutase (SOD), glutathione-S-transferase (GST) and glutathione peroxidase (GP_X) in heart tissues were observed. Histopathology of heart tissues revealed branching and anastomosing of cardiac myocytes, myocardial degeneration, necrosis, swelling and oedema, infiltration of inflammatory cells, coagulative necrosis of cardiac muscle fibres along with separation and disruption of cardiac muscle fibres. Treatment with metformin and extracts lowered blood glucose and per cent HbA1C. Reduction in blood glucose with ITK was comparable to metformin. GSME+ITK combination was effective in significantly to moderately reversing the hyperglycemia-induced oxidative stress and changes in heart architecture. Thus our ITK formulation seems to hold very promising potential against diabetes just like metformin.

F-EP-O5

EFFECT OF *Anthocephalus cadamba* AGAINST INDUCED OXIDATIVE STRESS BY SUB-ACUTE FIPRONIL TOXICITY IN RATS.

Singh Devendra, Anand Shweta, Sharma Pratistha, Swarnkar Rahul, Choudhary Abhishek, Boghia Sunil.
College of Veterinary and Animal Science, Navania, Udaipur, RAJUVAS, Bikaner, Rajasthan
E-mail: dr.devenchoudhary@gmail.com

The present study was conducted to evaluate the antioxidant potential of *Anthocephalus cadamba* (kadamba) against induced fipronil toxicity in rats. Rats of 100-250g were divided in four groups of six animals each; Group I served as control and received only corn oil. In Group II fipronil @ 10 mg/kg body weight was administered and in group III fipronil along with aqueous extract of *A cadamba* leaves @ 300/kg body weight were administered for 28 days. In Group IV *Anthocephalus cadamba* leaves aqueous extract @ 300 mg per kg body weight was administered. Lipid peroxidation, reduced glutathione, superoxide dismutase, catalase and glutathione reductase levels were estimated. Lipid peroxidation was significantly increased while reduced glutathione, superoxide dismutase, catalase and glutathione reductase levels were

significantly decreased in group II and III compared to control and group IV. There was significant decrease in lipid peroxidation level and increase in levels of reduced glutathione, superoxide dismutase, catalase and glutathione reductase in group III compared to group II. The study suggested that sub acute exposure of Fipronil in rats causes oxidative stress which can be ameliorated with administration of *Anthocephalus cadamba* extract @ 300mg/kg body weight orally.

F-EP-O6

EVALUATION OF HEPATOPROTECTIVE EFFICACY OF *PLATYCLADUS ORIENTALIS* IN PARACETAMOL TREATED RATS.

Saklani, S.[1] and Singh, S.P[-2]

1 MVScThesis work, 2 Prof & Head, Department of Veterinary Pharmacology & Toxicology,

CVASc, Pantnagar-263145

Hepatotoxicity refers to chemical or drug driven hepatic injury in man and animals. This study was to conducted with the objective to evaluate the hepatoprotective and antioxidant potential of hydroethanolic extract of (HEPO) *Platycladus orientalis* following its oral administration @ 200mg/kg and 400mg/kg b.wt. in paracetamol @500mg/kg b.wt treated rats and also comparing its efficacy with standard hepatoprotective drug silymarin @ 100mg/kg b.wt for 21days. Thirty rats were equally divided into 5 groups with 6 rats in each group. Group I served as control, group II rats were administered with PCM, group III with PCM @ 500mg/ kg b. wt. plus silymarin @100 mg/kg, group IV with PCM @ 500mg/ kg b. wt. plus HEPO @ 200mg/kg and group V with PCM @ 500mg/ kg b wt. plus HEPO @ 400mg/kg b. wt. for 21 days . Standard and widely accepted methodology was employed for the assessment of various parameters. The protocol for experimentation was approved by IAEC. There was no noticeable change in the appearance, behaviour and body weight in rats of all groups after 21 days. A significant ($P<0.05$) increase in values of, AST, ALT, ALP, bilirubin was observed in paracetamol treated group II, which were restored by HEPO towards normal in HEPO treated groups. Histopathological changes in liver from paracetamol treated groups were characterized by severe degree of congestion of central vein and sinusoids, severe degeneration and necrosis of hepatocytes which were of low and mild intensity in HEPO treated groups. Thus, it is concluded from the present study that hydroethanolic extracts of *P. orientalis* (HEPO) at daily oral dose @ 200 & 400 mg/ kg b. wt. produced hepatoprotective potential against paracetamol daily oral dose @ 500 mg/kg b. wt. induced hepatotoxicity toxicity after 21 days treatment in rats.

F-EP-O7

AMELIORATIVE EFFECT ON HAEMATOBIOCHEMICAL PROFILE FOLLOWING TREATMENT WITH *PLATYCLADUS ORIENTALIS* IN PARACETAMOL IN TOXICATED RATS

Saklani, S., Singh, S.P.*, Singh, Ahmad, A.H and Pant, Disha

Department of Veterinary Pharmacology & Toxicology, C.V.A.Sc., Pantnagar-263145.

The aim of this study was to evaluate the haematobiochemical parameters following oral administration of hydroethanolic extract of (HEPO) *Platycladus orientalis* @ 200mg/kg and 400mg/kg b.wt. in paracetamol @500mg/kg b.wt treated rats for 21days. Standard and widely accepted methodology was used for the determination of of various parameters. The protocol for experimentation was approved by IAEC. Five groups with 6 rats in each group were used n this study. Group I served as control, group II rats were administered with PCM, group III with PCM @ 500mg/ kg b. wt. plus silymarin @100 mg/kg, group IV with PCM @ 500mg/ kg b. wt. plus HEPO @ 200mg/kg and group V with PCM @ 500mg/ kg b wt. plus HEPO @ 400mg/kg b. wt. for 21 days. Paracetamol caused significant ($P<0.5$) reduction in Hb, PCV, TEC and TLC as compared to control group. HEPO group IV and group V showed significant ($P<0.5$) increase in these parameters in dose dependent manner. A significant ($P<0.05$) decline in total protein, albumin and globulin was observed in group II as compared to control. HEPO treated groups IV and V showed significant ($P<0.05$) amelioration in the level of total proteins as compared to group II and at par with silymarin. A significant ($P<0.05$) increase in values of triglycerides, cholesterol, creatinine, BUN, bilirubin was observed in paracetamol treated group II, which were restored by HEPO towards normal. Thus, it is concluded from the present study that hydroethanolic extracts of *P. orientalis* (HEPO) at daily oral dose @ 200 & 400 mg/ kg b. wt. produced ameliorative effects on haematobiochemical parameters against paracetamol daily oral dose @ 500 mg/kg b. wt. after 21 days treatment in rats.

F-EP-O8

PROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF *Tribulus terrestris* (NJERINJIL) IN CYCLOPHOSPHAMIDE TOXICITY ON REPRODUCTIVE SYSTEM OF MALE RATS

***Karthika P R[1], Sujith S[2], Pramod S[1], Usha P T A[3]**

[1]Veterinary Surgeon, Animal Husbandry Department, Kerala, [2]Assistant Professor, [3]Professor and Head, Department of Veterinary Pharmacology & Toxicology, College of Veterinary & Animal Sciences, Mannuthy – 680651

[*]Corresponding author: karthikapr.pr@gmail.com

The protective effect of *Tribulus terrestris* (Njerinjil) was evaluated in cyclophosphamide toxicity on reproductive system of male rats. Forty adult male Wistar rats were divided into five groups of eight

animals each. Group I served as normal control, while groups II, III, IV and V received cyclophosphamide orally at the rate of 15 mg/kg twice weekly for 30 days. Groups III, IV and V were supplemented with ethanolic extract of *T. terrestris* daily at the dose rates of 100, 250 and 500 mg/kg respectively orally. Body weight of all the animals was recorded on days 0, 15 and 30. Qualitative phytochemical analysis of the extract was performed to determine the active components. At the end of the experiment, all the animals were sacrificed. Testes weight, size and volume, semen parameters like sperm motility, count and morphology were estimated. Antioxidant assay of superoxide dismutase(SOD), reduced glutathione (GSH), lipid peroxidation (LPO) and functional marker enzyme levels such as acid phosphatise (ACP), alkaline phosphatise (ALP), lactate dehydrogenase (LDH) and sorbitol dehydrogenase (SDH) in the testes and epididymis were evaluated. Representative samples of testes, seminal vesicles, epididymis, liver, kidney and heart were subjected to histopathological screening. Phytochemical analysis of the extract revealed the presence of alkaloids, glycosides, phenolic compound, tannins, flavonoids, terpenes and saponins. Administration of cyclophosphamide significantly reduced body weight, testicular parameters, sperm motility, count, SOD, GSH, ACP and ALP. There was also significant increase in abnormal sperms, LDH, SDH and LPO levels. Abnormal sperm count was brought to normal level by extract administration. Extract treated groups showed decreased LDH levels and were comparable. *T. terrestris* dose dependently reduced SOD, GSH and SDH levels. Histopathological studies in testis, seminal vesicles and epididymis confirmed reproductive toxicity in the form of degeneration and loss of germinal epithelium within the seminiferous tubules. Marked hepatorenal injuries were also observed. *T. terrestris* extract supplementation significantly ameliorated above mentioned histopatholgical injuries. Hence, it could be concluded that ethanolic extract of *T. terrestris* has protective and antioxidant potential in cyclophosphamide induced reproductive toxicity in rats.

F-EP-O9

PROTECTIVE EFFECT OF *SPIRULINA* AGAINST IRON-INDUCED OXIDATIVE STRESS IN BROILERS

[1]Ramakrishnan V and [2]Gopala Reddy. A

[1]Assistant Professor, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Tirunelveli, TANUVAS, Tamil Nadu, [2] Professor and University Head, Department of Veterinary Pharmacology and Toxicology, Sri PVNR Telangana Veterinary University, Hyderabad.

ramakrishnanvet@gmail.com

Iron catalyzes the conversion of superoxide and hydrogen peroxide to free radicals, which attack cellular membrane, proteins and DNA leading tissue damage. High content of tissue iron has been reported to be associated with several pathological conditions. To study the protective anti-oxidant potential of *Spirulina* against iron-induced oxidative stress in poultry. 45 male broiler chicks of day-old age were divided into three groups of fifteen chicks in each. Group 1 was maintained on basal diet and 2 on FeSO4 @ 0.5% in feed for 6 wks. Group 3 was given FeSO4 diet for the first 4 wks and subsequently treated with *Spirulina* @ 0.1% in feed till the end of 6^{th} wk. The performance parameters were recorded at weekly intervals. Anti-oxidant enzymes were estimated at the end of 4^{th} and 6^{th} wk, while TBARS and GSH were estimated at the end of 6^{th} wk. Iron treatment resulted in significant ($p<0.05$) reduction in body weights and GSH (6^{th} wk), while TBARS (6^{th} wk), SOD and catalase, were significantly ($p<0.05$) increased at the end of 4^{th} wk in groups 2 and 3, and all these parameters exhibited similar trend at the end of 6^{th} wk in group 2. Following treatment, there was a marked improvement in all the above parameters in group 3 as compared to group 2 at the end of 6^{th} wk. Toxicity of iron was attributed to oxidative stress and supplementation of *Spirulina* prevented the toxic effects.

F-EP-O10

GENETIC ARCHITECTURE OF AYURVEDIC PHENOTYPICAL HUMAN BODY TYPES-'PRAKRITI' TYPES-'VATA, PITTA AND KAPHA'

O.Shyju[1], Bindya Liz Abraham[2], Suresh N Nair[3] and E.M.Muhammed[4]

[1]PhD Scholar, Department of Animal Genetics and Breeding, [2]Assistant Professor, Department of Animal Genetics and Breeding, [3]Assistant Professor, Department of Pharmacology and toxicology, College of Veterinary and Animal Sciences, Mannuthi, [4]Assistant Professor, Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Pookode, Kerala- 673 576, India..Email: drshyju@gmail.com

Ayurveda, an ancient Indian system of medicine documented and practiced in India since 1500 B.C has personalized approach towards management of health and disease. According to this system, every individual is born with his or her own basic constitution or phenotype, termed 'Prakriti'which, to a great extent determines inter individual variability in susceptibility to diseases and response to external environment, diet and drugs. Here we describe the biochemical and molecular basis of human body types in Ayurveda. Recent studies correlate Prakriti classification with genetic information and single nucleotide

polymorphisms (SNPs) in HLA-DRB1, CYP2C19, EGLN1, inflammatory and oxidative stress related genes, CD markers for various blood cells, DNA methylation alterations and risk factors of cardiovascular or inflammatory diseases. These studies have shown the association of specific genes with the phenotype of a particular Prakriti. The association of genomic variations with Prakriti classification describes 52 SNPs that are significantly different between 'Prakritis'. We argue that healthy individuals of contrasting 'Prakriti' types i.e. 'Vata, Pitta and Kapha' identified on the basis of Ayurveda exhibit significant differences at the gene and genome-wide levels.In conclusion, the phenotypic classification of individuals in India's traditional medicine has a genetic basis; and its *Prakriti*- dependent practice relates with personalized medicine.

F-EP-O11

A SIMPLE NOVEL *IN VITRO* METHOD TO ASSESS STABILITY OF ANDROGRAPHOLIDE IN RUMINAL FLUID

Srinivasan, M.R., Sriram, P., Ramesh, S., Sudhakar Rao, G.V. and Tirumurugaan, K.G.

Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai

seenubioinfo@gmail.com

Andrographolide is a major bioactive compound present in the *Andrographis 75aniculate* responsible for the various pharmacological properties of the herb, and used as liver tonic anthelmintic, febrifuge, anti-inflammatory and antineoplastic activities in veterinary practice. The stability of andrographolide in ruminal fluid is unknown and required to be assessed so as to allow oral administration of the herb or its extract in ruminants. Hence this study was conducted with the objective of assessing the stability of andrographolide in rumen fluid using modified Tilly and Terry method of *in vitro* 'MiniRumen' setup. The study was performed using pure andrographolide, commercial extract of *A. 75aniculate* (KalmCold^{TM}) and the powdered aerial part of the *A. 75 aniculate*. The concentration of andrographolide in the MiniRumen was analysed using a validated HPLC method at various time points up to 24 hours post exposure. Results showed that andrographolide was detectable in the rumen liquor till 24 hours though the concentration drastically decreased at 24 hours in pure andrographolide incubation. In case of extract and the powdered plant materials, the level of andrographolide was found to be increasing till 8 hrs after which the levels were slightly decreasing till 24 hrs. From the data it is concluded that the andrographolide is stable in the rumen

fluid for 24 hours and may be available for absorption from gastrointestinal tract. The results suggest the utility of MiniRumen as a novel *in vitro* system for predicting the stability of drugs / phytoconstituents.

F-EP-O12

EVALUATION OF THE ROLE OF SEAWEED SULPHATED POLYSACCHARIDES IN THE CONTROL OF HYPERCHOLESTEROLEMIA INDUCED INFLAMMATORY CHANGES

S.P.Preetha and Devaraj H*

Assistant Professor, Dept of Veterinary Pharmacology & Toxicology,

Madras Veterinary College, Chennai,

*Former Vice chairman, UGC & Senior Professor, Unit of Biochemistry, University of Madras

Hypercholesterolemia and associated cardiovascular diseases has increased worldwide. But certain coastal societies with regular intake of seaweeds have lower incidence of this disease. This may be attributed to the presence of sulphated polysaccharides in the seaweeds. To explore the efficacy of sulphated polysaccharides from native seaweeds against hypercholesterolemia induced inflammatory alterations. Male Wistar rats were fed high cholesterol diet for 14 days. The rats were then treated with sulphated polysaccharides extracted from native seaweed Sargassum wightii. Its efficacy was compared to the commercial sulphated polysaccharides (Sigma, USA). The abnormal inflammatory changes due to hypercholesterolemia were restored considerably on administration of seaweed sulphated polysaccharides. The sulphated polysaccharides from seaweeds were found to counter the detrimental inflammatory changes associated with hypercholesterolemic atherogenesis.

F-EP-O13

EVALUATION OF THE EFFECT OF *MORINGA OLEIFERA* LEAVES EXTRACT ON HAEMATOLOGICAL PARAMETERS IN MICE

M.Jayanthi and S.K.Garg*

Assistant professor, Department of Veterinary Pharmacology and Toxicology. Madras Veterinary College, Chennai. *Dean, DUVASU, Mathura

In this study, the effect of *Moringa oleifera* leaves extract (MOLE)on haematological parameters in mice were explored. Adult Swiss albino mice of either sex weighing between 18-25 gm were randomly

divided into three groups. Mice of group I were administered with saline, while groups II & III were orally administered with MOLE@125 & 250 mg/kg b.wt respectively. After 10 and 21 days of MOLE treatment, the haematological parameters were observed and recorded. Moringa oleifera at lower doses (125 mg/ kg) was found to possess an ability to induce leucocytosis and in particular the lymphocyte count.

F-EP-O14

NOVEL LIGAND-BASED DOCKING; MOLECULAR DYNAMIC SIMULATIONS APPROACH TO ANALYZE THE POTENTIAL XANTHINE OXIDASE INHIBITORY ACTIVITY OF *P.NIRURI* AGAINST GOUT DISEASE

Vikrama Chakravarthi,P, Murugesan,S, Arivuchelvan,A, Jagadeeswaran,A, Sukumar,K Arulmozhi, A, Lakshmanan and Karthikeyan, A.

Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal

drvikramvet@gmail.com

New herbal inhibitors of xanthine oxidase are discovered using molecular modelling techniques such as molecular docking and molecular dynamics simulations, which could be used therapeutically for gout disease in birds. With this objective, the present study was conducted in *Phyllanthus niruri* phytocompounds to test their xanthine oxidase inhibitory activity. *P.niruri* herb was collected locally and the alcoholic extract prepared for phytochemical analysis. Gas Chromatography Mass Spectrometry (GC-MS) was used to identify the bioactive components present in the *P.niruri* extracts. The GC-MS derived phyto compounds of *P.niruri* were docked with xanthine oxidase enzyme using Schrodinger Maestro software in comparison with standard drug allopurinol. The interpretation of results was carried out through GLIDE (Grid-based Ligand Docking with Energetics) extra precision (XP) Scoring, GLIDE Energy, MM-GBSA (Molecular Mechanics/Generalized Born Surface Area) energy and hydrogen bond and Pi-Pi interactions. Also molecular dynamics simulation carried out test by predicting *invitro* absorption, distribution, metabolism and excretion (ADME) of compounds. The results revealed that five phytocompounds (Octa decatrienoic acid, Isophytol, 2-methyl - Z, Z -3, 13-octadecadienol, 1, 2-15, 16 - Diepoxy hexa decane and Phytol) of *P.niruri* had superior scoring, high negative GLIDE energy value and high MM-GBSA energy than allopurinol. In conclusion, our study demonstrates that phytocompounds of *P.niruri* could be used as superior drug candidates for gout and further investigation using *in vivo* studies will validate the findings.

F-EP-O15

IMMUNOMODULATORY AND GROWTH PROMOTING POTENTIALS OF *ARTOCARPUS HETEROPHYLLUS LAM* HERB IN IMMUNOSUPPRESSED BROILERS

M. J. Raja, A. Arivuchelvan, A. Jagadeeswaran, K. Sukumar and S. Sivaseelan

Assistant Professor, Department of Veterinary Pharmacology and Toxicology,

Veterinary College and Research institute, Namakkal – 637 002.

Tamilnadu, India. *rajamj74@gmail.com*

In recent years, herbs are considered as alternatives throughout the world for their promising therapeutic effects in various ailments. The awareness on advantages of herbs has increased the usage as antioxidants, growth enhancers, antibacterials, antivirals and immunomodulators. This study was undertaken to explore the growth enhancing and immunomodulatory potentials of *Artocarpus heterophyllus Lam* leaves crude powder in broilers. A pilot study on dose fixation was conducted and 1% inclusion was selected. A total number of 120 broiler birds were used in this study, comprising of 10 birds in six groups with two replicates. Groups T_1 - T_4 were fixed as normal, positive (levamisole), negative (cyclophosphamide) and combined (levamisole + cyclophosphamide) controls respectively and groups 5 and 6 were fixed as immunized and immunosuppressed respectively with *Artocarpus heterophyllus Lam* leaves crude powder at 1% inclusion level in feed. Growth performance was assessed by evaluating weekly feed intake, feed conversion efficiency, body weight gain, and immunity was assessed by weekly hemagglutination inhibition (HI) titre against NDV LaSota antigen. In this study, T_5 group showed significant difference ($P< 0.05$) in producing body weight gain, feed intake and better FCR with T_1, T_3 and T_4 groups but it was not significant with T_2. T_6 treated with herb showed significant difference ($P< 0.05$) with T_3, but not significant with T_4. On immunity, similar results were noticed and the results revealed that the immunomodulatory and growth enhancing potentials of *Artocarpus heterophyllus Lam*. Since this herb is locally available and documented for being used traditionally as immunomodulator may become an alternative source for immunomodulation in poultry.

F-EP-O16

PHYTOCHEMICAL ANALYSIS OF *ANACYCLUS PYRETHRUM* CULTIVATED AT HERBAL GARDEN OF VETERINARY COLLEGE AND RESEARCH INSTITUTE, ORATHANADU

V. Ranganathan*, P. Senthil Kumar, K. Vijayakaran and A. Elamaran

Department of Veterinary Pharmacology and Toxicology,

Veterinary College and Research Institute, Orathanadu, Tamil Nadu

Anacyclus pyrethrum is a medicinal plant which has been shown to have immuno modulatory, sialogogue and insecticidal property in common and its effects have been shown to be attributed to their different phytochemical constituents. However, the plants, used for the study for the said properties have been resourced from places other than Thanjavur district of Tamil Nadu. Hence the present study has been undertaken to identify the presence of phytochemical constituents of this plant cultivated at the herbal garden, located in the Veterinary College and Research Institute, Orathanadu. The results showed the presence of alkaloids, triterpenes, steroids, flavonoids, saponins, tannins and amino acids.

F-EP-O17

STUDIES ON ANTI-NEOPLASTIC ACTIVITY OF PLANTS OF HIMALAYAN REGION

Pallavi Bhardwaj * and Shraya Thakur

Department of Veterinary Pharmacology and Toxicology, DGCN COVAS, CSKHPKV, Palampur, H.P.

*pallavivet@gmail,com

Cancer is one of the leading causes of death in companion animals such as dogs and cats. Their more complex heterogenous nature and multifactorial etiologies make them more challenging to control. Although many chemotherapeutical anti-neoplastic compounds have been discovered, but their poor efficacy and side effects restrict their use. Now a days naturally derived compounds have significant importance, due to their vast application as antioxidant and prevent the cellular organelles from oxidative destruction. It also impedes the cell signaling pathways by regulating proliferation of cell, apoptosis intuition and leads to oxidative destruction. The objective of the present study was to evaluate *in-vitro* antineoplastic activity of plants of himalayan region. On the basis of review of literature, twenty (20) plants of himalayan region were collected and got identified from the Dept. of Biodiversity, IHBT, Palampur. The plant samples were shade dried and aqua-ethanolic extracts were prepared. Lyophilized plant extracts were assesed for *in-vitro* antineoplastic activity at four different concentrations i.e. 20, 50, 100 and 200 µM by using cell culture technique, SRB Assay, on LC-540 (Rat Leydig cancer cell line) cell lines, procured from NCCS, Pune. *In-vitro* cytotoxicity studies revealed that among twenty different extracts, three extracts exhibited more than 40 % cytotoxicity and one extract (X) exhibited more than 60 % cytotoxicity. The plants exhibiting potent *in-vitro* anti-neoplastic activity could be explored for their *in-vivo* effects and further studies could be conducted to develop a therapeutic formulation against tumors.

F-EP-O18

HYDROETHANOLIC EXTRACT OF *TRIANTHEMA PORTULACASTRUM* LINN. RESTORES OXIDATIVE STATUS IN 7,12-DIMETHYLBENZ[A]ANTHRACENE INDUCED MAMMARY TUMORIGENESIS MODEL IN WISTAR RATS

Nirbhay Kumar,[1*] A. H. Ahmad and Anu Gopal

Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar – 263145, Uttarakhand, India

[1]Bihar Veterinary College, Bihar Animal Sciences University, Patna - 800014, Bihar, India

Email: drnkvet76@rediffmail.com

Trianthema portulacastrum Linn. has been known to possess various pharmacological properties and the plant has been traditionally used for treatment of cancer. Oxidative mechanisms play a potential role in different stages of carcinogenesis. Hence, the objective of the study was to investigate the role of hydroethanolic extract of the *Trianthema portulacastrum* (TPHE) on oxidative profile in 7,12-dimethylbenz[a]anthracene (DMBA) induced mammary tumour in Wistar rats. The tumours were induced in rats by administering the carcinogen, DMBA given 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 antioxidative effect of these extracts in DMBA induced mammary tumour model. There were extreme oxidative changes in DMBA induced cancer control group which were found to have improved in the TPHE treated animals. The significantly decreased LPO levels in the organs of TPHE treated groups may be due to reduction in the oxidant mediated stress in these tissues. The reduced levels of GSH in RBC and various organs of cancer control group can be considered as yet another confirmation of the oxidative damage due to DMBA induced carcinogenesis. The catalase and SOD activity in RBC and tissues also followed the same trend as GSH in cancer control group, probably as a response to carcinogenic stress. The elevated levels of these enzymes in TPHE treated group can be considered as an indication of the antioxidative property, which can in turn lead to inhibition in the progression of cancer. Based on the above findings, it can be concluded that *Trianthema portulacastrum* produces antioxidative activity by quenching and detoxifying the free radicals induced by DMBA. The attenuation of DMBA induced oxidative stress by TPHE could be attributed to the antioxidant activity of triterpenes, alkaloids and flavonoids of these plants, which are known to quench the free radicals by maintaining antioxidants levels.

F-EP-O19

EVALUATION OF ANTICOCCIDIAL POTENTIAL OF METHANOLIC EXTRACT OF TRIGONELLA FOENUM-GRAECUM (FENUGREEK) SEEDS AGAINST CAECAL COCCIDIOSIS

Divya P.D., Preethy John, Usha P.T.A., Shameem H. and Surya Sankar

Assistant Professor, Department of Veterinary Biochemistry,

College of Veterinary & Animal Sciences, Mannuthy, Thrissur, Kerala, India.

Email id: divyapd @kvasu.ac.in

Caecal coccidiosis caused by Eimeria tenella is a major threat to poultry industry. Anticoccidial drugs have played a major role in the effective control of avian coccidiosis, but the emerging drug resistance and the public concerns regarding drug residues in poultry meat necessitates sourcing of alternative strategies. Herbal anticoccidials open new perspectives and can serve as a substitute to the conventional treatment, particularly in countries with limited economic potential. Hence the present study was designed to evaluate anticoccidial potential of methanolic extract of Trigonella foenum-graecum (Fenugreek) seeds under in vitro and in ovo conditions. Plant extract was prepared using Accelerated Solvent Extractor based on a modified version of soxhlet method. Sufficient oocysts of E. tenella were recovered from the caeca of naturally infected chicks. The effects of plant extract on inhibiting sporulation of Eimeria tenella oocysts as well as its capacity to reduce the viability of sporulated oocysts were studied under in vitro conditions. The reduction in pathogenicity of the sporulated oocysts after treating with the herbal extract was confirmed by in ovo experiments. The efficacy of different concentrations of herbal extract such as 500, 250, 125, 62.5 and 31.25 mg/ml was tested for 72 hours at room temperature with amprolium 100 mg/ml as the positive control while 2.5 % potassium dichromate served as the negative control. Sporulation of E. tenella oocysts was found to be inhibited in a dose dependent manner with 500, 250 and 125 mg/ml of fenugreek extract which were statistically significant with that of standard drug control. The effect on sporulated oocysts of E. tenella was measured by counting the proportion of lysed sporulated oocysts with that of the sporulated unlysed oocysts. All the concentrations of fenugreek showed significant anticoccidial property with respect to the viability of oocysts and concentrations of 500, 250 and 125 mg/ml were statistically significant when compared to the positive control. Pathogenicity of the sporulated oocysts after treatment with fenugreek was validated by conducting in ovo experiments and the findings revealed great reduction in the viability of E. tenella oocysts in a dose dependent manner. The study thus recommends the use of fenugreek seeds as an herbal anticoccidial feed additive based on further scientific validations.

F-EP-O20

EFFECT OF DIETARY INCORPORATION OF TURMERIC RESIDUE ON GROWTH PERFORMANCE IN MALABARI KIDS

Jasmine Rani K., Ally K. and Thirupathy Venkatachalapathy. R

Department of Animal Nutrition, College of Veterinary and Animal Sciences, Mannuthy – 680 651, Thrissur, Kerala. Email-jasminerani@kvasu.ac.in

Turmeric (*Curcuma longa)* is known for its number of biological activities such as anti-inflammatory, antioxidant, antimicrobial, anti-diabetic and anti-ulcer properties. It has also been reported to improve the nutrients digestibility, metabolism, and prevent biliary disorders and anorexia in humans and farm animals. Turmeric residue is a by-product obtained during the extraction of curcumin from *Curcuma longa*. This residue is available in considerable quantity and discarded as a waste product. The level of incorporation and the effect of turmeric residue on growth in kids are not yet studied. Hence, an experiment was conducted on Malabari kids for a period of two months to assess the effect of dietary incorporation of spent turmeric residue on their growth, nutrient digestibility and blood biochemical profile. Fourteen healthy Malabari kids of six month age were selected from University Goat and Sheep Farm, Mannuthy and divided into two groups of seven each based on age, sex and body weight and allotted randomly to two experimental treatments T_1(control) and T_2 (kid starter containing 10 % turmeric residue). All the experimental animals were fed with pelletted complete feed and were fed as per ICAR standard (2013). The average body weight, body weight gain, dry matter intake, haematological and biochemical parameters were found to be similar in both the groups ($P > 0.05$). Digestibility of crude protein and ether extract were significantly improved in T_2. ($P < 0.05$). The occurrence of diarrhoea and respiratory infections were significantly lower in T_2 group ($P < 0.05$) than the control group. The results of the present study indicated that turmeric residue can be included in the kid ration up to 10% level without any adverse effect on their growth performance.

F-EP-O21

IN VITRO EVALUATION OF TOTAL MIXED RATION CONTAINING LOCALLY AVAILABLE UNCONVENTIONAL FEEDS USING IVGPT

Sajith Purushothaman[1], Shyama.K[2]. Ally.K[3] and Sunanda.C[4].

1. Assistant Professor, Department of Animal Nutrition, Veterinary College, Mannuthy, Kerala. 2. Associate Professor & Head, Department of Animal Nutrition, Veterinary College, Pookode, Kerala. 3. Professor & Head, Department of Animal Nutrition, Veterinary College, Mannuthy, Kerala, 4. Assistant Professor, Department of Statistics, Veterinary College, Mannuthy, Kerala sajith@kvasu.ac.in

The study was conducted to evaluate the effect of inclusion of unconventional feeds in total mixed ration and selection of two best total mixed rations for crossbred cattle. Thirty compounded concentrate feed mixture were prepared by using unconventional feed ingredients like cooked barley,dhanwantharam thailam residue,spent grapes,maize waste, tapioca waste, jack fruit seeds,mango seed kernel,spent rosemary,soya sauce waste,banana peels, ksheerabala residues, spent cumin, turmeric waste, elephant palm leaves, rain tree pods, azolla, rapeseed cake, desmanthus, gliricidia leaves, moringa leaves, coconut leaves, banana leaves , areca sheath, oil palm leaves , caliandra, jack leaves , venga leaves, spent coconut gratings, sugarcane baggase and agathi replacing the conventional ingredients and *in vitro* gas production technique (IVGPT) for 24 hour was done. Six crossbred animals maintained on TMR were used as rumen liquor donors. The concentrate to roughage ratio of the ration was maintained 50:50. The parameters observed were true dry matter digestibility (TDMD %), true organic matter degradability (TOMD %), methane%, microbial biomass production (MBP mg/200 mg), 83illimole83ble energy (ME MJ/kg DM) and total volatile fatty acid (TVFA 83illimole/L). Based on above observations cluster analysis of 30 TMRs were performed by considering the IVTMD, IVOMD, ME, MBP and TVFA production as positive factors and methane production capacity as negative factor and two best TMRs after analysis were selected. The results revealed that TDMD% ranged from 64.5 ± 0.29 to 80 ± 0.29, while TOMD% ranged from 62.45 ± 0.58 to 80.68 ± 0.38, methane % varied from 6.35 ± 0.03 to 13.5 ± 0.14, MBP (mg/200 mg) ranged from 21.28 ± 0.01 to 49.62 ± 0.01, ME (MJ/kg DM) ranged from 4.45 ± 0.03 to 5.55 ± 0.03 and TVFA (83illimole/L) ranged from 50.69 ± 0.06 to 94.78 ± 0.03, respectively. On cluster analysis the TMR containing rape seed cake and dhanwantharam thailam residues showed better fermentation characteristics and which can be included in crossbred cattle ration as a cost effective and nutritionally balanced ration.

F-EP-O22

PROTECTIVE EFFECT OF *PEDALIUM MUREX* EXTRACT IN CISPLATIN INDUCED NEPHROTOXICITY IN WISTAR RATS

Santosh Tambade, Sushma Shendre, Lalita Bhalerao

KNP College of Veterinary Science, Shirval

Maharashtra Animal and Fishery Sciences University, Nagpur

Chemotherapy and radiotherapy are the most common modalities of cancer therapy. Cisplatin is currently one of the most important chemotherapeutic agents used in the treatment of wide range of tumors. However the clinical usefulness of cisplatin is limited by its cumulative nephrotoxicity and neurotoxicity. This has been the rationale for development of new nephroprotective drugs and the search for

novel molecules has been extended to herbal drugs that offer better protection. Drugs of plants origin are gaining popularity and are being investigated for a number of disorders including nephrotoxicity induced by cisplatin. It purifies blood and also acts as a diuretic. *Pedalium murex* Linn. is a cooling tonic, aphrodisiac, improves appetite and is useful in strangely, urinary discharges, vesicular calculi, cough, asthma, pain, skin diseases, heart troubles, piles and leprosy. In the present study the ethanolic fruit extract of *P. murex* Linn (500 mg/kg and 1000 mg/kg body weight P.O) was studied for nephroprotective activity in wistar rats. The nephroprotective activity was studied in cisplatin induced nephrotoxicity using taurine as standard which showed significant nephroprotection. The extract at both preventive and curative doses of *P. murex* Linn. fruit extract had beneficial nephroprotective effect in rats.

F-EP-O23

IMMUNOHISTOCHEMICAL STUDIES OF KIDNEYS AND TESTIS IN RAT ACUTE KIDNEY INJURY AMELIORATED WITH NANO-QUERCETIN

K Ashok Reddy[1], K Adilaxmamma[1], Ravi Kumar P[2], Satish K[3] and Muralidhar Y[1]

[1]Dept. of Veterinary Pharmacology & Toxicology, CVSc, SVVU, Tirupati, Andhra Pradesh

[2]Buffalo Research Station, SVVU, Venkataramannagudem, WG, Andhra Pradesh

[3]Sheep & Goat Research Station, SVVU, Banavasi, Kurnool Dt.

Presenting author: dradilaxmamma@gmail.com

Acute kidney injury (AKI) is a sudden onset of potentially life-threatening kidney dysfunction and is a common disorder in dogs, cats and humans. The present study was conducted to evaluate the ameliorative effect of quercetin mediated zinc oxide nanoparticles (QZnO NPs) in AKI in rats. Rats were randomly categorised into seven groups, each group containing 3 males and 3 females. Rats of all groups were deprived of water for 24 hours before glycerol administration. Group I served as control group. AKI was induced by injecting 10 ml/kg body weight of 50% glycerol in sterile normal saline into the hind limbs. Half an hour after glycerol injection, the treatment groups III, IV, V, VI and VII received zinc oxide nanoparticles (ZnO NPs)@ 50 mg/kg, quercetin @ 50 mg/kg, quercetin mediated nanozinc (QZnO NPs) @ 10 mg/kg, QZnO NPs @ 25 mg/kg and QZnO NPs @ 50 mg/kg respectively. Rats of group II did not receive any treatment and served as AKI control. The treatments were given daily for 3 consecutive days and the animals were sacrificed on 4th day. Blood biochemical profile, antioxidant profile and histopathological studies of renal tissue were studied by H&E staining. In the present study, ZnO NPs and quercetin reduced glycerol

induced nephrotoxicity and treatment with QZnO NPs exhibited better nephroprotective action at low and medium doses tested when compared with quercetin, ZnO NPs and QZnO NPs at higher dose. The possible mechanism by which QZnO NPs exert their nephroprotection could be attributed to their free radical scavenging property.

F-EP-O24

EVALUATION OF ANTIMICROBIAL ACTIVITY OF ETHNO VETERINARY POLYHERBAL FORMULATION FOR BOVINE MASTITIS

R. Priya[1], P. Senthil kumar[2] and V. Ranganathan[3]

1.Veterinary Surgeon, Veterinary Dispensary, Vazhakkad , 2. Assistant Professor, Dept of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute Orathanadu, 3. Professor and Head, Dept of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute Orathanadu

The objective of the present study was to determine antimicrobial efficacy of locally available herbal plants, including *Aristolochia indica, Curcuma longa, Hydrophila auriculata, Tribulus terrestris* and *Boerhavia diffusa* against bovine mastitis. Phytochemical screening of the extracts revealed the presence of alkaloids, triterpenes, sterols, polyphenols, flavonoids and saponins. A total of 8 cows affected with clinical mastitis were selected from Vazhakkad panchayath of Malappuram district in Kerala. Bacteriological culture study was conducted in the milk collected from the selected animals to isolate pathogenic organisms. Milk somatic cell count was determined in the collected milk sample using Somatic Cell Counter in periodic time intervals up to 10 days. The polyherbal formulation I was prepared using whole plant (each 50 g) of *Hydrophila auriculata*, *Tribulus terrestris* and *Boerhavia diffusa* for oral administration. Polyherbal formulation II was prepared using 250g *Aristolochia indica* (whole plant) and 50 g *Curcuma longa* (rhizomes) for external application on the affected udder. Antimicrobial activity of herbal extract of five different plants and polyherbal formulation I and II were evaluated by agar well diffusion method and found that the plant extract of polyherbal formulation I and II showed higher amount of antimicrobial activity against *Salmonella aureus* compared to individual plant extracts. Thus, on the basis of antimicrobial susceptibility test and MIC, extract of polyherbal formulation I and II were selected for further clinical evaluation. The cows treated with polyherbal formulation I and II got cured within 6 to 7 days of treatment and the somatic cell counts of milk became normal within 8 days of treatment. Thus based on clinical evaluation and feasibility from farmers view, polyherbal formulation containing locally available herbal plants can be recommended to treat bovine clinical mastitis.

F-EP-O25

NANOCURCUMIN AMELIORATES *STAPHYLOCOCCUS AUREUS*-INDUCED MASTITIS IN MOUSE BY REDUCING OXIDATIVE STRESS AND BACTERIAL COLONIZATION IN MAMMARY GLAND

S. Suresh[1], P. Sankar[2], Avinash Gopal Telang[1]

[1]Division of Pharmacology and Toxicology, IVRI, Izatnagar-243 122, Bareilly, UP, India

[2]Department of Veterinary Pharmacology and Toxicology, VCRI, Namakkal, Tamil Nadu, India

Mastitis is the inflammation of the mammary glands caused by bacteria. It causes severe economic loss to dairy industry. Curcumin, a polyphenol obtained from turmeric, has considerable anti-inflammatory effect. Since it is rapidly eliminated from the body, its oral bioavailability is low. However, nanoformulation of curcumin significantly enhances its therapeutic efficiency by improving its oral bioavailability. We evaluated whether nanocurcumin could be more effective than normal curcumin against bovine *Staphylococcus aureus* mastitis in mouse model. Curcumin-loaded PLGA nanoparticles (CUR-NP) were prepared by solid-in-oil-in-water emulsion method. The mouse model of mastitis was induced by inoculation of a field strain of *S. aureus* (Bovine mastitis isolate) on the 9th day of parturition through the duct of the mammary gland. CUR-NP and curcumin were given orally for 7 days (day 2 to day 8 of parturition) prior to *S. aureus* inoculation. We determined the bacterial colonization and the level of lipid peroxidation and antioxidants in mammary gland. *S. aureus* infection increased the levels of bacterial colonization in mammary tissues. Both CUR-NP and curcumin significantly attenuated the levels of bacterial colonization and lipid peroxidation. However, comparatively, the ameliorative efficiency of CUR-NP was better than normal curcumin. *S. aureus* infection-reduced catalase and superoxide dismutase activity were significantly increased to the healthy control level by CUR-NP. Our study demonstrates that the nanoformulation of curcumin can reduce lipid peroxidation in *S. aureus*-infected mammary tissues by improving antioxidants. Besides, compared to normal curcumin, this nanoformulation appears to be a better alternative against murine mastitis.

S-EP-O1

ISOLATION OF α-TOCOPHEROL FROM *TETRASTIGMA LEUCOSTAPHYLUM* (Dennst.) Alston: A POTENTIAL ROLE IN THE HEALING EFFICACY OF THE PLANT IN BURNS

Mery S.J[1]., Adarsh Krishna T.P[1]., Darsana U[1]., Sreelekha K.P[1]. Nair S.N. [1], Ravindran R[2] Ajith Kumar K.G.[2] and Juliet S[1]

[1]Department of Veterinary Pharmacology and Toxicology, [2]Department of Veterinary Parasitology, CVAS, Kerala Veterinary and Animal Sciences University, Pookode-673576, Wayanad, Kerala, India.

Tetrastigma leucostaphylum (Dennst.) Alston is a woody climber, which belongs to the family, Vitaceae and is an important ethno-medicinal plant used among the tribal folklore in Wayanad district. The plant leaves are used by the traditional healers for the treatment of burn injuries. The present study was conducted to scientifically validate and isolate the compound that attributed most to its efficacy in burns. The plant material was subjected to extraction using petroleum ether in soxhlet extraction apparatus. The petroleum ether extract was tested for antimicrobial activity against most common organisms in burn wounds and wound healing activities. The petroleum ether extract was further fractionated using the column chromatographic system. The fractions thus obtained were further profiled by High pressure thin layer chromatography (HPTLC) and Gas chromatography and mass spectrometry (GC/MS). The petroleum ether extract did not possess significant antimicrobial activity but was efficacious in healing the wound. The HPTLC profiling of fraction one and GC/MS analysis detected the presence of high content of α-tocopherol. The results suggested that the healing efficacy of the plant in burn injuries was attributed to α-tocopherol in the extract which restored the attenuated levels of Vitamin E in burns.

S-EP-O2

EFFECT OF *TRIANTHEMA PORTULACASTRUM* IN ALLEVIATING CYCLOPHOSPHAMIDE INDUCED IMMUNOSUPPRESSION IN RATS

Aswathy P.U., Ahmad A.H., Pant D., Patwal P.C., Saklani. S., Singh V. and Narayan S. R.

Department of Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Pantnagar- 263145, email id - asharies999gmail.com

Trianthema portulacastrum Linn. commonly known as Horse purslane or Biskhapra is a weed distributed throughout India. This study was undertaken to explore the immunostimulatory efficacy of hydroethanolic extract of *Trianthema portulacastrum* (TPHE) in rats administered with cyclophosphamide. It was assessed by performing haemaglutination test, delayed type hypersensitivity reaction, phagocytic index, neutrophil adhesion test and total immunoglobulin estimation. In this study adult Albino Sprague Dawley rats were randomly divided into eight groups. Group I served as control, group II rats were administered with cyclophosphamide at the dose rate of 100 mg/kg p.o on 9^{th} and 16^{th} day of study; in group III levamisole was administered at dose rate of 50 mg/kg s/c daily for 28 days; in group IV both cyclophosphamide and levamisole were given at above mentioned doses, group V and VII were administered with TPHE at dose rates 200 mg/kg and 400 mg/kg p.o respectively, daily and group VI and VIII were given cyclophosphamide at dose rate 100 mg/kg p.o (on 9^{th} and 16^{th} day) along with TPHE at 200 mg/ kg and 400

mg/ kg respectively, for 28 days. A significant ($p<0.05$) decrease in haemagglutination titre, delayed type sensitivity reaction, phagocytic index, neutrophil adhesion and total immunoglobulin concentration was observed in group II as compared to that of control, in contrast, treatment with TPHE restored these parameters towards normal in the extract groups in a dose dependent manner. From the result, it can be concluded that the hydroethanolic extract of *Trianthema portulacastrum* has the potential to ameliorate immunosuppression induced by cyclophosphamide in rats.

S-EP-O3

CYTOTOXIC POTENTIAL OF CHLOROFORM EXTRACTS OF *MALLOTUS PHILIPPENSIS* IN MCF7 CELLS

Poonghuzhali R*, Dhanusha G*, Sujith S**

*MVSc Scholar, Dept of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy,

**Dept of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy.

The present study was carried out to evaluate the cytotoxic effect of chloroform extract of *Mallotus philippensis* in MCF7 cells. The qualitative phytochemical analysis of the extract was done and it revealed the presence of phenolics and tannins. MCF7 cells were maintained in RPMI media containing 10 per cent serum and one per cent antibiotic and antimycotic solution. The cells were harvested and seeded to 96 well plate at $1\times1\ 0^5$ cells/mL, incubated for 24 hours at 37˚C with 5 per cent CO_2. The cells were treated with 160, 80, 40, 20 and 10µg/mL of the extract for 24 hours and viability was assessed using MTT Assay. There was a dose dependent decrease in viability of cells exposed to chloroform extracts of *Mallotus philippensis*. The cells treated with 160µg/mL showed 70.3 per cent viability and IC_{50} was found to be 104.519.

S-EP-O4

COMPARATIVE STUDY ON DEWORMING POTENTIAL OF HERBAL DRUGS IN CROSSBRED HEIFERS AT AN ORGANISED FARM

Jith John Mathew., Priya, M.N., Deepak Mathew, D.K., Sreemol S Nair, Neelima Jayaraj, Nandana Chandrasekhar, Reshmi Raveendran and Syam Mohan, K.M

University Livestock Farm & FRDS, Mannuthy, Thrissur

Sustainable herbal alternatives to chemical deworming are being sourced from indigenous traditional knowledge to effectively counter resistance of worms against indiscriminate use of chemical dewormers. Eighteen heifer calves of the age twelve to eighteen month were selected for the study from University

Livestock Farm & FRDS, Mannuthy, Thrissur and were distributed as uniformly as possible with regard to age and body weight into three groups namely I, II and III. Pooled samples of feaces were taken from all the eighteen animals for analysing Eggs per gram (EPG), one day prior to them being subjected to treatments. Animals in Group I formed the control of the study and were provided with a chemical broad spectrum dewormer namely, Albendazole at prescribed rates i.e 10 mg/Kg body weight. Animals in Group II received the treatment with 80 grams of aloe vera mucilage mixed with 20 grams of palm jaggery and animals in Group III received deworming treatment with 1.5 grams of dried areca nut powder per kilo gram body weight of heifers. Pooled samples of feaces were again taken from all the eighteen animals for analysing EPG, one week after them being subjected to above treatments. Average EPG of control group was 225 before treatment which was reduced to 25 one week after the treatment. For Group II an average EPG of 333 was reduced to 166 where as for Group III an average of 333 was reduced to 58 after one week of treatment. A detailed study on deworming potential of above herbal preparations is further warranted to draw decisive conclusions.

ISVPT – 2019

TECHNICAL SESSION

ANTIMICROBIALS AND ANTIMICROBIAL RESISTANCE

Chairperson : Dr. A.M.Chandrasekharan Nair

Co-Chairperson : Dr. M.R. Srinivasan

Rapporteur : Dr. R Rashmi

College of Veterinary and Animal Sciences, Mannuthy

Pharmacogenomics in the development and validation of indigenous drugs
XIX ISVPT
CVAS MANNUTHY

LP-AMR-01

ANTIBIOTIC RESISTANCE BREAKERS

Dr. S.P.S.Saini

Professor of Veterinary Pharmacology and Toxicology, GADVASU, Ludhiana, India 141004

These are compounds that can increase the effectiveness of current antibiotics by combating the resistance mechanisms employed against them. ARBs may or may not have direct antibacterial effects and can either be co-administered with or conjugated to failing antibiotics.

Major classes of ARBs are:

1) Modifying-Enzyme Inhibitors

2) Membrane Permeabilisers

3) Efflux Pump Inhibitors (EPIs)

The idea of co-administering ARBs with conventional antibiotic sstems from dual antibiotic therapy, which has enjoyed success in the past through either synergistic or additive effects ofthe individual antibiotic agents (Kalan and Wright 2011), and several ARBs have enjoyed lengthy clinical use including the β-lactamase inhibitors (BLIs) (Drawz and Bonomo 2010). Successful co-administered ARBs should enhance the effects of antibiotics by combatting the bacterial resistance mechanisms employed against the latter, allowing lower doses of antibiotics to be used.

The minimum inhibitory concentration (MIC), the minimal concentration required of a compound to prevent visible growth of the pathogenic species under defined conditions(Wiegand, Hilpert and Hancock 2008), is a useful term in thisregard; the more successful ARBs achieve greater reductions in the MICs of antibiotics versus antibiotic monotherapy. Such potentiation is an attractive prospect, both because reduced antibiotic selection pressure could slow the onset of resistance and because widening of the therapeutic window may allow for the alleviation of side effects experienced by patients on antibiotic monotherapy.

A. Modifying Enzyme Inhibitors

Modifying enzyme inhibitors are used to disrupt bacterial detoxification enzymes, increasing the effectiveness of a co-administered antibiotic. Two major classes are the BLIs and aminoglycoside-modifying enzymes.

a) β-lactamase inhibitors

The most successful class of ARBs is arguably the BLIs. β-lactam antibiotics function by interfering with bacterial cell-wall synthesis, binding to and inactivating the C-terminal transpeptidase domain of

penicillin-binding proteins which are responsible for the cross-linking of the peptidoglycan chains in thecellwall (Fisher et al. 2005). The β-lactams include several frequently prescribed families of antibiotics such as the penicillins and cephalosporins. They remain the most widely used classof antibiotics, reported to comprise 65% of the global antibiotic market in 2004 (Elander 2003), while broad-spectrum penicillins and cephalosporins were reported to be the two most consumed drug classes globally in 2010 (Van Boeckel et al. 2014).β-lactamases (EC 3.5.2.6) are bacterial enzymes that hydrolyse the β-lactam rings such drugs possess, inactivating them. Modification of β-lactam drugs is the major defence mechanism for Gram-negative pathogenic bacteria, with β-lactamases differing in their mechanisms and their substrate specificities.

Eg: clavulanic acid, sulbactam, tazobactam, brobactam, AAI101(a novel penicillanic acid sulfone similar instructure to tazobactam, is an ESBL inhibitor active against someclass A and D carbapenemases),non-β-lactam BLIs is the diazabicyclooctanes (DABCOs) -avibactam, relebactam, zidebactam, nacubactam, Boronic acid transition state inhibitors (BATSIs) –vaborbactam, are a novel class of BLIs with activity against serine β-lactamases.the electrophilic natureof the boron atom imitates the electrophilic carbonyl centre ofa β-lactam ring, but nucleophilic attack by the catalytic serineresidue of a β-lactamase generates a tetrahedral enzyme-BATSIadduct, inhibiting the enzyme in a competitive, reversible manner(Rojas et al. 2016).

b) Aminoglycoside-modifying enzyme inhibitors

The aminoglycosides are a family of bacterial protein synthesis inhibitors that bind to the A site of the prokaryotic 70S ribosomeand possess bactericidal activity. Aminoglycoside resistance is a major concern because of theseveral important uses of aminoglycoside antibiotics, includingtreatment of infections of Mycobacterium tuberculosis.

The three groups of aminoglycosidemodifying enzymesare the aminoglycoside acetyl transferases (AACs), aminoglycoside nucleotidyl transferases and aminoglycoside phosphotransferases (APHs). AACs function by catalysing the acetylation ofprimary amine groups within the aminoglycoside molecules,using acetyl coenzyme A (CoA) as a donor substrate. Aminoglycoside nucleotidyl transferases are responsible for mediatingthe transfer of an adenosine monophosphate group to ahydroxyl group in the aminoglycoside molecule, using ATP asa donor substrate, while APHs catalyse the transfer of a phosphategroup to the aminoglycoside molecule (Ramirez and Tolmasky2010).

Use of bisubstrate analogues, consistingof the aminoglycoside antibiotic and CoA , was successfully carried out with gentamicin; the bisubstrates lowered inhibitory activity of aminoglycoside acetyltransferases (AACs).

B. Membrane Permeabilisers

Gram-negative bacteria are intrinsically resistant to severalantibiotic classes because of the presence of a second, OM comparedto Gram-positive bacteria which these antibiotics cannotpenetrate. The Gram-negative bacterial envelope consists of three components; an inner membrane which surrounds theorganelles, an OM and a periplasmic region between the two membranes containing a peptidoglycan layer (Silhavy, Kahneand Walker 2010). The OM consists mainly of lipopolysaccharides(LPS), which are made up of three parts; a polysaccharide referred to as the O-antigen, a core domain consisting ofan oligosaccharide component and a lipid region referred toas lipid A (Raetz and Whitfield 2002). This LPS layer is stabilisedby cross-linking, enabled by divalent cations such as Mg2+ and Ca2+ (Zabawa et al. 2016). The OM contains porins,water-filled protein channels that facilitate entry of hydrophilicmolecules into the bacterial cell; mutations in Gram-negativebacteria resulting in reduced porin expression can reduce influxof hydrophilic drugs into these bacteria. This method of antibacterial resistance has been confirmed in several clinically relevantbacterial species, such as *P. aeruginosa.*

Besides directly damaging the cell membrane, various othermethods have been suggested to increase rates of antibioticinflux in bacterial cells, such as the use of liposomal drug preparations (Torres et al. 2012). However, it is the use of membrane permeabilisers, compounds that make the Gram-negative OMmore permeable to facilitate increased antibiotic influx, thatwillbe reviewed herein. Membrane permeabilisers can function bychelating and removing divalent cations from the OM and/or (inthe case of permeabilisers with a net cationic charge) association with the negatively charged OM to disrupt it, causing a breakdown of OM structure (Zabawa et al. 2016). The effectiveness of putative membrane permeabilisers can be assessed by measuringthe level of uptake of substances that would not normally beable to penetrate the Gram-negative OM, such as a hydrophobic probe. The fluorescent dye N-phenyl-1-napthylamine (NPN) is used for this purpose; an increase in fluorescence indicatesincreased incorporation of NPN into the OM of the pathogenand thus increased OM permeability (Lee et al. 2004). Besidesenabling increased influx of antibiotics, membrane permeabilization alone can be sufficient to cause bacterial lysis; as such, several of the compounds mentioned in this section also havedirect antibacterial activity (Zabawa et al. 2016).

Polymyxins- Polymyxins, including polymyxin B andpolymyxin E (colistin), are antibiotics that function throughdisruption of the Gram-negative OM. Polymyxins are pentacationic lipopeptides consisting of a cyclic peptide attached to a long fatty acid chain. Colistin itself was available for treatment of Gram-negative bacterial infections from 1959(Ross, Puig and Zaremba 1959), though clinical use decreased from

the 1970s to the 1990s because of reports of neurotoxicity and nephrotoxicity. However adverse events related to current polymyxin use are less frequent than reported in older literature, possibly due to better understanding of appropriate dosing regimen and the avoidance of simultaneousadministration of nephrotoxic and/or neurotoxic drugs (Falagas and Kasiakou 2006), increased colistin use in recentyears has been primarily driven by the onset of resistance toβ-lactams, aminoglycosides and quinolones in Gram-negatives (Livermore 2002). Polymyxins interact electrostatically with theOM to displace Mg2+ and Ca2+ cations from their binding sitesto disrupt membrane integrity, causing cell damage and alsofacilitating the influx of othermolecules, including other antibiotics (Landman et al. 2008). Lin et al. found that azithromycin,ineffective against Gram-negative rods, showed synergy withcolistin; the combination was effective against MDR-isolates of*P. aeruginosa*, *K. pneumoniae* and *A. baumannii* (Lin et al. 2015).Synergistic combinations of colistin with other drugs have alsobeen reported; Lee and co-workers found that the combination of colistin and rifampicin at clinically-relevant concentrationswas additive or synergistic against MDR strains of *A. Baumannii* and suppressed the emergence of colistin resistance (Lee*et al*. 2013).

The membrane permeabilising effects of colistin towards pandrug-resistant Gram-negative bacteria have been investigated.Pandrug-resistant bacteria refers to bacteria thatare resistant to all anti-pseudomonal drugs (penicillins,cephalosporins, carbapenems, monobactams, quinolones andaminoglycosides) except the polymyxins.

Polymyxin derivatives- Efforts to develop polymyxin derivatives as ARBs to potentiatethe actions of antibiotics has started. **Polymyxin B nonapeptide (PMBN),** lacks the fatty acid and terminal diaminobutyric acid moieties of polymyxin B required for bactericidal activity (Vaara 1988), but it does retain the OM permeabilising character of the latter. It is capable of enhancing penetrationof hydrophobic antibiotics, including erythromycin, clindamycin, rifampicin, fusidic acid, novobiocin and cloxacillin, in most polymyxin-susceptible Gram-negative bacteria, including MDR *E. coli*, *K. pneumoniae and P. aeruginosa.*

Antimicrobial Peptides - It is umbrella term encompassing adiverse array of compounds produced by a variety oforganismsto combat infections of pathogenic microorganisms, have beeninvestigated for use as ARBs.AMPs have been identified at most sitesof the human body that are normallyexposed to microbes, such as the skin, intestinal mucosa, oral mucosa, lung,eye and reproductive tract. It became clear that, while some AMPs are constitutively expressed, the majority of these peptides are induced during infection or by inflammation or injury. The specific sites of expression and the strict regulation of AMP expressionare key

to understanding how they work. This may explain why AMPs, asevolutionarily ancient gene products, remain as effective antibiotics, while pharmaceutically derived antibiotics can rapidly become useless due to the development of bacterial resistance

Mechanism of action: The ability of AMPs to kill bacteriausually depends upon their ability tointeract with bacterial membranes orcell walls. Generally, AMPs exhibit anet positive charge and a high ratio of hydrophobic amino acids, allowing themto selectively bind to negatively chargedbacterial membranes. Binding of AMPs to the bacterial membrane leads to non-enzymatic disruption. Selectivity for specific species is due to differences in the membrane composition of different microbes and cell types.

Temporins - the first 10 membersof which were isolated from the skins secretions of the European common frog *Rana temporaria*, are a notable example; Giacometti *et al.* (2005) investigated the activity of temporin A against *Enterococcus faecalis* and reported both direct activity and synergism with imipenem and co-amoxiclav (Simmaco et al. 1996;Giacometti et al. 2005).

LL-37, a cathelicidin class antimicrobial lpeptide found in humans, it can potentiate the macrolide antibiotic azithromycin and the combinationto be synergistic at sub-MIC concentrations against MDR strains of *P. aeruginosa, K. pneumoniae and A. baumannii.*

C. Efflux Pump Inhibitors

Bacterial efflux pumps act to decrease intracellular concentrations of antibiotics by pumping antibiotics out of bacterialcells, thereby reducing their effectiveness. The presence of efflux systems has been confirmed in prokaryotic species, archaea and both inferior and superior eukaryotic species. Their main function is the extrusion ofundesirable compounds from cells; these include heavy metals(Nies 2003), organic solvents (Ramos et al. 2002), dyes such as ethidium bromide (Kaatz, Seo and Ruble 1993), amphiphilicdetergents (Ma et al. 1994), biocides (Costa et al. 2013), quorum sensing molecules (Pearson, Van Delden and Iglewski 1999)and metabolites (Van Dyk et al. 2004) in addition to antibiotics. The presence of efflux pumps and their clinical significance in contributing towards AMR has been confirmed in many bacteria, including *M. tuberculosis* (Ainsa et al. 1998) and *P. aeruginosa.* The latter species possesses which together can extrude fluoroquinolones, tetracycline, chloramphenicol and some β-lactams to achieve a multidrug resistant phenotype (Piddock 2006). Efflux systems havealso been implicated in biofilm formation in a number of different bacterial species. Prokaryotic efflux systems can be categorised resistance-nodulation-division (RND) family,the major facilitator superfamily (MFS), the ATP-binding cassette(ABC) superfamily, the small multidrug resistance (SMR)family and the multidrug and toxic compound extrusion

(MATE) family (Sun et al. 2014). A popular approach to combating bacterial efflux systems has been the development of EPI compounds.

Efflux Substrate Competition. Competition for pump binding betweendiscrete EPI and antibiotic molecules - Major classes of EPI : Catechingallates (green tea extracts), Abietane diterpines (herb *Rosmarinus officinalis*), Methoxylated flavones and isoflavones (Baicalein, isolated from the leaves of *Thymus vulgaris*), Homoisoflavonoids–Bonducellin and digyna purified from the roots of Caesalpinia, Trimethoprim andsertraline - The combination of trimethoprim, a dihydrofolatereductaseinhibitor, and sertraline, a selective serotonin reuptake.Inhibitor(SSRI), is synergistic with three conventional antibiotics. (levofloxacin,piperacillin and meropenem) against *P. aeruginosa*, Proton pump inhibitors - omeprazole and lansoprazole, have inhibitory activity towards NorA in S. aureus, Calcium channelblockers - Verapamil, a drug used to treat cardiac disorders through inhibiting mammalian efflux transporters such as P-glycoprotein.

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LP-AMR-02

DESIGN AND IMPLEMENTATION OF ANTIMICROBIAL STEWARDSHIP PROGRAMS IN VETERINARY MEDICINE BASED ON ONE HEALTH PRINCIPLES

Rudrappa S.G.*, Simon T. and Prayaga K.

*Technical Manager, Zoetis India Ltd,

Email: Shashidhara.rg@zoetis.com

Antimicrobial resistance (AMR) has become a major concern in both human and veterinary medicine. Innovation of novel antibiotics is lagging in human medicine. Antibiotics are not available to treat multi-drug resistant (MDR) bacteria, serious community and hospital-acquired infections. It is increasingly accepted as a fact that human use is most important contributor to AMR in human pathogens. In veterinary medicine AMR is not prevalent as human medicine, AMR is critical in some key pathogens such as MDR Escherichia coli in all species, MDR methicillin-resistant Staphylococcus skin infections in dogs. Primary concern about AMR in veterinary medicine is the spread of resistant bacteria from animals to human through direct transmission or through the food chain. One Health is a collaborative, multisectoral and trans-disciplinary approach to achieve optimal health outcomes based on the interaction between people, animal, plant and their shared environments. In line with One Health principles, regulatory agencies have categorized antimicrobials based on their importance in human medicine. Medically important antimicrobials are being phased out from use in feed for growth promotion in food animals. Veterinary oversight is recommended for therapeutic use of all medically important antimicrobials. Overall, the regulatory guidelines on responsible use of antimicrobials in food animals and emphasis on preventive tools such as vaccination, timely and accurate diagnosis of disease will ensure production of safe food and sustainable use of antimicrobials in humans and animals.

F-AMR-O1

EVALUATION OF IN VITRO ANTIBACTERIAL AND IMMUNOMODULATORY ACTIVITY OF *ACHYRANTHES ASPERA* EXTRACT IN BROILER CHICKS

ShraddhaNety Monika, K. M. Koley, Kranti Sharma and S. M. Tripathi

Department of Veterinary Pharmacology and Toxicology,

College of Veterinary Science & Animal Husbandry,

Anjora, Durg, (C.G.) 491001.

The present study, was conducted to evaluate the Phytochemical analysis, antibacterial and immunomodulatory activity of *Achyranthesaspera* extract against Pathogenic Microrganism such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella gallinarum* and *Pseudomonas aeruginosa*. Methanolic

extract of aerial part of *Achyranthesaspera* were prepared and tested by "Disc Diffusion Technique". Ciprofloxacin was used as positive, Extract of *Achyranthesaspera*revealed antibacterial activity against both gram-positive bacteria (*S. aureus*) and gram-negative bacteria (*E. coli, Salmonella gallinarum and P.auriginosa*). The immunomodulatory effect of extracts of *Achyranthusaspera* aerial parts was done at the dose rate of 0.4g/ liter in drinking water as alternative to antibiotic growth promoter in broiler chicks. For cell mediated immunity study, 18 chicks were divided into 3 treatment groups (T1, T2 and T3) 6 chicks in each replicates. Similarly, for humoral immunity study, 18 chicks were divided into another 3 treatment groups of 6 chicks in each replicates. Each bird of different groups was individually identified by using leg band. T1 (Control diet), T2 (Standard growth promoter; BMD @ 0.05% in feed), T3 AAE @ 0.4g/L) in drinking water daily for consecutive 42 days. The *Achyranthesaspera* extract group was significantly higher skin thickness in DNFB skin sensitization test both at 24 hours and 48 hours after sensitization as compared to the control group. However, did not showed stimulation of humoral response. Inconclusionmethonolic extract of *Achyranthusaspera* found to have potent antibacterial activity against various pathogenic organism andimmunomodulatory activity in broiler chicks.

F-AMR-O2

SCREENING OF MILK BORNE *STAPHYLOCOCCUS AUREUS* FOR RESISTANCE AGAINST BETA LACTAM ANTIBIOTICS

B. Anurag, S. Ramesh, T. Ramasamy, L. Kalaiselvi, S.J. Deepak, R. GokulaKannan

Department of Veterinary Pharmacology and Toxicology,

Madras Veterinary College, TANUVAS, Chennai - 600007

Antibiotic resistance is one of the growing concerns facing the veterinarians in the treatment of mastitis. A study was carried out to screen milk borne *Staphylococcus aureus* for resistance against Beta lactam antibiotics. A total of 45 milk samples were collected over a period of three months from outpatient unit of MVC, Chennai. Upon collection of samples, ABST was carried out to see the antibiotic sensitivity patterns of commonly used antibiotics in mastitis. Phenotypic screening of *Staphylococcus* species was carried out by streaking of inoculum in Mannitol Salt Agar. Genotypic screening for *Staphylococcus* screening was done with the help of PCR by using nuc gene primer. The mecA gene indicative of MRSA species was also checked using PCR technique. MIC for ceftriaxone and cloxacillin was carried out with the samples that were found positive for *Staphylococcus aureus*. The antibiotic sensitivity pattern is presented: Fluoroquinolones (87.5% sensitive), aminoglycosides (72.5% sensitive), Amoxyclav (72.5% sensitive). The other classes were less sensitive. The MSA screening revealed 40 positive samples for *Staphylococcus*

species. The MSA positive samples were subjected to molecular identification with the help of PCR. The results revealed 10 samples positive for *Staphylococcus aureus* and 5 among them positive for mecA gene. The MIC results were as follows: MIC_{50}-10.95µg/ml and MIC_{90}- 87.510.95µg/ml for ceftriaxone and MIC_{50}-43.75 µg/ml and MIC_{90}- 87.5µg/ml for cloxacillin. The MIC results are significant indicating development of resistance for cephalosporins and cloxacillin which are commonly used in the treatment of mastitis. However, further studies are required in a larger sample size that can help us to attain more conclusive results.

F-AMR-O3

MONITORING OF ENROFLOXACIN RESIDUE IN CHICKEN MEAT FROM TAMILNADU, KARNATAKA, AND KERALA

GokulaKannan, R., Ramesh, S., Kalaiselvi, L. and Sriram. P.

Department of Veterinary Pharmacology and Toxicology,

Madras Veterinary College, TANUVAS, Chennai 600007

The poultry business is one of the fastest and largest developing agro industries in the world. In order to increase the production, poultry farmers are using many antibiotics which may be detrimental from the residue point of view. Present research was focussed to study the prevalence of antibiotic residues in chicken meat by screening for the fluoroquinolone antibacterial commonly used in chicken, enrofloxacin. 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 Enrofloxacin by High Performance Liquid Chromatography (HPLC) method. The mobile phase was isocratic with orthophosphoric acid with a pH of 2.5 and the detection was made at 278 nm. The method had a sensitivity of 30 ppb, which is enough to test for the MRL of 100 ppb for enrofloxacin in chicken meat. Totally, 436 samples were collected at fourteen districts of Tamilnadu, Kerala, and Karnataka. All the chicken meat samples were evaluated by HPLC method and all were negative for enrofloxacin. This type of monitoring is essential to provide safe foods free of contaminants.

F-AMR-O4

MONITORING OF OXYTETRACYCLINE RESIDUE IN CHICKEN MEAT FROM TAMILNADU, KARNATAKA, AND KERALA

Ramesh, S., GokulaKannan, R., Kalaiselvi, L. and Sriram. P.

Department of Veterinary Pharmacology and Toxicology,

Madras Veterinary College,TANUVAS, Chennai 600007

Tetracyclines are broad-spectrum antibiotics, commonly used in livestock to treat and prevent the disease and as growth promotion. Continuously, small amount of drug is administered for longer time known as drug residues. With increasing buyer awareness and international trade restrictions, monitoring becomes a very important activity. The current study was carried out to study the prevalence of OTC in chicken meat by screening for Oxytetracycline (OTC). Chicken meat samples were collected from retail consumer points of chicken and farms where broiler chicken are reared. The meat samples were brought to the laboratory and processed for assay of OTC. Assay of OTC was carried out using a sensitive commercial ELISA kit. The ELISA kit had a sensitivity of 1.5 ppb whereas the MRL for OTC was 100 ppb. Samples were collected from fourteen districts of Tamilnadu, Kerala and Karnataka. Out of 174 samples tested, 41 samples were found to be positive but none of the samples were above MRL. The concentrations ranged from 1.5 to 55.99 ppb with a mean concentration of 16.54. The study suggests the requirement of continuous monitoring of residues of oxytetracycline from the point of view of public health.

F-AMR-O5

SCREENING OF ANTIBIOTIC-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM COW MILK AND THEIR ANTIBIOTICS SUSCEPTIBILITY PATTERNS TO COMMONLY USED ANTIMICROBIAL AGENTS

Addison M. Pule and SarathchandraGhadevaru

Pharmacovigilance Laboratory for Animal Feed and Food Safety,

DCAHS, TANUVAS, Chennai - 600051, Tamil Nadu, India,

E-mail: richeaddson@gmail.com

The aim of this study was to investigate the occurrence, antibiotic susceptibility profiles, and determinants of *S. aureus* gene isolated from milk obtained from Madras Veterinary College Teaching Hospital. To achieve this, 84 samples of fresh milk were collected from all four mammary quarters. *S. aureus* was isolated and positively identified using morphological (gram staining), biochemical (catalase, fermentation of mannitol salt agar) tests and molecular (nuc gene specific PCR) methods. The antimicrobial resistance profiles of the isolates were determined using the phenotypic agar diffusion method. Among all the samples examined, 37 of 84 milk samples (44.04%) were isolated based on cultural and biochemical properties. Susceptibilities of the isolates to a panel of 6 different antimicrobial agents that include tetracycline (30 µg), amoxicillin-clavulanic acid (30 µg), gentamicin (10 µg), enrofloxacin (10 µg), ampicillin (10 µg) and ampicillin/cloxacillin (10 µg) were then determined. Based on antibiotic sensitivity test, the isolates were resistant to ampicillin (89.18%), amoxicillin-clavulanic acid (78%) and

ampicillin/cloxacillin (75.67). On the other hand, the isolates were found to be sensitive against enrofloxacin (86.49 %), tetracycline and gentamicin (94.6 % in both the cases). The indiscriminate use of antibiotics/antimicrobials agents for prophylactic as well as other therapeutic purpose could be the reasons for increased antimicrobial resistance of *S. aureus*. This study highlights the need for continuous surveillance of antibiotic sensitivity pattern of *Staphylococcus aureus* with a view to selecting appropriate therapy.

F-AMR-O6

DEVELOPMENT OF POLYHERBAL NANO-EMULGEL FARM GATE THERAPEUTIC KIT FOR BOVINE MASTITIS

SenthilKumar,P., V.Ranganathan, K.Vijayakaran and A.Elamaran

Veterinary College and Research Institute, Orathanadu-614625, Tamil Nadu, India.

Email: p.senthilvet@gmail.com

Bovine mastitis, an inflammation of the mammary gland in cows, is a major disease challenge for the dairy industry worldwide. The lack of therapeutic success against mastitis has prompted a reevaluation of treatment strategies using herbal plants. Most of the biologically active constituents of extracts, such as flavonoids, tannins, and terpenoids, are highly soluble in water, but have low absorption, because they are unable to cross the lipid membranes of the cells, have excessively high molecular size, or are poorly absorbed, resulting in loss of bioavailability and efficacy. Hence, it was planned to formulate and evaluate the herbo-mineral nanoemulgel therapeutic kit for bovine mastitis containing *Daturametel, Aloe vera, Curcuma longa* and calcium hydroxide. Phytochemical screening revealed the presence of alkaloids, glycosides, phenols, flavonoids, terpenoids, carbohydrates, tannins and protein. Antibacterial activity of extract of *Daturaalba, Aloe vera, Curcuma longa* and herbo-mineral formulation were studied against pathogens isolated from cow infected with bovine mastitis. The agar well-diffusion method showed significant zone of lysis against isolated pathogens and the results were comparable to the conventional antibiotic cefotaxime. Herbo-mineral formulation was converted into nanoemulsion using cremophor-EL as oil phase and tween 20 as aqueous phase by ultrasonicator with mean particle sizes of 218.6±17.24 nm. Then nanoemulsion was made into nanoemulgel preparation using carbapol940 and was evaluated for rheological studies, spreading coefficient studies, skin irritation studies, *ex vivo* release studies. The nanoemulgel formulation was also clinically validated in cows affected with mastitis. The results shown significant effect in clinical cases of mastitis and it was comparable with conventional antibacterial agent cefotaxime.

F-AMR-O7

IN VITRO DETECTION OF BENZIMIDAZOLE RESISTANCE BY LARVAL DEVELOPMENT ASSAY

Asha Rajagopal[1], Lucy Sabu[1], Radhika R.[1], Devada K.[1], Gleeja V.L.[2] and Usha P.T.A.[3]

[1]Department of Veterinary Parasitology, [2]Department of Statistics,

[3]Department of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy, Thrissur, Kerala,

Email: asha@kvasu.ac.in

Larval development assay (LDA) was done for *in vitro* detection of benzimidazole resistance in two organized goat farms and small holder farmers' flocks in Palakkad district. The assay was done in flat-bottom 96 well plates using the nematode eggs extracted from the pooled faecal samples of goats. Thiabendazole was used at effective concentrations of 0.05, 0.03, 0.02, 0.01, 0.005 and 0.001 µg/ml in the assay to which egg suspension diluted 1:1 with amphotericin B was added to give a final count of 50 eggs per well. This was followed by the addition of 20 µl of the nutrient medium containing yeast extract and Earles Balanced Salt Solution. Ten per cent DMSO was added to the control wells instead of the drug. The assay plates were incubated at 25ºC for seven days in a BOD incubator, after which, the number of mature third stage larvae (L_3)and unhatched eggs were counted in each well. The number of L_3 in the test wells were adjusted to that in the control wells and this adjusted proportion was fitted into a sigmoid curve (dose-response curve) after logarithmic transformation from which the LC_{50} was calculated using statistical software SPSS. Larval development assay was also interpreted using the discriminating dose criterion in which the percentage of L_3 which developed at the discriminating dose of 0.02 µg/ml of Thiabendazole (P_{dd}) was determined. The LD_{50} values were 0.044 and 0.011 µg/ml in the two organized farms and 0.002 µg/ml in the small holders' flocks. P_{dd} values were 0.1 and 0.737 in the organized farms and 0.128 in the small holder goat flocks. P_{dd} values were found to be significantly correlated with percentage of larvae with homozygous resistant genotype indicating that it is a better criterion for resistance detection than LD_{50} in LDA.

F-AMR-O8

ASSESSMENT OF AMITRAZ RESISTANCE IN *HAEMAPHYSALIS* SPP. USING BIOASSAY

Tambe Kajal Annasdaeb, Bindu Lakshmanan, Sunanda, C, Asha Rajagopal, Indu V Raj , Suresh N Nair and Devada, K

Department of Veterinary Parasitology,

College of Veterinary and Animal Sciences, Mannuthy, Kerala, Email: bindul@kvasu.ac.in

Tick borne pathogens inflict considerable loss in small ruminant sector. The establishment and spread of acaricide resistance has been a major impediment in tick control strategies in the state. Hence, an attempt was made to assess the status of acaricide resistance among ticks infesting goats in Kerala using bioassays. Larval packet test (LPT) and adult immersion test (AIT) were used to assess the resistance to amitraz in *Haemaphysalis*spp. of ticks infesting goats, in Thrissur and Palakkad districts. Log probit analysis was done to derive the LC50 and LC90 of resistant and susceptible isolates. All the tested population *viz.*, Mundupalam, Poomala, Vadakkanchery and Choondal isolates of *Haemaphysalis* spp. were found susceptible to amitraz by LPT. The LC50 calculated for Vadakkanchery and Choondal isolates were37.97 ppm and 25.99 ppm, respectively, by LPT. Adult tick mortality , reproductive index and inhibition of oviposition were also tested using AIT indicating that the isolates in this study were susceptible to amitraz. Amitraz caused a considerable reduction in reproductive efficiency of ticks as indicated by reproductive index and inhibition of oviposition. The results of the study suggested that amitraz resistance has not emerged among *Haemaphysalis* spp. on goats in the sampling location.

F-AMR-O9

QUANTITATIVE DETERMINATION OF RESIDUAL TETRACYCLINES IN FIELD SAMPLES OF BUFFALO MEAT USING RP-HPLC

S. Kalpana and M.Muthukumar

Chemical Residues Laboratory, National Research Centre on Meat, Hyderabad-500092, E-mail: kalpananrcm@gmail.com

Antimicrobial agents like tetracyclines are most widely used in bovine therapy to combat infectious diseases. Although irrational use of oxytetracycline and chlortetracycline in buffaloes carries the risk of their presence in edible tissues which can be responsible for deleterious effects in consumers, studies related to residue levels in field samples has received only minimal attention. Hence, there is an urgent need for quantitative determination of antimicrobials in field samples of meat. After extraction of the buffalo meat samples for these two tetracyclines, the analysis of the antimicrobials was carried out using a reverse phase HPLC.The chromatographic separation was accomplished with an isocratic mobile phase consisting of 0.1M oxalic acid, methanol and acetonitrile (70:15:15, v/v).A UV detector was operated at a wavelength λmax 360nm.The linearity, recovery, selectivity, intraday as well as interday variation and precision of the modified method were evaluated from buffalo meat samples at drug concentrations ranging from 25-1000 ng/g. Mean extraction recoveries of oxytetracycline and chlortetracycline were in the range of 90-97 %.The

limits of quantification for oxytetracycline and chlortetracycline were 38.30 and 36.63 µg/kg, respectively. About two hundred and fifty buffalo meat samples were collected and analyzed for the levels of oxytetracycline and chlortetracycline in this study.

S-AMR-O1

EVALUATION OF ANTIBACTERIAL ACTIVITY OF CINNAMON OIL AND CLOVE OIL AGAINST BACTERIA

Humbal, B.R., Sadariya, K. A., Prajapati, J.A., Bhavsar, S.K. and Thaker, A. M.

Dept of Veterinary Pharmacology & Toxicology, College of Veterinary Science and Animal Husbandry, AAU, Anand:humbalbrijesh@gmail.com

The study was planned to evaluate *in vitro* antibacterial activity of cinnamon oil and cove oil. Screening of antibacterial activity was done by the disc diffusion method against *Streptococcus agalactiae*(ATCC 13813), *Listeria Monocytogenes*(ATCC 1911), *Staphylococcus aureus*(ATCC 6538P), *Pseudomonas aeruginosa*(ATCC 19154), *Escherichia coli* (ATCC 10799) and *Salmonella typhimurium*(ATCC 23564). It was performed using an 18 h culture at 37°C in 10 ml of Muller Hinton Agar (for *S. agalactiae*5% defibrinated sheep blood was added). The test suspension was standardized to match 0.5 McFarland turbidity standards. The cinnamon oil and clove oil were suspended in a solution containing 10% dimethyl sulfoxide and 0.5% tween 80. Under aseptic condition, empty sterilized discs were impregnated with 50 µl of different concentrations (1:1, 1:2, 1:5, 1:10 and 1:20) of cinnamon oil and clove oil and placed on the agar plate surface. Paper disc moistened with vehicle (DMSO plus tween 80) was placed on the seeded petriplate as a vehicle control. Standard disc containing antibacterial drugs (cefotaxime, ampicillin, tetracycline and gentamicin) were used as reference control. The petri plates were incubated at 37°C for 18 h. After the incubation period, the zone of inhibition was measured. The results of the present study revealed that the cinnamon oil and clove oil showed antibacterial activity. Both gram positive (*Staphylococcus aureus*, *Listeria monocytogenes*and *Streptococcus agalactiae*) and gram negative (*Salmonella typhimurium*and *Escherichia coli*) bacteria were sensitive to the cinnamon and clove oils. *Pseudomonas aeruginosa*was found sensitive to the clove oil (between 15.33±0.88 to 18.33±0.33mm zone of inhibition) but resistant to cinnamon oil. There was no inhibition in growth of bacteria with the vehicle control.

S-AMR-O2

ANTIBACTERIAL ENHANCING EFFECT OF SILVER NANOPARTICLES USING *BIOPHYTUM SENSITIVUM* WITH ANTIBIOTICS IN BACTERIAL ISOLATES FROM BOVINE MASTITIS

Arya Mohan., Nisha A.R., Keerthika V., Preethy John., Haima J.S., Vishnu Soman., Adheena Xavier., Vishnu K., Sujith.S., Usha P.T.A

Department of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy,Thrissur.

Nanoparticle synthesis using plant extract is a growing field in nanotechnology. The green synthesis of nanoparticle using plant extract is eco-friendly, economic and an alternate source for the chemical synthesis of nanoparticles. The objective of the present study was to analyse the antimicrobial properties of *Biophytum sensitivum* nano particle against bacterial isolates from bovine mastitis clinical samples. In the present study disc diffusion antimicrobial assay was conducted by Kirby Bauer assay and minimum inhibitory concentration by microdilution was done in isolates from mastitis milk samples. Biofilm assay was performed by Congo red method. The bacterial isolates used in this study were *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella sp*.The present study used methanolic extracts *Biophytum sensitivum* and silver nanoparticles synthesised from *Biophytumsensitivum* as a reducing agent for the synthesis of silver nanoparticle. The formation of silver nanoparticles was confirmed by UV-Visible Spectroscopy. Phytochemical analysis of the methanolic whole plant extract showed the presence of alkaloids, flavonoids, tannins and saponins. The potentiating activity of silver nanoparticles from *Biophytum sensitivum* is compared with the whole plant extract in combination with various antibiotics. In this study it was found that the silver nanoparticle as well as plant extract showed a potentiating effect on the resistant antibiotics. The isolated bacteria did not produce any biofilm. In this reduced size, biosynthesized silver particles can easily enter inside bacterial cells and thereby by affecting the cellular metabolism of bacteria.

S-AMR-O3

IN VITRO ANTIBACTERIAL AND ANTIBIOTIC RESISTANCE MODIFYING EFFECT OF THYME OIL ON CLINICAL ISOLATES OF CEFOXITIN RESISTANT *STAPHYLOCOCCUS AUREUS*

Adheena, X.[1], Suja, R.S.[1], Haima, S. [1], Jisha S. [1], Arya, M. [1], Nisha, A.R. [1], Nair, S.N.[1], and Usha, P.T.A.[1]

[1]Department of Veterinary Pharmacology and Toxicology,

College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala,

Email: adheenachiitu@gmail.com

Alarming rise in the number of methicillin resistant *Staphylococcus aureus* (MRSA) strains in the recent years has become a significant clinical and epidemiological crisis, as resistance to methicillin implies resistance to all β-lactam antibiotics. *S. aureus, especially MRSA* is one of the most common pathogens

associated with bovine clinical and subclinical mastitis. Surveillance of epidemiology, prevalence and incidence of MRSA is thus essential to develop strategies for prevention of economic loss for dairy producers and also for safeguarding the human health based on the "One Heath" policy. However, cefoxitin disk diffusion assay is a better detector of methicillin resistance in *S. aureus* since it is a more potent inducer of the *mecA*regulatory system than the penicillins. Furthermore, current studies have demonstrated the role of essential oils in efficiently combating the bacterial resistance mechanisms and confer better efficacy of the conventional antibiotics in combination with the oil.Therefore, the aim of the study was to investigate the antibacterial as well as antibiotic resistance modifying activities of thyme oil obtained from *Thymus vulgaris* (Family:Lamiaceae) against cefoxitin resistant (methicillin resistant) *S.aureus* isolates from bovine mastitis. The antibacterial activity of the thyme oil against resistant *S.aureus* was investigated using agar disc diffusion assay, either alone or in combination with cefoxitin. Antimicrobial effect of thyme oil tested at different concentrations revealed moderate activity against the resistant *S. aureus* strains, whereas thyme oil in combination with cefoxitin modulated the resistance and potentiated the sensitivity of the isolates to cefoxitin. Thus, the results of the study indicated antibacterial and antibiotic resistance modifying effect of thyme oil on cefoxitinresistant (methicillin resistant) *S. aureus* isolates from clinical and subclinical bovine mastitic milk samples and may be ascribed to the future therapeutic alternativesagainst MRSA infection.

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Pharmacogenomics in the development and validation of indigenous drugs
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ANTIBIOTIC RESISTANCE: A RUNDOWN OF A GLOBAL CRISIS

Suresh Kumar Sharma and Swatantra Kumar Singh*

College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (Punjab), *College of Veterinary Science & AH, NDVSU, Rewa (M.P.)

Antibiotics are used for treatment or prevention of bacterial infection. Nearly all classes of antibiotic are based on the structure of antibiotics naturally found in environmental microorganisms; with many of the antibiotics in widespread use being synthetic derivatives of these natural structures (Demain, 1999). Ever since penicillin was introduced into medical therapy in 1942, hundreds of other antibiotics have been isolated or synthesized for the treatment of human and animal infections. Antibiotics played a significant role in the increase in life expectancy witnessed in the second half of the 20th century. Antibiotics transformed modern agriculture and livestock industries, the latter of which used antibiotics for prophylaxis, meta-prophylaxis, treatment for infection, and as a growth promoter to enhance feed efficiency in healthy livestock. The overuse and misuse of antibiotics stimulated the more rapid emergence of antibiotic-resistant bacteria (ARB) and antibiotic resistant genes (ARGs), reducing their therapeutic potential against human and animal pathogens. World Health Organization characterises antimicrobial resistance as a global public health crisis that must be managed with the utmost urgency (WHO 2015).

Global alarm of Antimicrobial resistance

In 1945, Alexander Fleming shared the Nobel Prize for Physiology or Medicine in recognition of his part in the discovery of penicillin. In his Nobel Lecture, he warned that "it is not difficult to make microbes resistant to penicillin," and that people may die as a result of bacteria becoming resistant to antibiotics in the future. Fleming's prediction has come true (Simon, 2014). New surveillance data released today by the World Health Organization (WHO) reveals widespread and in some cases high levels of antibiotic resistance across the globe in the most common bacterial infections. In the first report from the WHO's Global Antimicrobial Resistance Surveillance System (GLASS), data from 22 countries and more than 500,000 isolates show that *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae,* and *Salmonella* spp are the most commonly reported resistant bacteria.

India, the antibiotic capital of the world

Antimicrobial Resistance (AMR) is a major public health concern in India. The emergence of resistance is not limited to the older and more frequently used classes of drugs. The One Health concept

highlights the importance of inter-dependence of human, animal and environmental parameters for the containment of AMR. The same holds true for India wherein the rates of AMR in all these three sectors have been rising disproportionately in the past decades. Another issue is the lack of sufficient research and paucity of data that not only hampers the estimation of exact rise and extent of AMR in India but also prevents a nation-wide comparison. Out of 2152 studies published by Indian institutions on AMR, 1,040 (48.3%) were on humans, while only 70 (3.3%) on animals, 90 (4.2%) on environment and 11 (0.5%) on One Health (Taneja and Sharma, 2019).

Although the per capita consumption of antibiotics in India (10.7 units per 10 capita) was lower than that seen in many other countries (e.g. 22 units per capita in USA), the overall population and infection load led to higher total consumption. With respect to consumption of antimicrobials in food animals, the global consumption was estimated to be 63,151 (±1,560) units in 2010; India accounts for 3% of the global consumption and is the fourth highest in the world, behind China (23%), the United States (13%) and Brazil (9%). The consumption of antimicrobials in the food animals sector in India is expected to double by 2030 (Inter-Ministerial Review Meeting on Antimicrobial Resistance, 2016).

Dynamics of Developing Antibiotic Resistance

Antibiotic use is a key driver for antibiotic resistance. In fact, the introduction of a new antibiotic is often followed by reports of emerging resistance within months or a few years. Human antibiotic consumption is reported to have increased by 36% globally between 2000 and 2010. The variation in resistance rates within and between countries reflects the variations in antibiotic use, which is in turn related to socioeconomic factors, cultural differences, and remuneration incentives. Antibiotic misuse and overuse causes selection of resistant strains. It has been estimated that as much as 50% of antibiotic prescriptions are inappropriate. In many countries outside Europe and North America non-prescription use is still common. Such usage has been reported to account for as much as 19% of all consumption, in some cases even up to 100%.

Antibiotic resistance is of increasing concern worldwide, but initiatives to curtail inappropriate use have had little success. Studies of several antibiotic combinations, such as meropenem and sulbactam, have reported no additional advantage over their individual constituents, and have been reported to cause toxic reactions and promote resistance (Ahmad et al, 2016).The regulation of fixed dose combinations (FDCs) of antimicrobials in India is important from a global perspective. The growth of worldwide trade and travel has allowed resistant microorganisms to spread rapidly to distant countries and continents. New Delhi metallo-β-

lactamase, an enzyme that causes bacteria to be resistant to antibiotics, was first reported in India in 2008 and is now found worldwide. Moreover, FDCs that have been banned in India have been reported to be exported to other African and Asian countries. Such exports are a setback for the individuals or organisations trying to implement antimicrobial stewardship initiatives in these settings. Inadequate knowledge of physicians about FDCs is also an exacerbating factor in antibiotic resistance. Injudicious use of antibiotic FDCs could lead to emergence of bacterial strains resistant to multiple antibiotics. Approximately 118 antibiotic FDCs are available in India (Ahmad et al. 2016; Shankar et al. 2016).

Antibiotic usage is also not exclusive to humans. Every day, antibiotics are used to treat livestock and fish to prevent infections. Similar to overuse in humans, uncontrolled use of antibiotics creates a reservoir of bacteria that could become resistant, thus rendering the antibiotic useless. As a result of cities becoming more densely populated, people are exposed to more pathogens all the time. Hospitals and clinics are seeing more and more patients with infections, and it is not always possible to curb the spread of a pathogen in a population. Identification, isolation or treatment of all infectious diseases are not often feasible, resulting in the addition of more pathogens to the local community Coupled with lack of hygiene and poor sanitation, urban centers become and ideal breeding ground for bacteria. Several social factors have been associated with inappropriate antibiotic use in India among the general public and formal healthcare providers. Among the general public, such factors include self-medication, access to antibiotics without prescription, use of pharmacies and informal healthcare providers as sources of healthcare, and lack of knowledge about when to use antibiotics. Self-medication is mainly to avoid the financial burden of expensive allopathic medical visits and is compounded by the availability of drugs without a prescription. The major sources of self-medication are previous doctors' prescriptions and leftover medicines from previous illnesses. Self-medication with antibiotics is a common practice for infections such as the common cold, indicating a lack of knowledge of when to use antibiotics. In rural areas, when there is a lack of healthcare services in their village, people may want to avoid the travel cost to get allopathic services and instead approach informal healthcare providers and chemists or pharmacists at pharmacy stores. In urban areas, doctor fees and diagnostic investigation charges may prevent people from visiting formal healthcare providers.

In recent years, India has emerged as a global hotspot for antibiotic resistance (ABR), with increasing resistance rates to most antibiotics in common pathogens and rising number of treatment failures. Apart from the human health sector an additional area of concern in India is the rampant use of antibiotics in the food-animal production sector. There are few regulations governing the use of antimicrobials for cattle, chicken, and pigs raised for domestic consumption in India, with no stringent implementation protocols even when

there are regulations. Several studies show the use of antimicrobials as growth promoters is quite widespread. Non therapeutic usage of antibiotics has been especially common in poultry production.

Antimicrobial resistance (AMR) is a major global health concern and pharmaceutical pollution is posing dangers to ecosystems and human health worldwide. The devastating effects are already evident in India and China, where most active pharmaceutical ingredients (API) are manufactured. The Indian pharmaceutical industry supplied 20% of generic drugs, with an estimated US$15 billion in revenue in 2014 (Nordea Asset Management 2015). However, the waste water effluents from the antibiotic manufacturing units contain a substantial amount of antibiotics, leading to contamination of rivers and lakes (Larsson et al. 2007; Gothwal and Shashidhar 2017). The existing good manufacturing practices (GMP) framework (WHO 2016) is restricted to drug safety and does not include environmental safeguards. The development of new antibiotics has been successful in significantly reducing morbidity and mortality. With increasing use there has occurred an increase in antibiotic resistance but not a parallel increase in new agents with significantly improved spectrum of activity. Without concerted action from the pharmaceutical industry, physicians, academia, health care providers, and governments, the prospects look gloomy.

The link between sanitation, or lack thereof, and antimicrobial resistance (AMR) is primarily to do with two factors: the level of antibiotic resistant bacteria in a person's gut, and the level of AMR in the environment. The argument that resistance starts in a hospital and then spreads into the community or environment is often inaccurate. Antibiotic resistant bacteria (and the antibiotics themselves) are excreted with effluents and sewage into the environment, and from there re-contaminate humans and animals via drinking water or food. Many studies on antibiotic resistance (AR) focus on hospital infections yet in developing countries where sanitation is so poor, the continuous recycling of antibiotic resistant bacteria in poor communities invariably impacts on the health of those communities, the life span of the individual and represents a financial burden. More studies are urgently needed examining the risk factors for carriage of AR bacteria and their impact on human infections and wellbeing. Greater political commitment is required and a global awareness campaign encapsulating a "one world health" message, as, invariably, it is an issue that has global ramifications.

Surveillance and monitoring for antimicrobial use and resistance

Surveillance and monitoring are widely acknowledged as critical components of the response to antimicrobial resistance. To be most effective, surveillance systems should be coordinated and complementary. They should cover human and animal populations and food as well as, when supported by

scientific evidence and risk assessment, plant production and relevant aspects of the environment. They should also, as far as possible, provide harmonized – or equivalent – data that can be easily compared, exchanged, used or aggregated locally, nationally and globally. The Global Antimicrobial Resistance Surveillance System (GLASS) is being launched to support a standardized approach to the collection, analysis and sharing of data on AMR at a global level, in order to inform decision-making, drive local, national and regional action, and provide the evidence base for action and advocacy.

In 1997, the World Health Organization for Animal Health (OIE) proposed standards pertaining to resistance surveillance. In order to monitor the status of AMR, the first model of national surveillance program was the *Danish Integrated Antimicrobial Resistance Monitoring and Research Programme* (DANMAP), initiated by the Danish Government in 1995. On similar pattern, *National Antimicrobial Resistance Monitoring System (NARMS) was constituted in 1996 with joint efforts* of the United States Department of Agriculture, FDA and the Centers for Disease Control and Prevention (CDC). In 2009, European Medicines Agency (EMA) launched the *European Surveillance of Veterinary Antimicrobial Consumption* program to monitor AMU in animals from member states. The *Canadian Integrated Program for Antimicrobial Resistance Monitoring Surveillance was designed in light of a* 2002 report from the Advisory Committee on Animal Uses of Antimicrobials, and Impact on Resistance and Human Health. In Asia, the *Japanese Veterinary Antimicrobial Resistance Monitoring system was initiated in 1999. Korean Nationwide Surveillance of Antimicrobial Resistance was established* in 1997 in South Korea. In Indian context, there are no regulations for the use of antibiotics in food animals. The Global Antibiotic Resistance Partnership (GARP) was established in the year 2009 to develop actionable policy recommendations for spread of AMR, specifically relevant to low and middle-income countries, including India. In India, the issue of AMR came to the attention of policymakers with the 2010 discovery of NDM-1 and the controversy over its name. Subsequently, AMR-related policies were initiated in 2011 by publishing the National Policy on Containment of AMR. In addition, other nongovernmental initiatives such as the Chennai Declaration were published to create a roadmap to tackle the AMR problem. Surveillance of antibiotic resistance in priority bacteria from humans, food animals, and environmental sources is an essential component of a national action plan on antibiotic resistance.

Strategies to Contain the Development and Consequences of Resistance

AMR requires ownership and active participation by several stakeholders, some of which are: the Ministry of Health, the Ministry of Agriculture and Rural Development, the Ministry of Education, the national and regional regulatory authorities, medical, laboratory and veterinary professional bodies, medical

and veterinary institutions, national medical and veterinary research institutions, health facilities in public, private and other sectors, international agencies, NGOs, mass media, community and faith based organizations, professional associations, consumer organizations, and other concerned bodies. The main objectives of the AMR Strategy are to: 1) Strengthen the infrastructure needed to address the AMR situation through adequate support of the inter-sectoral coordinating mechanism (ICM), appropriate legislation and strengthening of relevant surveillance and feedback systems in Human and Animal Health as well as the environment. 2) Foster improved awareness and education on AMR among healthcare professionals, veterinary professionals and the public, as well as the measures needed to prevent it. 3) Introduce overarching measures to ensure appropriate antibiotic prescribing and use in community, hospitals and veterinary practice and in both Human and Animal Health sectors. 4) Improve Infection Prevention and Control (IPC) through national coordination and oversight, implementation of effective IPC multi-modal strategies in all healthcare facilities and foster hygiene standards in farms to prevent cross-transmission of animal pathogens. 5) Encourage and support innovation, research and networking in areas relevant to AMR.

Conclusion

Countries and networks at the forefront of AMR should make efforts to engage additional stakeholders in developing such an agenda, recognizing that an effective strategy will have far-reaching benefits in minimizing the impact of this urgent problem on human and animal health, the environment, the global economy, and national and global security.A global, multidisciplinary, long-term approach toward novel diagnostic development and identifying the critical control points is required. Control of AMR should be taken as a **"global priority"** before it becomes too grim. A One Health approach integrating human, animal and environmental whole-genome sequencing surveillance data is essential to getting to the root of AMR and developing effective prevention and control strategies

"*Encourage the use of alternative treatment and prevention options including vaccines and the development and use of affordable diagnostics tests in human and veterinary medicine*", to reduce the burden of antibiotics and resistance.

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LP-CR-02

CURRENT REGULATORY LANDSCAPE FOR VETERINARY DRUG APPROVAL

Supritha Sinha

Indian Immunologicals

Indian Pharmaceutical market has its comprehensive role in shaping public health outcomes as well as enormous contribution to Indian economic growth. This industry supplies over 50 per cent of global demand for various vaccines, 40 per cent of generic demand in the US and 25 per cent of all medicine in UK. Contributing significantly in improving the public health by providing universal healthcare at affordable price.India's pharmaceutical exports stood at US$17.27 billion in FY18 and have reached US$ 19.14 billion in FY19 [1] [2]. The India Veterinary Healthcare market is expected to register a CAGR of around 10% during the forecast period, 2018 to 2023[3]. Indian veterinary drug market is emerging and expected to dominate as fastest growing market segment.

Drug development process embraces several steps; exploratory stage, preclinical stage, IND application, Clinical phase, Pharmacovigilance etc. During all these stages multiple regulatory approval are required. The most extensive and critical phase of drug development is Clinical phase where the investigational drug is tested on target population in the field condition. This stage has multiple stringent regulatory stipulations which needs to be critically followed for successful licensing of drug. Some of which comprises of compliance with VICH guidelines, Institutional Animal ethical approvals, Institute Biosafety Committee Approvals etc. [4] Recently pharmacovigilance has also been included as a new step into the drug approval process for veterinary drug in India.

Different countries function through their own regulatory authority to enforce the rules and regulations and issue the guidelines to regulate the marketing of the drugs. It is necessary to have knowledge about regulatory requirement for marketing authorization application of each country. The regulatory agency for USA and INDIA is a single agency i.e.USFDA and CDSCO respectively, whereasin EUROPE, there are three regulatory agencies, they are EMEA, CHMP and NATIONAL HEALTH AGENCY[5].

IND (Investigational New Drug) application is filed by sponsor to regulatory body be forestarting the clinical phase of study in target animals. IND application shall provide high quality data of preclinical and safety studies. After the approval the clinical phase of the study is conducted which includes phase I as exploratory study and phaseII and III asassesment of safety and efficacy of the investigational drug. Phase

IV of the study or Pharmacovigilance then comes into picture. The information is collected from the veterinary health care personal and customer *via* pharmacovigilance agreement (PVAs) as well as other sources. The necessary reports for assesment of adverse event related to drug in true target population of animals is then assessed in terms of Risk-benefit ratio.

A New Drug Application (NDA) can be filed only when the drug successfully passes clinical trials and comprises all animal data, data analyses, pharmacokinetics of drug and its manufacturing and anticipated labelling. These study data along with risk-benefit anaysis is then scrutinized crucially by the scientific committee of the regulatory body. If clinical studies confirm that a new drug is comparatively safe and effective, and will not pose risks to target animal population, the approval is given.

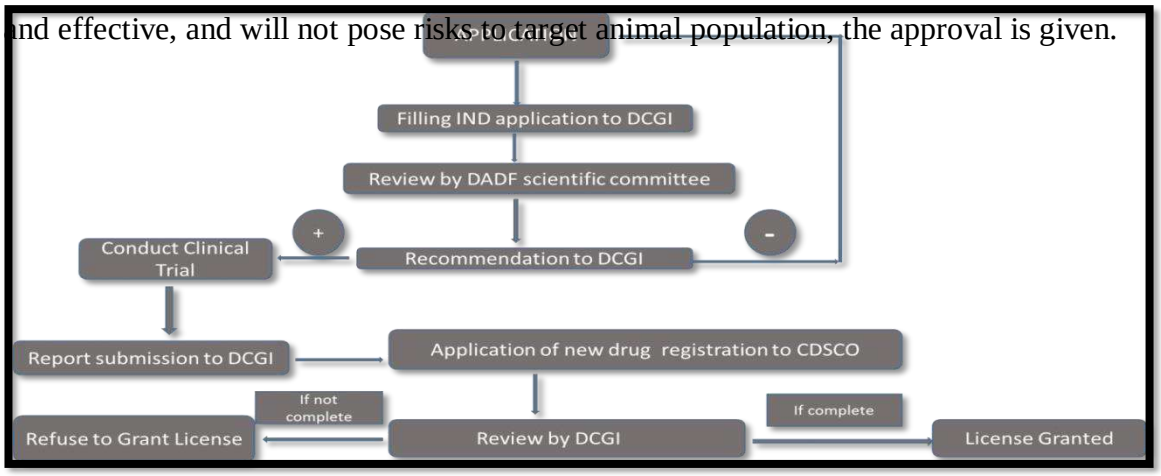

FIG:1 - DRUG APPROVAL PROCESS IN INDIA [6]

The study pertaining to quality, safety and efficacy of drug is submitted and scrutinized by regulatory agency for drug approval, these documental information submitted to regulatory authorities are almost the same in all countries. To harmonize, the International Conference on Harmonization (ICH) has taken major steps for recommendations in the uniform interpretation and application of technical guidelines and requirements. Through the International Conference on Harmonization (ICH) process, the Common Technical Document (CTD) guidance has been developed for United States, European Union, Canada, Japan and other countries. Hence, India also tracks the same.

VICH (Veterinary International Committee on Harmonization) is multinational programme, established in April 1996. Prerequisite guidelines for approval of veterinary medicinal product from regulatory bodies for marketing has been defined by VICH. The Central Drugs Standard Control

Organization (CDSCO) is the national regulatory body for Indian pharmaceuticals. Within the CDSCO, the Drug Controller General of India (DCGI) regulates pharmaceutical and medical devices, under the gamut of Ministry of Health and Family Welfare. For Approval process of veterinary drugs the DCGI is advised by DADF (Department of Animal Husbandry Dairying & Fisheries).

These regulatory guidelines has a number of positive attributes including clarity in regulatory standards, which makes it easier to apply and to spot instances of non-compliance. However, such a system also requires considerable awareness regarding standards to be maintained during preclinical and clinical trials of drug development process. With an aim of gaining a global position in new veterinary drug discovery there is a colossal need to focus on setting standards in each and every step of drug discovery starting from there search studies being conducted in academics till the post marketing surveillance. For the benefit of animal health and veterinary sciences the academics and veterinary drug industry should accord as per the regulatory requirements.

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LP-CR-03

REGULATORY APPROVAL PROCESS FOR VETERINARY DRUGS

Dr. P. Sriram,

Professor and Head, Department of Veterinary Pharmacology and Toxicology,

Madras Veterinary College,

Chennai 600 007.

Animal healthcare products market is a growing economy not only in India but also in the rest of the world due to the demand for the production of the quality animal products and care for the companion animals. The drug discovery and development for the veterinary drugs are similar to the human drug discovery and development, however there are differences in the studies requirements as these drugs will be used in various species as compared to the single species in humans. Hence the target animal efficacy and safety studies are required in addition to the studies required in laboratory animals. There is an additional safety concern for the animal health products used in food animals as the possibility of the presence of residues in the animal food product which will enter in to the human food chain. Hence the studies required to ensure the safety of the veterinary drugs to humans are mandatory as these drugs are consumed by humans as the residues present in the animal food products. There are separate agencies for the approval of veterinary drugs in many countries such as Centre for Veterinary Medicine regulated by US-FDA. There is an international guideline, Veterinary International Cooperation on Harmonization (VICH) with the objective of establishing and implementing harmonized technical requirements for the registration of veterinary medicinal products in the VICH regions, which meet high quality, safety and efficacy standards and minimize the use of test animals and costs of product development. In India, the agency, CDSCO which approves the human drugs is the regulatory agency which approves the new animal drugs. However there is a need for the Veterinary Pharmacologist and Toxicologist to be present in the Veterinary Division of the CDSCO to review the technical dossier submitted by the companies to this regulatory agency for the approval. In this presentation, further information on the studies required for the approval of veterinary drugs in USA, ICH countries and India will be discussed.

F-CR-O1

ANTI-INFLAMMATORY POTENTIAL OF CINNAMON OIL IN FEMALE WISTAR RATS

Prajapati, J.A., Humbal, B.R., Sadariya, K. A., Bhavsar, S.K. and Thaker, A. M.

Department of Veterinary Pharmacology & Toxicology

College of Veterinary Science and Animal Husbandry AAU,

Anand - 388 001, Gujarat, INDIA.

Email: skbhavsar@aau.in

The present study was planned to evaluate *in vivo* anti-inflammatory activity of cinnamon oil *(Cinnamomumzeylanicum)* following single dose oral administration (50, 100 and 200 mg/kg) in female wistar rats. Carrageenan induced paw edema model was used for the *in-vivo* anti-inflammatory activity of cinnamon oil in female wistar rats. All rats were injected subcutaneously with 0.1 ml of a 10% w/v carrageenan suspension (0.1 ml of a 1% suspension in 10% saline) s/c in the sub-planter region of the left hind limb as a local acute oedema inducer after 30 minutes subsequent to oral administration of clove oil. As a standard drug control indomethacin was administered at the dose rate of 10 mg/kg female wistar rats. Rats of control groups were kept untreated. Other three groups were treated with cinnamon oil at the dose rate of 50, 100 and 200 mg/kg b.wt., respectively. Volume of edematous paw will be measured by using plethysmometer (PLM-01 plus, Orchid) at 0 hr (before treatment), 1, 2, 4, 6, 12 and 24 hours after treatments. Increase in paw thickness and per cent inhibition was calculated. Cinnamon oil showed dose dependent anti-inflammatory effect at various doses in female wistar rats. The anti-inflammatory effect of cinnamon oil was highest at 3h (30.58%) at the dose of 200mg/kg. It was lower than anti-inflammatory effect of standard drug indomethacin at 3h (42.99%). The highest anti-inflammatory activity was observed at 3- hour post oral administration of cinnamon oil @ 50,100 and 200 mg/kg b.wt. in female wistar rats.

F-CR-O2

EFFICACY OF IVERMECTIN AGAINST BRUGIAN MICROFILARIAE IN DOGS- A CASE REPORT

Deepa Chirayath, Parvathy J., Ashi R. Krishna and Usha N. Pillai

Department of Veterinary Clinical Medicine, Ethics & Jurisprudence,

College of Veterinary and Animal Sciences, Mannuthy,

Kerala Veterinary and Animal Sciences University,

Email: deepachirayath@kvasu.ac.in

Filarial parasites of the genus *Brugia* are prevalent in dogs in Kerala. Brugian filariae identified in dogs in Kerala are *Brugia malayi and Brugia pahangi*. Adult worms of Brugia genus reside in lymphatics and female worms release hundreds of fully formed sheathed microfilariae into the lymphatics and then into general circulation. The present paper report the therapeutic efficacy of ivermectin against Brugian microfilariae in dogs. A two year old female Rottweiler was presented with reduced weight gain and ulcerated nonhealing wound on the paw of right hindlimb. Physical parameters were in the normal range. Wet blood film examination revealed the presence of moving microfilariae (++++). Laboratory examination revealed leucocytosis, hyperglobulinemia, hypoalbuminemia, and sheathed microfilaria in thick blood smear. The animal was treated with Ivermectin 200 mcg/kg bodyweight for three days. On review after two weeks, wet film was positive (++) and blood smear was positive for sheathed microfilariae. In the next review after two more weeks, both wet film and blood smears were negative.

F-CR-O3

COMPARATIVE EVALUATION OF DIFFERENT HERBS FOR LIPOTROPIC POTENTIAL IN RATS

Mathew J.[1], Suja R.S.[1], Sujith S.[1], Usha P.T.A.[2],Nair N.D.[3] and Sanis, J.[1]

[1]Department of Veterinary Pharmacology and Toxicology, CVAS, Pookode, Wayanad, Kerala,

[2]Department of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy, Thrissur, Kerala,

[3]Department of Veterinary Pathology, CVAS, Pookode, Wayanad , Kerala,

E mail: sujarani@kvasu.ac.in

The escalating prevalence of fatty liver in both humans and domestic animals surged the development of safe and potent alternative therapeutic agents against fatty liver. The present study was undertaken to identify safe and potent lipotropic herbs which can be suggested for treatment of fatty liver. The lipotropic potential of dried leaf powders of *Cassia fistula, Leucas indica, Phyllanthus emblica* and *Sidar homobifolia* was evaluated at 100 mg/kg b.wt. dose level for a treatment period of 14 days in CCl_4 induced fatty liver model. The CCl_4 control rats showed significant increase in serum and hepatic lipids and lipid peroxide (LPO) levels, indicating effective induction of fatty liver, hyperlipidemia and oxidative stress while histopathological examination showed moderate to severe diffuse fatty infiltration in the liver. Amongst the different experimental herbs tested, *P. emblica, S. rhombifolia* and *L. indica* significantly ameliorated hepatic lipids concentration with a distinct reduction in degree of hepatic steatosis histologically, depicting their lipotropic potential. Furthermore, the levels of hepatic LPO and serum cholesterol, ALT, AST and ALP

showed a significant attenuation after treatment with these selected lipotropes, endorsing its hepatoprotective effect. Within these lipotropic herbs identified, *P. emblica* showed comparatively the maximum reduction in hepatic lipids in the CCl_4 induced fatty liver model. Subsequently, these lipotropic herbs were found to be safe upto 2000 mg/kg rat body weight in acute oral toxicity study and phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, glycosides, phenolic compounds and saponins, while steroids were present only in *P. emblica*. Thus the present findings suggest that *P. emblica, L. indica,* and *S. rhombifolia* are safe and effective therapeutic agents for the management of fatty liver in humans and domestic animals.

F-CR-O4

IMMUNOHISTOCHEMISTRY AND HISTOCHEMISTRY OF THE GUT-ASSCOCIATED LYMPHOID TISSUE IN LARGE INTESTINE OF GOATS

Indu V. R., Lucy K. M., Maya S., Ashok N., and Chungath J.J

Department of Veterinary Anatomy, CVAS, Mannuthy, Thrissur, Kerala 680651, India

Immunohistochemical and histochemical studies were conducted on the gut-asscociated lymphoid tissue (GALT) in large intestine of six crossbred male goats of six months of age. The GALT in large intestine were distributed in the proximal colon and in rectum along the whole intestinal circumference in the rectal sinus near the anorectal junction. Histologically, the lymphoid nodules in the patches occurred in two morphologically different forms, *viz*. propria nodules and lymphoglandular complexes (LGC). Goblet cells were strongly PAS-positive for glycogen and acidic and neutral mucopolysaccharides. In the FAE above the dome surface, PAS positive goblet cells could not be seen. In the dome crypts, acidic mucopolysaccharides were more abundant than neutral in the combined PAS and alcian blue technique. In the lamina propria, acid phosphatase gave a reticular reaction in the fibroblastic reticulum cell (FRC) in the parafollicular or internodular regions and linear reaction in the capsule of lymphatic nodules. The FRC gave alkaline phosphatase activity in the form of reticular staining in the centre of lymphatic nodules and internodular area and linear reaction in the capsule. The FITC-conjugated lectin from *Ulex europaeus* (UEA-I) bound intensely to the apical membrane of M-cells. In the peroxidase anti-peroxidase (PAP) technique, strong positive reaction for cytoplasmic IgG bearing B-lymphocytes was noticed within the germinal centre of lymphoid nodules. Since the lymphoid tissue was well developed in the rectal patches, they could be exploited as targets for rectal vaccines for the induction of mucosal immune response in goats.

F-CR-O5

THERAPEUTIC EFFICACY OF TOPICAL SELAMECTIN IN FELINE OTOACARIOSIS

Ambily.V.R.,Kamalu.S.Kumar, Usha .N. Pillai and Ajith Kumar,S.

Department of Veterinary Clinical Medicine Ethics and Jurisprudence,

CVAS,Mannuthy

Email : ambily.vr@kvasu.ac.in

Otodectic mange due to *Otodectes cynotis*, is the most commonotoacariosis in cats and is highly contagious.*Otodectes cynotis* is usually found in the ear canal but it can also be found on the skin surface and cats become infested by direct contact with infested animal. A study was conducted on the therapeutic efficacy of topical selamectin in six cats infested with *Otodectes cynotis* , presented to small animal dermatology unit attached to Teaching Veterinary Clinical Complex, Mannuthy is discussed. Clinical signs include a brown waxy exudate from the external ear canal, intense aural irritation, head-shaking, and scratching at the ears. Diagnosis is made with microscopic evaluation of ear cytology, revealing the presence of the *Otodectescynotis* in all the cases. All the six cases were successfully treated with selamectin spot on preparation @ 6 mg/kg body weight, as single dose applied topically on the dorsal aspect of neck above the shoulder blades. A single administration of a spot-on formulation of selamectin is safe and highly efficacious in the treatment of otodectic mite infestations in cats.

S-CR-O1

PREPARATION, CHARACTERIZATION AND ANTIBACTERIAL EVALUATION OF CEFOTAXIM INCORPORATED λ-CARRAGEENAN HYDROGEL

Haima J. S.[1], Suresh N Nair[2], Usha P. T. A.[3], Suja Rani S.[2], Jisha S[1], Aathira K. K.[1], Dhanusha G.[1], DabholkarAkshay Rajkumar[1], Arya Mohan[1]

1. MVSc Scholar, Dept. of Veterinary Pharmacology and Toxicology, CVAS Mannuthy
2. Assistant Professor, Dept. of Veterinary Pharmacology and Toxicology, CVAS Mannuthy
3. Professor and Head, Dept. of Veterinary Pharmacology and Toxicology, CVAS Mannuthy

Email: drhaimajshajahan@gmail.com

The hydrogels are three dimensional hydrophilic networks composed of either homopolymeric or heteropolymeric chains, which have the ability to absorb high amount of water and drugs within its polymeric structure without itself getting dissolved in water. Hydrogel film was prepared by dissolving λ-carrageenan in distilled water, followed by heating and stirring until a homogenous solution was obtained.

The solution was solidified and dried at room temperature to a constant weight. Film was cross-linked by immersing the prepared film in 5% glutaraldehyde solution in presence of 0.1N HCl as catalyst. The cross-linked film was cured at 110^0C for 25 minutes. The obtained film was air dried at room temperature to a constant weight. Cefotaxim loaded hydrogel discs were prepared for assessment of antibacterial evaluation by incorporating the cefotaxim at a concentration of 1µg/disc, 2µg/disc, and 4µg/disc. Drug diffusion was analysed using agar disc diffusion method. The glutaraldehyde cross-linked λ- carrageenan hydrogel was characterized for its gel fraction percentage, swelling index and equilibrium water content.The FTIR spectra and the swelling index of the obtained hydrogel showed that carrageenan was polymerized. Antibacterial activity was measured by comparing the zone of inhibition produced by hydrogel discs with standard sterile discs. It showed hydrogel discs had an increased zone of inhibition than the standard sterile discs indicating its superiority in antibacterial activity. From the results obtained show that crosslinked λ-carrageenan hydrogel can be effectively used as a novel drug delivery polymer system for topical antibacterial activity.

S-CR-O2

EFFICACY OF MODIFIED UNIVERSITY WISCONSIN-MADISON PROTOCOL FOR MANAGEMENT OF CANINE MULTICENTRIC LYMPHOMA IN TWO DOGS

Anjaly M. V.[1], MadhavanUnnyN.[2], Sindhu K Rajan[3], Usha Narayana Pillai[3]

[1,]MVSc scholar, [2] Associate professor, [3]Assistant professor, [4]Professor and Head,

Dept of Veterinary Clinical Medicine, Ethics and Jurisprudence,

College of Veterinary and Animal Sciences, Mannuthy,

Email:anjaly01@gmail.com

Canine lymphoma is most common neoplastic condition affecting middle aged to older dogs. It is a lymphoid malignancy that orginates from lymph nodes, spleen and liver. Two cases of canine multicentric lymphoma presented to university veterinary hospital, Mannuthy were evaluated for the present study. Both cases were presented with history of swelling of neck, forelimbs, hindlimbs and anorexia. Clinical examination of both cases revealed pale conjuctival mucous membrane, elevated temperature and enlarged lymphnodes. On haematological examination leucocytosis, lymphocytosis and monocytosis were observed.Survey radiographs revealed hepatomegaly and spleenomegaly. Cytology of suprascapularlymphnodes revealed anisocytosisand poikilocytosis in lymphoid population with numerous blast cells. Anaplastic cells with multiple nuclei, higher nuclear to cytoplasmic ratio and numerous mitotic figures could be detected in both cases. Based on history, clinical signs, physical examination and cytology

the cases were diagnosed as multicentric lymphoma with secondary involvement of spleen and liver. Chemotherapy was initiated with modified UW-Madison lymphoma protocol for 19 weeks with vincristine, prednisolone, cyclophosphamide and doxorubicin. Both cases showed complete remission clinically after nineteen week chemotherapy protocol and treatment was discontinued. However, after one month of discontinuing therapy, the one animal become dull and on presentation had enlarged superficial lymphnodes and moderate anaemia. Reinduction of remission or rescue protocol was started with new cycle of Modified UW Madison chemotherapy protocol in one case. Furthur details of the case will be discussed.

ISVPT – 2019

TECHNICAL SESSION

TOXICOLOGY OF XENOBIOTICS

Chairperson : Dr. N. Punniamurthy

Co- Chairperson : Dr.Suresh N. Nair

Rapporteur : Dr. Prakash V. Sogalannavar

College of Veterinary and Animal Sciences, Mannuthy

Pharmacogenomics in the development and validation of indigenous drugs
XIX ISVPT
CVAS MANNUTHY

LP-TOX-O1

RECENT ADVANCES IN TOXICOKINETICS OF PYRETHROIDS

J.K. Malik[1], S.K. Bhavsar[2], A.M. Thaker[3] and A.K. Srivastava[4]

[1]Former Joint Director (Research), IVRI, Izatnagar-243122; [2]Department of Pharmacology & Toxicology, COVS&AH, AAU, Anand-388001; [3]Former Dean, COVS&AH, AAU, Anand-388001; [4]Member, ASRB, New Delhi-110012 [E-mail: jkmalik48@gmail.com]

Pyrethroids are becoming the first choice for use as insecticides both in agriculture and in the house, and in medicine as they have broad-spectrum activity with nonpersistent nature, low mammalian toxicity and rapid rate of degradation. Obviously, the use of pyrethroid insecticides has increased constantly in recent years. The characterization of disposition and toxicokinetics of pyrethroid insecticides including absorption, distribution, metabolism and excretion plays a pivotal role in evaluating their dosimetry, biological response and risk assessment in human beings exposed to these compounds. Toxicokinetic studies provide highly relevant information on the amount of toxicant delivered to its target site as well as differences in biological response. The pertinent information relating to the fate of an insecticide in food animals is necessary in determining its selectivity and toxicity. [1].

The toxicokinetic profiles of pyrethroid insecticides have been characterized in a variety of species at various dose levels and following different exposure routes. Several factors including dose, nature of vehicle, route of exposure and species markedly influence the toxicokinetic behavior of an insecticide. Compartmental pharmacokinetic/toxicokinetic models which do not require information on anatomic structure and physiology cannot predict tissue concentrations. In order to examine chemical distribution in specific tissues, a more sophisticated approach is needed. The physiologically based pharmacokinetic (PBPK) models which devide the body into several physiologically representative compartments with a mass balance for each compartment can predict tissue concentrations of toxicants [1].

The toxicokinetic properties and bioavailability of pyrethroid insecticides permethrin (10 mg/kg body wt) and deltamethrin (0.75 mg/kg body wt) have been investigated in broiler chickens following intravenous and intracrop administration. Following intravenous permethrin administration, the t1/2β was 4.73 h, whereas the t1/2β, and bioavailability of permethrin following intracrop administration were 5.54 h and 0.11, respectively [2]. The t1/2β, MRT and AUC values of deltamethrin after intravenous administration were 4.0 h, 4.65 h and 702 ng.h/ml, respectively. The respective values of t1/2β and bioavailability after intracrop application were 7.27 h and 21.8% [3]. Deltamethrin was shown to rapidly hydrolyze and accumulate in pig tissues. After oral administration of deltamethrin (5 mg/kg body wt) in miniature pigs, the Cmax, Tmax and

AUC0-72 h of deltamethrin were reported to be 17.8 ng/ml, 6 h and 555 ng.h/ml, respectively [4]. The toxicokinetic parameters of fenvalerate have been determined in buffalo calves after its oral administration at 1 mg/kg body wt, and the t1/2β, MRT and AUC values were 8.74 h, 12.6 h and 69 μg.h/ml, respectively [5].

Toxicokinetic profiles of pyrethroids are extremely useful for the development of a biomarker predicting risk to health and, consequently, for interpreting the results of human biomonitoring studies. Measurement of pyrethroid urinary metabolite levels is being used to identify and quantify the internal exposure of humans to pyrethroid insecticides and to provide an integrated assessment of exposure from all sources and routes. The metabolites trans-chrysanthemum dicarboxylic acid, cis- and trans-3-(2,2-dichlorovinyl)-2, 2-dimethylcyclopropane carboxylic acid (cis- and trans-DCCA), cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid (cis-DBCA), 3-phenoxybenzoic acid (3-PBA) and 4-fluoro-3-phenoxybenzoic acid (4F3PBA) in human urine are the commonly used biomarkers for an exposure to pyrethrins and pyrethroids [6,7]. The kinetics of biomarkers of exposure to permethrin has recently been documented in agricultural workers exposed to permethrin following typical exposure conditions in the field. A progressive rise in excretion values was reported with a single peak being reached 29 h following the onset of the 3.5 h exposure and ensuing elimination with a half-life of 6.4 h for trans-DCCA and 8.7 h for 3-PBA [8].

The kinetics of cypermethrin has been shown to be similar to that of permethrin in orally exposed humans and their common biomarkers of exposure may be used for an overall assessment of exposure. The apparent plasma elimination half-lives for trans-DCCA, cis-DCCA and 3-PBA of 5.1, 6.9 and 9.2 h, respectively, following cypermethrin treatment as compared to 7.1, 6.2 and 6.5 h after permethrin dosing have been reported. Corresponding values obtained from urinary rate time courses were apparent elimination half-lives of 6.3, 6.4 and 6.4 h for trans-DCCA, cis-DCCA and 3-PBA, respectively, following cypermethrin treatment as compared to 5.4, 4.5 and 5.7 h after permethrin dosing [9]. Following dermal application, lambda-cyhalothrin absorbed in the body was rapidly cleared in humans. Based on the cis-3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethylcyclopropane carboxylic acid and 3-PBA plasma profiles, calculated mean apparent absorption half-lives were 3 and 7.3 h, respectively, and corresponding mean apparent elimination half-lives were 11.2 and 7.6 h [10].

A human data-based toxicokinetic model of both permethin and cypermethrin has been developed that relates absorbed doses to common biomarkers of exposure, cis- DCCA and trans-DCCA and 3-PBA, as a function of the exposure route and temporal scenarios [11]. Recently, a life-stage PBPK model, supported by in vitro to in vivo extrapolation was developed to predict age-dependent changes in target tissue

exposure of eight pyrethroids namely deltamethrin, cis-permethrin, trans-permethrin, esfenvalerate, cyphenothrin, cyhalothrin, cyfluthrin and bifenthrin. This approach was shown to provide a robust framework for evaluating age-related differences in toxicokinetics and internal target tissue exposure in humans for pyrethroids [12]. This presentation will provide a comprehensive overview of the current knowledge on the toxicokinetics and more recent applications of toxicokinetic models in health risk assessment of pyrethroid insecticides.

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F-TOX-O1

DEVELOPMENT OF SIMPLE AND RELIABLE HPLC METHOD FOR ESTIMATION FLUBENDIAMIDE IN PLASMA

Mandil R.[a], Prakash A[b], Rahal A[c] and Garg S.K[b]

[a]Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Sardar Vallbhbhai Patel University of Agriculture & Technology, Meerut-250110, [ba]Department of Veterinary Pharmacology and Toxicology , College ofVeterinary Science and Animal Husbandry, U.P. Pt. Deen Dayal Upadhyay Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go- Anusandhan Sansthan (DUVASU), Mathura-281001, India, [c]Central Institute for Research on Goat (CIRG), Makhdoom, Farah, Mathura, Uttar Pradesh-281122, India, rajesh_mandil@rediffmail.com

Aim of present study to develop a simple, efficient and reliable HPLC method of flubendiamide estimation in plasma. For this study fifty-four male Wistar rats (130-150 g) were divided into nine groups of six animals each. Saline (I), corn oil (II) and α-tocopherol (@100 mg/kg) (III) were controls groups, next three groups were orally exposed with copper sulphate (@33 mg/kg)(IV), flubendiamide (@200 mg/kg)(V) and copper sulphate (@33 mg/kg)+ flubendiamide + (@200 mg/kg)(VI),respectively. Three more groups were concurrently treated with Copper sulphate +α-tocopherol(VII), flubendiamide + α-tocopherol (VIII) and Copper sulphate +flubendiamide + α-tocopherol (IX), respectively. Duration of exposure period for all xenobiotics groups were 90 days. Plasma sample (0.25 ml) and 0.75 ml hexane: acetone (8:2) mixture was vortexed for 1 min and centrifuged at 12000 rpm for 20 min. The top layer contains clear supernatant was collected and filtered through Millipore 0.22 µm cellulose acetate membrane filter and an aliquot of 20 µl of sample was injected into Rheodyne manual loop injector of HPLC system. Flubendiamide (10.36 min) in plasma shows linear standard curve in the range of 0.039-5.0 $\mu g.ml^{-1}$withcorrelation coefficient (R^2) 0.999. Intra-day and inter-day coefficient of variance were found to be 2.70% and 3.50% respectively, and the mean recovery was 100.00% by using C_{18} reverse phase column as a stationary phase and mixture of acetonitrile: water (70:30, v/v) as mobile phase with flow rate of 0.5 $mlmin^{-1}$ at 210 λ. LOD and LOQ was estimated to be 0.039μgml^{-1}.Significantly (*P<0.05*) higher level of flubendiamide(0.22 ± 0.03 µg/ml) was observed in alone treated group compared to copper + flubendiamide exposed group and simultaneous treatment ofα-tocopherol significantly(*P<0.05*) decreased flubendiamide (0.09 ± 0.02 µg/ml) level in rats of flubendiamide + α-tocopherol. Copper + flubendiamide + α-tocopherol, no significant alteration in flubendiamide residual level was observed as compared with copper + flubendiamide group. Result of this study concluded that this HPLC method of flubendiamide estimation is very simple, reliable and repeatable can be used in estimation of residual level of flubendiamide in biological samples also.

F-TOX-O2

EFFECT OF CONTINUOUS HEATING ON THE 2,3,7,8 TETRACHLORODIBENZOP-DIOXIN (TCDD) CONTENT OF PACKAGED MILK

Sujith S*,Usha PTA*, Juliet, S, Bibu John Kariyil**, Raji, K^.and Shynu, M#**

*Dept. of Veterinary Pharmacology & Toxicology, CVAS Mannuthy, ** Dept. of Veterinary Pharmacology & Toxicology, CVAS Pookode,^ Dept. of Veterinary Physiology, CVAS Mannuthy, # Dept. of Veterinary Biochemistry, CVAS Mannuthy, e-mail:sujith@kvasu.ac.in

The effect of continuous heating on the 2,3,7,8 tetrachlorodibenzo-p-dioxin levels in packaged milk was studied in twelve different brands of milk available in and around Mannuthy. The milk packets were purchased from local market and sampled for detection of TCDD. The same packets were kept in a simulated boiler for 3hours at a temperature not less than 90°C and TCDD was extracted using liquid: liquid extraction. The sample was cleaned up using D-tube and activated carbon silica and the extract was injected in a Gas Chromatograph Mass Spectrometer. The results of the study revealed that eight out of the twelve samples contained TCDD with values ranging from 3.084 and 36.054 ng/g of fat. Six out of these eight samples were tested negative for TCDD when analysed before heating. The results of the study concluded that there was effect of heat on leaching of dioxins into milk and the effect may be due to the low quality of the packets in which milk were packed.

F-TOX-O3

SAFETY ASSESSMENT OF *CINNAMOMUM ZEYLANICUM* OIL IN MALE AND FEMALE WISTAR RATS

Prajapati, J.A., Humbal, B.R., Sadariya, K. A., Bhavsar, S.K. and Thaker, A. M.

Department of Veterinary Pharmacology & Toxicology, College of Veterinary Science and Animal Husbandry AAU, Anand - 388 001, Gujarat, INDIA., skbhavsar@aau.in

The aim of the present study was to evaluate safety of cinnamon oil (*Cinnamomum zeylanicum*) in male and female wistar rats. Forty wistar rats divided into eight groups, each group contains 5 males and 5 females. Group I & V served as vehicle control for male and female, respectively. Cinnamon oil was administered orally at dose of 50, 100 and 200 mg/kg body weight once daily for 28 days in male rats of group II, III and IV as well as in female rats of group VI, VII and VIII, respectively. There was no significant difference observed in body weight and feed consumption in rats of group II, III and IV as compare to group

I (male control) as well as female rats of group VI, VII and VIII as compared to group V (female control). No significant changes have been observed in hematological parameters like Hb, RBCs, PCV, TLCs, MCV, MCH and MCHC as well as no significant changes observed in serum creatinine, BUN, bilirubin, AST, ALT, total cholesterol, total protein and albumin in cinnamon oil treated male and female rats as compared to male and female control rats. Gross and histopathological examination of kidney, liver, spleen and heart from cinnamon oil treated male and female rats did not show any marked gross or histopathological changes. Results of the present study suggest that cinnamon oil is safe following repeated oral administration @ 50, 100 and 200 mg/kg b.wt. for 28 days in male and female wistar rats.

F-TOX-O4

ALTERATIONS IN HEPATIC BIOCHEMICAL AND ANTIOXIDANT BIOMARKERS ON SUBACUTE EXPOSURE OF QUINALPHOS ALONE AND IN CONJUNCTION WITH ARSENIC IN WISTAR RATS

Singh Parvinder, Sharma Priyanka, Verma PK*, Raina Rajinder, Sood S, Pankaj NK

Division of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Science and Animal Husbandry, R S Pura, Jammu, 181102, INDIA , drpawankv@yahoo.co.in

The present study was undertaken to evaluate the alterations in hepatic and antioxidant biomarkers in liver of Wistar rats following repeated co-exposure of commercial preparation of quinalphos and arsenic. Fifty four adult Wistar rats of either sex were randomly allocated into nine groups of six rats. Group I served as control receiving only distilled water, group II and III received orally quinalphos at $1/100^{th}$ and $1/10^{th}$ of LD_{50} (19.9mg/kg) respectively whereas group IV and V received arsenic @ 50 and 100 ppb respectively in drinking water. Group VI and VII received quinalphos @ $1/100^{th}$ and $1/10^{th}$ of LD_{50} along with arsenic in drinking water at the concentration of 50 ppb. Similarly the animals of group VIII and IX received quinalphos @ $1/100^{th}$ and $1/10^{th}$ of LD_{50} along with arsenic in drinking water at the concentration of 100 ppb. The animals were provided toxicants daily for 28 days. Hepatotoxicity was manifested with significantly ($P<0.05$) elevation of plasma transferases (alanine and aspartate) and alkaline phosphatase activity following cisplatin administration. Significantly ($P<0.05$) declined antioxidant biomarkers in hepatic tissue viz. total thiols, catalase, superoxide dismutase, glutathione peroxidase, glutathione-s-transferase, glutathione reductase along with increased ($P<0.05$) malondialdehyde levels indicate increased oxidative damage in liver following repeated administrations of quinalphos at either dose levels or arsenic at the concentration of 100ppb. The alterations in the antioxidant parameters was observed to be more pronounced in co-administered groups as compared to rats exposed to single toxicant. These finding are corroborated with

moderate to severe histopathological alterations in hepatic tissue were observed in all the rats exposed to toxicants and such changes were more pronounced in groups of rats co-administered arsenic and quinalphos. Alteration in histopathological and antioxidant biomarkers in hepatic tissue by each of toxicant indicate their hepatotoxic effects and such effects are potentiated with the co-exposure of these toxicants.

F-TOX-O5

QUERCETIN ALONG WITH CURCUMIN PROTECTED THE BRAIN CORTEXAND HEART FROM HARMFUL EFFECTS OF CADMIUM

Makwana C. N., Patel U. D., Shreesha S Rao, Ladumor V. C., Patel H. B., Modi C.M.

Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry
Junagadh Agricultural University, Junagadh - 362 001

The experiment was carried out to evaluate the toxicity of cadmium after sub-acute exposure in rats and its amelioration by quercetin, curcumin and both in combination. Rats of group C1 were kept as normal control. Rats of toxic control group (C2), vehicle group (C3), quercetin treatment group (T1), curcumin treatment group (T2) and, quercetin and curcumin in combination treatment group were administered with cadmium in drinking water (100 ppm) for 28 days. Rats of vehicle group (C3) were administered with corn oil (vehicle). Rats of group T1, T2 and T3 were orally administered with quercetin (50 mg/kg, P.O.), curcumin (100 mg/kg, P.O.) and both quercetin and curcumin in combination, respectively for 28 days.Adaptation to sub-acute cadmium exposure might be responsible for less ROS production and acquired cadmium tolerance in rats exposed to 100 ppm cadmium through oral route. The oxidative damage following sub-acute cadmium exposure at 100 ppm level through oral route was mainly due to increased level of nitric oxide in rats. Sub-acute cadmium exposure at 100 ppm level through oral route significantly increased lipid peroxidation in brain and heart of rats. Combination treatment significantly decreased cadmium-induced high level of MDA in brain cortex and heart. Sub-acute cadmium exposure at 100 ppm level through oral route resulted in histopathological alterations in brain cortex and cardiac muscle in rats. Quercetin and curcumin in combination showed partial protection to the brain cortex and heart against cadmium induced alterations.

F-TOX-O6

ACUTE ORAL TOXICITY STUDY OF ANILOFOS IN WISTAR RAT

Bagri P., Kumar V. and Singh P.

Department of Veterinary Pharmacology and Toxicology

Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana

The oral maximum tolerated dose (MTD) of anilofos in rat was determined in pilot dose range finding study. Rats were observed for toxic signs and symptoms after single oral administration of anilofos at a dose equal to MTD in 48 hours and 14 days study. The 14 days study was taken to assess delayed toxic effect of anilofos. The body weights of animals were recorded on alternate day for 14 days. Animals were sacrificed after 48 hrs and 14 day, and changes in haematological parameters (Hb, TEC, TLC and DLC) and relative organ weight of various body organs (heart, liver, spleen, kidney, testis, epididymis and brain) were evaluated.

The oral MTD of anilofos in rat was determined to be 750 mg/kg body weight. The signs and symptoms of acute toxicity shown by animals were dullness, depression, respiratory dyspnoea, tremor and altered gait. Animals also showed ataxia, stiffness and fasciculation of muscles, head drop and protrusion of eye ball. Anilofos treatment resulted in decreased body weight gain as compared to the control group up to 14 days. The changes in haematological parameters were not significant between anilofos treated and control group rats in both 48 hrs and 14-day study. The relative weight of liver and spleen of treated group was significantly different at 48 hrs study and the relative weight of liver in 14-day study as compared to their respective control groups. The study revealed that there was no significant delayed toxic effect of anilofos considering its effect on body weight, relative organs weight except liver and haematological parameters in rat.

F-TOX-O7

ARSENIC REDUCES THE ANTIPYRETIC ACTIVITY OF PARACETAMOL IN RATS: MODULATION OF BRAIN COX-2 ACTIVITY AND CB_1 RECEPTOR EXPRESSION

Vijayakaran K[1], Kannan K[1], Kesavan M[2], Suresh S[2], Sankar P[3], Tandan SK[2], Sarkar SN[2], Ranganathan V [1]

Division of Pharmacology and Toxicology, Indian Veterinary Research Institute, Izatnagar, Bareilly, UP

1. Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute Orathanadu.
2. Division of Pharmacology and Toxicology, Indian Veterinary Research Institute, Izatnagar, Bareilly, UP
3. Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute Namakkal,Tamil Nadu.

A study was conducted to examine the subacute arsenic exposure can reduce paracetamol-mediated antipyretic activity by affecting COX pathway and cannabinoid CB_1 receptor regulation. Rats were preexposed to elemental arsenic (4 ppm) as sodium arsenite through drinking water for 28 days. Next day pyrexia was induced with lipopolysaccharide and paracetamol's (200 mg/kg, oral) antipyretic activity was assessed. The activities of COX-1 and COX-2, the levels of PGE_2, TNF-α and IL-1β and expression of CB_1 receptors were assessed in brain. Arsenic inhibited paracetamol-mediated antipyretic activity. COX-1 activity was not affected by any treatments. Paracetamol decreased COX-2 activity, levels of PGE_2, TNF-α and IL-1β and caused up-regulation of CB_1 receptors. Arsenic caused opposite effects on these parameters. In the arsenic-preexposed rats, paracetamol-mediated effects were attenuated, while CB_1 receptor up-regulation was reversed to down-regulation. Results suggest that elevated COX-2 activity and reduced CB_1 expression could be involved in the arsenic-mediated attenuation of the antipyretic activity of paracetamol.

F-TOX-O8

TO STUDY THE INFLUENCE OF α-TOCOPHEROL ON CU, FE, ZN AND MN LEVELS IN LIVER FOLLOWING 90 DAY'S EXPOSURE OF FLUBENDIAMIDE AND COPPER TO WISTAR RATS

Mandil R[a], Prakash A[b], Rahal A[c], Koli S[b] and Garg S.K[b]

[a]Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Sardar Vallbhbhai Patel University of Agriculture & Technology, Meerut-250110

[b]Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, U.P. Pt. Deen Dayal Upadhyay Pashu ChikitsaVigyan Vishwavidyalaya Evam Go- Anusandhan Sansthan, Mathura-281001

[c]Central Institute for Research on Goat (CIRG), Makhdoom, Farah, Mathura, Uttar Pradesh-281122, India

rajesh_mandil@rediffmail.com

Present study was designed to study the effects of α-tocopherol on the levels of Copper (Cu), Iron (Fe), Zinc (Zn) and manganese (Mn) in liver ofcopper and/or flubendiamide exposed rats. Fifty four male rats were randomlydivided into nine groups containing six animals in each:groups I (deionized water), II (corn oil) and III (α-tocopherol @ 100 mg/kg) served as negative controls while the remaining six groups, namely-IV (copper sulphate-@ 33 mg/kg), V (flubendiamide-@ 200 mg/kg), VI (flubendiamide-@ 200 mg/kg + copper sulphate- @ 33 mg/kg), VII (copper sulphate-@ 33 mg/kg + α-tocopherol-@ 100 mg/kg), VIII (flubendiamide-@ 200 mg/kg + α-tocopherol-@ 100 mg/kg) and IX (flubendiamide-@ 200 mg/kg +

copper sulphate-@ 33 mg/kg + α-tocopherol-@ 100 mg/kg) were treatment groups. The oral exposure period for xenobiotics and α-tocopherol was 90 days. Animals were humanly sacrificed on 91st day. One gram liver tissue sample was kept with equal volume of nitric-acid overnight and, after this 10 ml mixture {(containing: 2 ml HNO_3 (sub-boiling, 65 %) + 2 ml H_2O_2 (suprapure, 30 %) + 0.5 ml HCl (suprapure, 30 %) + 5.5 ml H_2O (ultra pure)} was pour into Microdigestion systemMultiwave 3000 with high performance rotor 16XF100 (100 ml PTFE-TFM vessels, 60 bar) for digestion. Whatman 40 filtered paper samples were used for estimation of Cooper, Iron Zinc, and Manganese by Atomic Absorption Spectrophotometer. Copper and iron levels were markedly higher in copper,flubendiamide alone and combined exposed groups while manganese level was low in the xenobiotics exposed groups. No significant alteration was observed in level of zinc in any of the xenobiotics exposed as well as xenobiotics + α-tocopherol treatment groups. α-tocopherol-treatment improve the values of copper, iron , zinc and manganese towards the levels of control groups indicates antioxidant potential of α-tocopherol.

S-TOX-O1

DETERMINATION OF ORGANOCHLORINE PESTICIDE RESIDUES IN MILK FROM LIVESTOCK INSTRUCTIONAL FARM, POOKODE

Vidyavarsha S.J[1]., Jasna C[1]., Shijin M.S[1]., Saranya C[1]., Megha M[1]., Mery S.J[1]., Suraj S.V.[1], Sanjay B.M.[1], Ranjith D[1]., Bibu J.K[1]., Benjamin E.D[2]. and Juliet S[1]

[1]Department of Veterinary Pharmacology and Toxicology, [2]Livestock Instructional Farm, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala- 673576. E mail i.d.: vidyavarshavarsha@gmail.com

Organochlorine pesticides have been extensively used in agriculture, sanitation and animal husbandry because of their efficacy and low cost. These class of compounds have always been a cause of worldwide concern because of their chemical stability, bioaccumulation and persistence in the environment. A preliminary screening of milk samples from Livestock Instructional Farm, Pookode was undertaken for the presence of organochlorine pesticide residue which would serve as an indicator of any exposure to them.A total of 17 milk samples collected in sterile screw cap vialswere extracted according to standard protocols. The processed samples were analyzed ina Shimadzu GC-MS QP2010 gas chromatograph with auto injector AOC-20s. The column used for the analysis was a 30 m x 0.25 µm x 0.25 mm i.d. RX1 SILMS. An aliquot of 1 µl of the standard in the split mode (split ratio 1:10) and final sample volume were injected into the GC-MS system. Helium was used as the carrier gas with a flow rate of 1 mL /min.The analytes were separated

from the milk by solid phase extraction. All the samples were screened for the presence of 20 major organochlorine compounds *viz*,α-lindane, β-hexachlorocyclohexane, γ-lindane, δ-lindane, δ-endosulphan, heptachlor, aldrin, heptachlor epoxide, chlordane (cis), chlordane (trans), p,p'-DDE, dieldrin, endrin, endosulfan II, mitotane, endrin aldehyde, endosulfan sulfate, p,p'-DDT, endrin ketone and methoxychlor.The calibration graphs of various analytes were established with correlation co-efficient and the recovery percentage of organochlorine pesticide residues were calculated. The results revealed that the analytes in the all milk samples analyzed were below the detection limit. However, further studies with large sample size are also necessary to rule out the possible health hazard caused by pesticide residues.

S-TOX-O2

ORAL SUBACUTE TOXICITY STUDIES ON BETULINIC ACID AND URSOLIC ACID IN RATS

Madhu C Lingaraju[1], Mohanapriya K[1], Aneesha V Appukuttan[1], Naresh K[1], Karikalan Mathesh[2], Dhirendra Kumar[1], Thakur Uttam Singh[1], Dinesh Kumar[1]

[1]Division of Pharmacology and Toxicology, [2]Centre for Wildlife Conservation Management and Disease Surveillance, ICAR-Indian Veterinary Research Institute, Izatnagar, 243 122.

Betulinic acid (BA) and ursolic acid (UA) at 30 mg/kg doses were found to improve the kidney function in the adenine model of CKD in rats by attenuating the development of fibrosis in our previous study. In this study we sought to evaluate BA and UA at 0, 30, 60 and 120 mg/kg doses for the safety in normal rats. Rats were administered with the said doses of BA and UA, orally for 28 days once daily. Results of this study showed that body weight gain was significantly unchanged between the doses. Likewise, relative organ weights viz. liver, lung, kidney, spleen, hear and brain were similar between the doses with no significant differences. PCV and WBC counts also did not alter. BA and UA at any doses used in this study did not pose significant effect on the levels of urea, creatinine, A/G ratio, SGOT, SGPT, CK-MB, triglycerides, LDL and HDL cholesterols revealing the safety of BA and UA on kidney, liver and heart. Histomorphology of lung, liver, kidney, brain, spleen and heart tissues were normal at the entire doses as revealed by normal arrangement of epithelia, cellular architecture with no signs of any haemorrhage or infiltration of inflammatory cells. From the results of this study it is revealed that use of BA and UA was safe on different important organs which were reflected by unaffected organ weights, their functional biochemical parameters and histological analysis in rats.

ISVPT – 2019

TECHNICAL SESSION

ANIMAL WELFARE AND ALTERNATE ANIMAL USE

CHAIRPERSON : DR. P.SRIRAM

CO-CHAIRPERSON : DR. RAJDEEP KAUR

RAPPORTEUR : DR. JAYANTHI M.

College of Veterinary and Animal Sciences, Mannuthy

Pharmacogenomics in the development and validation of indigenous drugs
XIX ISVPT
CVAS MANNUTHY

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DETERMINATION OF SAMPLE SIZE IN EXPERIMENTS – 3R CONSIDERATIONS

Dr. S. Ramesh

Professor, Laboratory Animal Medicine, TANUVAS, Chennai 600051

The 3Rs – replacement, Reduction and Refinement- remain the hallmark of animal welfare in a biomedical research setting even after 60 years of their declaration by Russell and Burch (1959). As awareness about animal welfare in research is growing, the use of minimal number of animals in research is being continuously emphasised. On the other hand, the importance of optimal number of animals for better repeatability and reproducibility, need not be overemphasised. The use of too many animals leads to a waste of precious resources (including animals, chemicals etc.) and is also unethical. At the same time if too few animals are used, the experiment lacks the power to be statistically reliable, which is, again, a waste of precious resources since the results are not going to be relied upon for logical conclusion. Thus, the need for an optimal number of animals (read: sample size) is a very important tool to begin the experiment.

Readers are introduced here to the guidelines proposed by the international community, the PREPARE guidelines *(**P**lanning **R**esearch and Experimental Procedures on **A**nimals: **R**ecommendations for **E**xcellence)*. This guideline suggests a 15 -point checklist as an effort to reduce waste and increase the reproducibility of animal research and testing. Out of the 15 point checklist, Experimental design and statistical analysis occupies an important role in the implementation of 3R principle to in vivo research.

Readers are also introduced to the ARRIVE guidelines (Animal Research – Reporting of in vivo experiments). The ARRIVE guidelines were developed as an initiative by NC3Rs to improve the design, analysis and reporting of research using animals.

The common pitfalls cited in the experimental design include:

- Subjective bias (Lack of randomisation and blinding)
- Inadequate sample size

In this lecture, some of the simple procedures to arrive at appropriate sample size in animal experiments are discussed.

Power analysis:

The term Power of the experiment refers to the probability of finding an effect of the treatment when the treatment is effective. For biological experiments the ideal power is fixed as > 0.8. Researchers wish to fix the power between 0.8 and 0.9 (80% to 90%). It is important to fix the power before start of the experiment and calculate the sample size.

Criteria for fixing sample size:

1. Effect size: This is the difference between the means of the groups used in the study. This is called the SIGNAL. This is obtained by taking the expected difference in the mean. Otherwise the means can be taken from previous literature for the parameter that is the most important. Eg. BP, Cell count. etc.
2. Standard deviation: The reported deviation in previous literature can be used. This is called the NOISE.

   The ratio of signal to noise (S/N ratio) can be used straightaway to determine sample size using a table.

   As the table indicates, a higher S/N ratio will result in fewer animals
3. Significance: Usually fixed at 0.05. This value is arbitrary and even lower values can be fixed.
4. Power: Power is fixed by the investigator. A power of 0.8 or 0.9 is usually fixed for biological experiments.

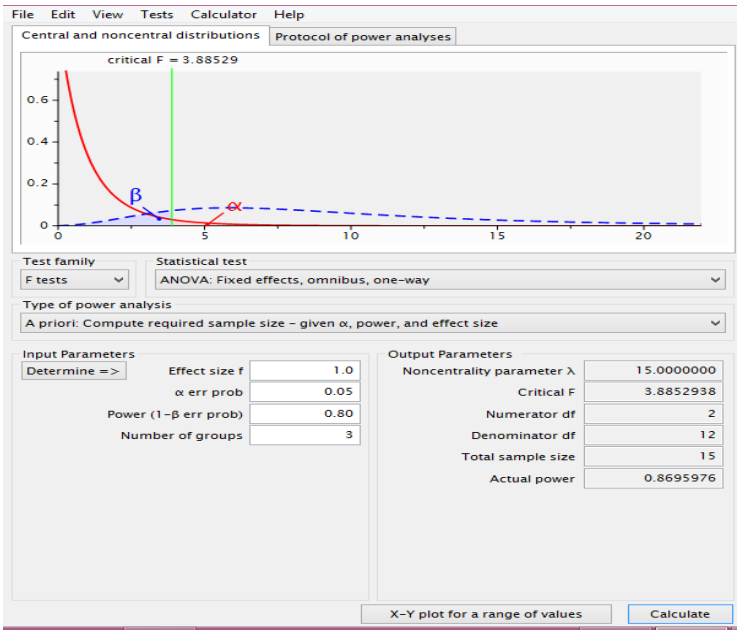
   The data represent the number of animals that can constitute the group size given the S/N ratio and fixing the power

Group size as a function of S/N ratio (5% sig., 2-sided)

SN ratio	90% power	80% power
0.2	526	393
0.4	132	99
0.6	59	45
0.8	34	26
1.0	22	17
1.2	16	12
1.4	12	9
1.6	9	7
1.8	8	6
2.0	6	5
2.2	6	4
2.4	5	4
2.6	4	4
2.8	4	3
3.0	4	3

Source: http://www.3rs-reduction.co.uk/html/6__power_and_sample_size.html

5. Statistical test: the above table is good for 't' tests. However, for more complex tests such as ANOVA, sample size can be calculated using software such as *Gpower*.



A screen shot of Gpower where the values of Effect size (S/N ratio), no. of groups, power % and type of test (ANOVA) were fed and the output gives total sample size. Gpower is a downloadable software and is a freeware.

6. Expected Death or Attrition: After calculating sample size, additional requirement can be calculated giving consideration to the risk of death during the study, as can happen in surgical procedures/ tumour trials etc.

   Corrected sample size: Sample size / (1 – (death%/100))

Resource equation: If the investigator does not have any idea of the values such as signal, noise (or) if the investigator is not able to decide the most important parameter, then Resource equation can be used. The value 'E' is calculated as follows. The value should be between 10 and 20.

E = Total no. of units – Total no. of groups.

Eg: If 30 animals are used at 6 animals / group, then E = 24. Since 24 will be higher than desirable estimate of 'E', the same can be modified; the total number can be reduced to 24 to yield a value of 18.

Resource equation is not a very robust method. It can at best be used in pilot trials which can be later on followed up with regular trials, for which power analysis can be used.

The readers are also referred to the EDA software (experimental design assistant) which is an interactive online tool which incorporates all the features of experimental design and suggests a very good design based on the users inputs. The EDA includes functionalities that support blinding, randomization and sample size calculation.

Further reading:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3826013/

http://www.lawrencemoon.co.uk/pdfs/festing_et_al_2002.pdf

http://www.3rs-reduction.co.uk/html/6__power_and_sample_size.html

https://www.nc3rs.org.uk/experimental-design-assistant-eda

https://norecopa.no/prepare.

https://www.nc3rs.org.uk/arriveguidelines#:~:targetText=ARRIVE%20guidelines,published%20and%20minimising%20unnecessary%20studies.

LP-AWA-02

ALTERNATIVES TO THE USE OF ANIMALS IN REGULATORY AND BIOMEDICAL RESEARCH IN INDIA

Dr Ankita Pandey[1], Dr Dipti M. Kapoor[2] and Dr Manilal Valliyate[3]

[1]Research Associate, People for the Ethical Treatment of Animals (PETA) India, New Delhi

[2]Science Policy Advisor, People for the Ethical Treatment of Animals (PETA) India, New Delhi

[3]Chief Executive Officer, People for the Ethical Treatment of Animals (PETA) India, New Delhi

According to the 2017–18 report of the Department of Science and Technology (which is primarily a policymaking body for India's science and technology sector), the country's gross expenditure on research and development has been consistently increasing over the years and more than quadrupled in just over a decade, from Rs 24,117.24 crores in 2004–05 to Rs 1,04,864.03 crores in 2016–17. Of all the research funding from the Indian regulatory and governing agencies for biomedical research and regulatory policy development, a portion goes towards animal experimentation, even though an increasing number of studies show – and ever more scientists acknowledge – that the results of experiments using animals are often not reproducible or translatable to humans. A great deal of scholarly research shows that animal studies are flawed, diverting economic and intellectual resources from methodologies better suited to curing human disease. While multiple factors contribute to the failure of animal experimentation to predict human outcomes reliably, intrinsic biological and genetic differences between species contribute significantly to problems in extrapolating results from non-human animals to humans. A 2014 BMJ article by Pandora Pound and Michael Bracken observed that "if research conducted on animals continues to be unable to reasonably predict what can be expected in humans, the public's continuing endorsement and funding of preclinical animal research seems misplaced." Internationally, an evolving trend has shifted efforts away from animal-based research toward the new and rapidly evolving field of human-relevant, non-animal methods.

In this presentation, a number of strategic priorities, which PETA India has already discussed with the union ministers of Science and Technology and of Environment, Forest and Climate Change, will be discussed. The strategic priorities include the following:

- Immediately eliminate animal use in areas in which animals have already been shown to be poor and unreliable predictors for human reactions and have impeded progress.
- Conduct critical scientific reviews of animal use to identify the areas in which their use has failed to advance human health and should therefore be phased out.

- Work with agencies and bodies globally to harmonise and promote international acceptance of non-animal testing methods for regulatory toxicity testing requirements.
- Divert funds from animal studies towards the development of non-animal methods, including areas in which further development, validation, and implementation of non-animal methods are required.

Through these strategic priorities, we offer a robust blueprint to translate the restriction of animal use and an increase in the availability of human-relevant biotechnology into actions aimed at eliminating animal use in regulatory and biomedical research in India.

F-AWA-O1

EFFECT OF 2,3,7,8 TETRACHLORODIBENZOP-DIOXIN (TCDD) ON TOTAL CHOLESTEROL OF H295R AND MCF-7 CELLS

Sujith S*, Usha PTA*, Bibu John Kariyil, Raji, K^.,Shynu, M#and Juliet, S**.**

*Dept. of Veterinary Pharmacology & Toxicology, CVAS Mannuthy, ** Dept. of Veterinary Pharmacology & Toxicology, CVAS Pookode, ^ Dept. of Veterinary Physiology, CVAS Mannuthy, # Dept. of Veterinary Biochemistry, CVAS Mannuthy, e-mail:sujith@kvasu.ac.in

Dioxins are persistent organic pollutants that modulate steroidogenesis in cells by affecting their production and metabolism. The effect of 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) treatment on total cell cholesterol was studied in H295R and MCF-7 cell lines as cholesterol is the precursor of all steroid hormones. H295 R cells were grown in DMEM-F12 media fortified with one percent serum, ITSplus premix and gentamicin where as MCF-7 cells were grown in RPMI media containing 10 per cent serum and one percent of antibiotic antimycotic solution. The cells were grown in six well plates and exposed to 1, 3.1 10 and 100 nM concentrations of TCDD for 96 hours. The cells were harvested every 24 hours, the total cholesterol isolated using isopropanol:n-hexane and was quantified by spectrofluorimetry using Amplex Red assay kit. The results of the study revealed that the total cell cholesterol levels were maximum in cells treated with 3.1nM TCDD after 96 hours of treatment in both H295 R and MCF-7 cells. The cholesterol concentration in cells was lowest in cells treated with 10nM concentration of TCDD. The cells treated with 100nM concentration of TCDD did not survive for more than 24 hours. Hence it could be concluded that higher concentrations of TCDD caused a decrease in total cell cholesterol whereas lower concentrations caused deposition of cholesterol in cells.

F-AWA-O2

IN SILICO SCREENING OF CHEMO PREVENTIVE EFFECT OF PHYTOCOMPOUNDS FROM INDIAN MEDICINAL PLANTS FOR CANINE MAMMARY TUMOUR

V. Ramakrishnan[1], P. Ravikumar[2], T. Raja[2], K. Vijayarani[2], S.P. Preetha[1] and A. Arivuchelvan[1]

[1]Department of Veterinary Pharmacology and Toxicology, [2]Bioinformatics Centre and ARIS Cell, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai – 600 007, Tamil Nadu, India, ramakrishnanvet@gmail.com

Canine mammary tumours are among the most predominant neoplasms and happen all the more usually among unblemished females which are not spayed at an early age. The previous study carried out at

Madras Veterinary College reported that out of the 14,326 clinical cases presented in an eight months study, 61 cases were mammary tumours. The current study was aimed to screen chemo preventive effect of phytocompounds of Indian medicinal plants for Canine mammary tumours. Mammaglobin-B was taken as a target protein and it was modeled using I-Tasser. Around 920 phytocompounds were collected from different Indian medicinal plants using Dr. Duke's database. In which, after checking Lipinski Rule of five, 132 compounds were selected for this study. The 3D structure of all the phytocompounds were retrieved from PubChem database. Docking studies were done using Discovery Studio 4.0.From the results, the phytocompounds Homocapsaicin (Libdcok score: 102.27), Homodihydrocapsaicin (Libdcok score: 101.55) and isositsirikine (Libdcok score: 99.19) showed the best Libdcok score. Hence, the present study was concluded that the phytocompounds Homocapsaicin and Homodihydro capsaicin from *Capsicum annuum* and isositsirikine from *Catharanthusroseus* had potentialchemo preventive effect for Canine mammary tumours.

F-AWA-O3

IN SILICO MOLECULAR DOCKING OF ACTIVE PRINCIPLES OF *ANDROGRAPHIS PANICULATA* WITH NEWCASTLE DISEASE VIRAL PROTEINS

Nagajothi S, Mekala P*, Raja A, Raja MJ andSenthilkumar P

Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal.

Tamil Nadu Veterinary and Animal Sciences University

Email: mekskathir@gmail.com

The present study focused on molecular docking analysis of active principles of *Andrographis paniculata* with NDV2K35 proteins, a strain of Newcastle disease virus. The 3D structures of NDV2K35 proteins (receptors) *viz.* Haemagglutin-Neuraminidase protein (HN), Fusion protein (F), Matrix protein (M), Phosphoprotein (P), Large polymerase protein (L) and Nucleocapsid protein (N) were downloaded from Uniprot database,modelled using Swiss model analysis and validated by RAMPAGE: Ramachandran plot analysis. The active principles of *Andrographis paniculata* (ligands) *viz.* andrographolide, neoandrographolide and 14-deoxyandrographolide were downloaded from PUBCHEM, a database for chemical molecules. The docking analysis was carried out using Accelrys Discovery Studio 4.0 Client software. The results revealed that andrographolide interacted with all except M protein. The dock score of neoandrographolide was more than andrographolide with all the proteins but it failed to interact with F and M protein. The compound 14-deoxyandrographolide interacted with all proteins except F protein but the

dock score was comparatively less. The active principles were further screened for *in silico* pharmacokinetic and pharmacodynamic properties using Swiss ADME, a standard online tool and PASS online server. The active principles exhibited good GI absorption with bioavailability of 55 % and acted as substrate for p-gp. Of the three,neoandrographolide inhibited CYP3A4 which indicate the possibility for drug interactions. Toxicity analysis also showed comparatively higher toxicity for neoandrographolide. Thus andrographolide can be considered as a better choice for developing antiviral drug against Newcastle disease virus.

F-AWA-O4

EFFECTS OF POLYHERBEL OINTMENT FORMULATION ON WOUND HEALING IN DIABETIC RATS

Dipshikha, Prasad, R*, Singh, P., Manisha and Singh, K.K.

Corresponding author:- drrajuprasad@gmail.com (Raju Prasad)

Background: Wound healing in diabetic patients is delayed due to increased production of ROS through glucose auto oxidation enhances cellular damage. The herbal plants in combination (*Clerodendron infortunatum + Aloe vera + Curcuma longa*) acts synergistically to hasten wound healing process due to their anti-oxidant and anti-inflammatory properties.

Objective: To investigate the time dependent effects of polyherbal formulation on topical application in experimentally induced excision wound model in diabetic rats.

Methods: The ointment was prepared in different extracts of herbal plant *C. infortunatum*, *A. vera and C. longa* at the ratio of 4 %: 7%: 1% in ointment base (88%) respectively. The excisional wound was created (400 mm^2) after induction of diabetes by the intra-peritoneal injection of streptozotocin in the all 30 rats which was divided into two groups, namely group I (Control) and group II (Treatment) (N= 15). The Gr. 1 and Gr. II also further subdivided into three groups at each times point of sample collection (granulation tissue) and analysis (n=5 (day 3, 7, 15).

Results: Polyherbal formulation treated wounds revealed significantly higher ($p<0.05$) percent wound contraction on day 7 and 15 as compared to diabetic control. The levels of SOD, CAT and GSH in the treated group was significantly ($p<0.05$) higher on day 3, 7 and 15 post wounding in a time dependent manner as compared to control group where as lipid peroxidation (MDA level) was significantly lower in treated group. The level of hydroxyproline were significantly ($p<0.01$) increased in polyherbal formulation treated wounds on day 3, 7 and 15 as compared to diabetic control rats. The histopathology study of tissue

section of treated rats revealed better and organized collagen deposition, more neovascularisation, faster re-epithelialisation, as compared to control rats.

Conclusions: From above finding, it can be concluded that the polyherbal formulation has great potential to hasten the wound healing process in diabetic rats

F-AWA-O5

PARASPORAL PROTEINS OF NOVEL *BACILLUS THURINGIENSIS* STRAIN INDUCE NECROTIC CELL DEATH IN JURKAT CELL LINE

Beena V., Ramnath V., Karthiayini K., Sreekumar K. P., Philomina P.T., Sunanda C. and Girija D

College of Veterinary and Animal Sciences,

Kerala Veterinary and Animal Sciences University, Pookode, Wayanad

beenav@kvasu.ac.in

The non-haemolytic parasporal inclusion proteins of *Bacillus thuringiensis* (Bt) showing preferential cytotoxicity to human cancer cells at the same time non-toxic to normal cells are known as Parasporins. The cytotoxicity of these proteins to cancer cells was either reported to be due to necrosis or apoptosis. This study was undertaken with the non haemolytic Bt strain KAU 375, collected from Western Ghats. A loopful of culture was introduced in 50 mL of T3 medium and incubated at 30°C with continuous shaking at 200rpm in an orbital incubator shaker for 2 to 5 days to induce sporulation. Sporulated cultures were harvested by centrifuging the culture medium and washing with 2% Triton X-100 in 0.5*M* NaCl, followed with 0.5*M* NaCl and finally with deionized water. The inclusion proteins were solubilised on an alkaline solubilising reagent of pH 10 and after incubation for 1 h at 37°C supernatant containing solubilised proteins was collected. Quantification of solubilised proteins was done by Lowry's method using bovine serum albumin as standard. Solubilised inclusion protein was subjected to proteolytic activation by proteinase K. Human leukemic T cells, Jurkat and normal lymphocytes were cultured in RPMI 1640 containing 10 per cent foetal bovine serum and Kanamycin sulphate 30 µg/mL. One dose cytotoxicity assay was carried out by Cell Titer 96 Aqueous Non Radioactive Cell Proliferation Assay Kit. Solubilised and proteolytically activated inclusion protein of this strain was found to possess potent cytotoxicity against Jurkat cells at the same time non toxic to normal lymphocytes. Cytotoxicity was measured in triplicates for eight different two fold serial dilutions and 50 per cent effective concentration (EC_{50}) value was deduced from log probit analysis. Necrotic cell death was confirmed by dual staining with Acridine orange / Ethidium bromide.

F-AWA-O6

IN-VITRO ANTHELMINTIC ACTIVITY OF FRUIT EXTRACTS OF *DURANTA ERECTA* L.

Krishnaprasad G. K.*, Sujith S.[b], Preethy John[b], Priya M. N[c], Devi S. S. [d], Usha P T. A[b], Surya S.[b]

*Product Manager, Petcare Divn. Cargill India Pvt. Ltd. Bangalore, Karnataka, INDIA b. Department of Veterinary Pharmacology and Toxicology, c. Department of Veterinary Parasitology d. Department of Veterinary Pathology, CVAS, Mannuthy, Thrissur, Kerala, INDIA. Correspondence author: E mail Id: kpvet.kgvk@gmail.com

The present study was aimed to evaluate the *in vitro* ovicidal, larvicidal and adulticidal activity of methanolic extract and its n-hexane, chloroform, n-butanol and aqueous fractions from fruits of *Duranta erecta* against strongyle ova, larvae and adult amphistomes. The phytochemical analysis revealed the presence of flavonoids in the extract and all fractions while tannins, glycosides and diterpenes were absent in n-hexane fraction of *D. erecta*. The extract and fractions were diluted serially in distilled water to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 1.95 mg/mL. Ivermectin and thiabendazole each at 10 µg/mL acted as positive control and distilled water as negative control. The chloroform fraction was highly active against the ova with IC_{50} of 3.0 mg/mL. The n-butanol fraction was potent in inducing larval mortality with IC_{50} of 0.04 mg/mL while chloroform fraction was more efficient in inhibiting larval migration (IC_{50} of 16.30 mg/mL). Amphistomes were highly sensitive to chloroform fraction of *D. erecta* which possessed IC_{50} of 1.354 mg/mL and histopathology revealed morphological changes in tegument, syncytium and parenchyma. Based on IC_{50} values, the n-butanol fraction was found to be most potent among various extracts assayed. GCMS analysis of n-butanol fraction revealed the presence of phenolics which may have contributed for the anthelmintic activity. The acute oral toxicity study revealed mild vascular changes in liver. From the present study, it can be concluded that chloroform fraction possessed maximum activity against ova, larvae and adult stages of gastrointestinal nematodes indicating a broad spectrum activity.

S-AWA-O1

IN VITRO ANTIBACTERIAL EFFECT OF SILVER NANOPARTICLES USING *TECTONA GRANDIS* WITH ANTIBIOTICS IN BACTERIAL ISOLATES FROM BOVINE MASTITIS

Keerthika V., Nisha A.R., Arya Mohan., Preethy John., Poonghuzhali R., Dhanusha G., Jisha S., Suja Rani S., Suresh N Nair., Usha P.T.A

MVSc Scholar, Department of Veterinary Pharmacology and Toxicology, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences,Mannuthy,Thrissur.

Nanoparticle synthesis using plant extract is a growing field in nanotechnology. The green synthesis of nanoparticle using plant extract is eco-friendly, economic and an alternate source for the chemical synthesis of nanoparticles. The objective of the present study was to analyse the antimicrobial properties of *Tectona grandis* nano particle against bacterial isolates from bovine mastitis clinical samples. In the present study disc diffusion antimicrobial assay was conducted by Kirby Bauer assay and minimum inhibitory concentration by microdilution was done in isolates from mastitis milk samples. Biofilm assay was performed by Congo red method. The bacterial isolates used in this study were *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella sp*. The present study used methanolic extracts *Tectona grandis* and silver nanoparticles synthesised from *Tectona grandis* as a reducing agent for the synthesis of silver nanoparticle. The formation of silver nanoparticles was confirmed by UV-Visible Spectroscopy. Phytochemical analysis of the methanolic whole plant extract showed the presence of alkaloids, and tannins. The potentiating activity of silver nanoparticles from *Tectona grandis* is compared with the leaf extract in combination with various antibiotics. In this study it was found that the silver nanoparticle as well as plant extract showed a potentiating effect on the resistant antibiotics. The isolated bacteria did not produce any biofilm. In this reduced size, biosynthesized silver particles can easily enter inside bacterial cells and thereby by affecting the cellular metabolism of bacteria.

S-AWA-O2

ANTIPROLIFERATIVE POTENTIAL OF METHANOLIC EXTRACT OF *DURANTAERECTA* IN MDAMB231 CELLS

Mukesh U, Dhanusha G, Aathira, K.K and Sujith S.

*MVSc Scholar, Dept of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy, **Dept of Veterinary Pharmacology and Toxicology,CVAS,Mannuthy.

The present study was carried out to evaluate the antiproliferative activity of methanolic extract of *Durantaerecta* in MDA-MB-231 cells. The qualitative phytochemical analysis of the extract was done and it revealed the presence of steroids, alkaloids, flavonoids, glycosides, saponins, tannins, cardiac glycosides, phenolics, triterpenes and diterpenes. MDA-MB-231 cells were maintained in RPMI media containing 10 per cent serum andone per cent antibiotic and antimycotic solution. The cells were harvested and seeded to 96 well plate at 1×10^5 cells/mL, incubated for 24 hours at 37°C with 5 per cent CO_2. The cells were treated with 160, 80, 40, 20 and 10µg/mL of the extract for 24 hours and viability was assessed using MTT Assay. There was a dose dependent decrease in viability of cells exposed to methanolic extracts of *Durantaerecta* with a viability of 15.55±0.193 for cells treated with 160µg/mL. The IC_{50} was found to be 91.36±0.87.

S-AWA-O3

CYTOTOXIC AND ANTIPROLIFERATIVE POTENTIAL OF METHANOLIC EXTRACTS OF *ASPARAGUS RACEMOSUS* IN MDA-MB-231 CELLS.

Dhanusha G[1], Sujith S[2], Usha P T A[2], Aathira K K[1], Dabholkar Akshay Rajkumar[1], Haima J S[1], Akhil G H[1], Poonghuzhali R[1] and Mukesh U[1]

*MVSc scholar, Dept of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy

** Dept of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy

Naturally occurring compounds derived from plants, having chemical structures similar to oestrogen are classified as phytoestrogens and have potential application in human cancers and other diseases. The present study was carried out to evaluate the cytotoxic and antiproliferative activity of methanolic extract of *Asparagus racemosus* in MDA-MB-231 cells. The qualitative phytochemical analysis of the extractand it revealed the presence ofsteroids, alkaloids, flavonoids, glycosides, saponins and diterpenes. MDA-MB-231 cells were maintained in RPMI media containing 10 per cent serum and 1 per cent antibiotic and antimycotic solution. The cells were harvested and seeded to 96 well plateat 1×10^5 cells/mL, incubated for 24 hours at

37˚C with 5 per cent CO_2. The cells were treated with 160, 80, 40, 20 and 10µg/mL of the extract for 24 hours and viability was assessed using MTT Assay. Also cells were plated in 6 well plates at concentrations 3×10^5 cells/mL and exposed to 80, 40, 20 and 10µg/mL of the extract of *Asparagus racemosus* foracridine orange ethidiumbromide (AO/EB) staining. There was a dose dependent decrease in viability of cells exposed to methanolic extracts of *Asparagus racemosus* with a viability of 15.55±0.193 per cent for cells treated with 160µg/mL.The IC_{50} was found to be 91.36± 0.87 µg/mL. The AO/EB staining revealed that most of the cells were dead in extract treated with 80µg/mL where more than 90 per cent of cells were live when treated with 10µg/mL of the extract.

ISVPT – 2019

TECHNICAL SESSION

MOLECULAR AND NEUROPHARMACOLOGY

CHAIRPERSON : DR. N. GOPAKUMAR

CO-CHAIRPERSON : DR. NIRBHAY KUMAR

RAPPORTEUR : DR. SUJA RANI S

College of Veterinary and Animal Sciences, Mannuthy

Pharmacogenomics in the development and validation of indigenous drugs
XIX ISVPT
CVAS MANNUTHY

LP-MNP-01

MELATONIN -MOLECULAR PHARMACOLGY AND THERAPEUTIC SIGNIFICANCE

N. Prakash

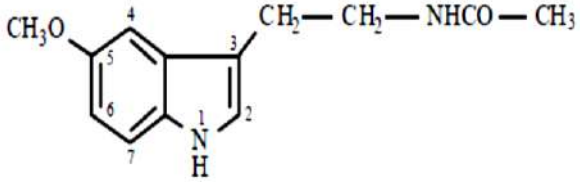
Veterinary College, Vinobanagar, Shivamogga-577204, KVAFSU (K.S)

pnadoor@rediffmail.com

Melatonin (N-acetyl-5-methoxyindolamine) is a neurohormone produced by pineal gland which is synthesized from tryptophan via serotonin. In addition, other tissues like lymphocyte, retina, salivary gland, platelet, skin, and developing brain are also involved in melatonin production. Its physiological actions include control of circadian and circannual rhythms, sleep induction, regulation of seasonal reproduction, food intake, immune enhancement and also biological modulator of mood, sleep, and sexual behavior. Melatonin is used to reduce jet lag, adjust sleep cycles and in the treatment of general insomnias in man. Melatonin is considered as a health supplement in the United States of America and governed by US-FDA regulations (NCCIH, 2016). However, many countries including Australia, Finland, Norway market melatonin against prescription only.

Structure and Chemistry

Chemically melatonin is an indoleamine consisting of an indole heterocycle with two side chains namely, 5-methoxy group, and 3-amide group. The molecular formula of melatonin is $C_{13}H_{16}N_2O_2$. The molecular weight and melting point is 232.83 and 116 - 118℃ respectively. The water solubility of melatonin 2 $g.L^{-1}$ at 20˚C; 5 $g.L^{-1}$ at 50˚C (European Commission, 2010).



The core structure for melatonin required to scavenge free radicals is the indole heterocycle due to its indole rich moiety with resonance stability and electro-reactivity determines melatonin's potent free radical scavenging capacity. The carbon atoms at positions 2, 3, 4, 6 and 7 of the indole heterocycle are suitable sites for hydroxyl radical or nitrogen radical reactions. The methoxy group (at C5) as well the aminoacyl group (at C3) of the indole moiety in the melatonin molecule, were essential for melatonin to display potent OH· scavenging activity.

Receptors and mode of action

The biological functions of melatonin are mediated through two major signaling cascades comprising of receptor-and non-receptor-mediated activities. There are two subtypes of transmembrane G protein-

coupled receptors viz., MT_1 and MT_2. Modification of functions of adenylate cyclase, guanylate cyclase, phospholipase C, as well as calcium and potassium channels are involved in the cellular signaling of melatonin receptors. Activation of MT_1 receptors results in several biologic effects, that are mediated by the suppression of cyclic adenosine monophosphate (*c*AMP) as well as enhancement of cytosolic calcium through Gq_{11}, and stimulation of MT_2 receptors can cause *c*AMP and cyclic guanosine monophosphate (*c*GMP) inhibition.

A third subtype of melatonin receptor is a *quinone reductase*- II, which has been found to be associated with the xenobiotic metabolism of the cell. Melatonin binding sites in the nucleus are mediated by orphan receptors from the retinoid orphan receptor -α and retinoid -*Z* receptor family. MT_1 receptors are involved in metabolic functions, vasoconstriction, and reproductive activities and MT_2 receptors are associated with regulation of circadian rhythms as well as dopamine release (Srinivasan *et al.*, 2015).

Antioxidant properties

Melatonin exerts a potent antioxidant and free radical scavenging property *via* several means. It possesses a specific electron reduction potential by forming a melatonin cation (melatoninyl) radical which then reacts with $O_2^{\bullet -}$ to form N_1-acetyl-N_2-formyl-5-methoxykynuramine (AFMK) (Hardeland *et al*., 1993). Burkhardt e*t al*, (2001) observed the ability of AFMK to protect against free radical mutilation of DNA equivalent that of melatonin. However, its protection against lipid peroxidation was reported to be less than that of melatonin.

Further 6-hydroxymelatonin, a hydroxylated metabolite of melatonin (hepatic biotransformation), also possesses free radical scavenging property due to similar structure. According to Turjanski *et al*, (1998) melatonin might donate a hydrogen atom from the -NH structure of the pyrrole ring to generate a neutral melatonin radical which can scavenge $O_2^{\bullet -}$ to form end product AFMK similar to that of melatoninyl cation radical. Additionally melatonin also acts as a power full electron acceptor.

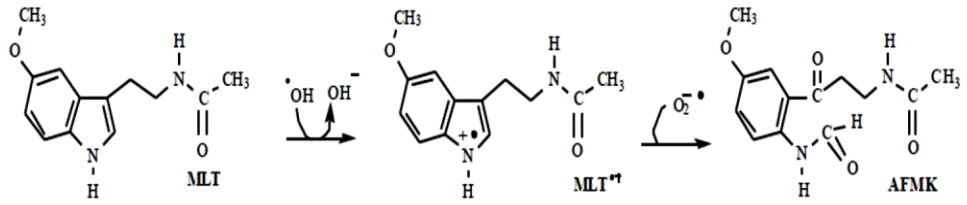


Fig.: Pathway of melatoninyl cation radical formation

MLT: Melatonin, $MLT^{\cdot +}$: Melatoninyl cation radical, AFMK: N1-acetyl-N2- formyl-5-methoxy kynuramine

Melatonin can also act as powerful electron acceptors by abstracting 'H' atoms from most molecules. In this path way one melatonin molecule scavenges two –OH groups to form a thermodynamically stable end product called cyclic 3- hydroxymelatonin. Further, after intra-molecular arrangement the unpaired electron appears to shift to the carbonyl structure adjacent to the nitrogen and acting as a member of the newly formed 5-membered ring. This structure scavenges the second OH· to form cyclic-3-hydroxymelatonin which later interacts with ROS to form AFMK.

Pharmacokinetics

Melatonin is an amphiphilic molecule and many endogenous substances share the structure and chemical properties of melatonin. In rats the bioavailability following *per oral* or *i.p* administration (10 $mg.kg^{-1}$) was 53.5 and 74.0 *per cent* respectively, suggesting lack of substantial first-pass hepatic metabolism (Yeleswaram *et al*., 1997). Melatonin has very low affinity constants for both plasma albumin and α-1-acid glycoprotein and not likely to displace the binding of other drugs as per the Australian public assessment report for melatonin (Aus-PAR, 2009). Gibbs and Vriend, (1981) reported plasma half-life of 27 and 17 min. for melatonin in rats after assaying the samples using either tritiated or iodinated radioimmunoassay, respectively. The metabolism of melatonin in man involves hydroxylation at the 6-position by a hepatic cytochrome P_{450}, predominantly through CYP_{1A2} isoform. 6-hydroxymelatonin thus formed get conjugated with sulfate and to a lesser extent with glucuronic acid, and the conjugates excreted in urine. However, in some mouse strains melatonin has been shown to be metabolized to 6-glucuronylmelatonin rather than 6-sulfatoxymelatonin.

Exogenously administered melatonin can pass through the blood- brain barrier and accumulates in the CNS at substantially higher levels than exist in the blood (Tan, 2010). In the brain tissue the degradation of melatonin occur *via* oxidative pyrrole-ring cleavage leading to formation of N_1-acetyl-N_2-formyl-5-methoxykynuramine (AFMK) which subsequently deformylated by either arylamine formamidase or hemoperoxidase to N_1-acetyl-5-methoxykynuramine. In man, about 90 *per cent* of orally administered exogenous melatonin gets cleared in a single passage through the liver, while a small amount excreted *via* urine and saliva (Buscemi *et al*., 2004).

Neuroprotective properties

By virtue of chronobiotic and chronobiological regulator properties of melatonin, low levels due to disturbances in its secretion of nocturnal melatonin is involved in several neurological diseases, including insomnia, stroke, depression, Alzheimer's disease, Parkinson's disease, as well as migraine and headache. Melatonin offers neuroprotection in a number of neurodegenerative diseases and is widely

accepted as an alternative approach to ameliorate the symptomatic features of Parkinson's disease in experimental animals. Melatonin can easily cross the blood–brain barrier and enter the subcellular compartments, lacks toxicity as compared with many other neuroprotective agents, and possesses effective combating efficacy against free-radical-induced neuronal injury (Farzaei *et al.*, 2019).

In neurodegenerative disorders loss of melatonin receptors have been mainly described. In the early stages of Parkinson's disease, melatonin is not typically reduced but the expression of MT_1 and MT_2 was found to be declined in the substantia nigra and amygdala. In Alzheimer's disease changes are much more pronounced and the densities of MT_1 and MT_2 are progressively decreased in the cortex and pineal gland, specially MT_1 in the cerebrovascular system and, most importantly, in the suprachiasmatic nucleus, and of MT_2 in the hippocampus and retina (Savaskan *et al.,* 2007; Srinivasan *et al.,* 2015).

Anti-inflammatory activity

Melatonin is reported to have antioxidant and antioxidative stress function which is mediated by diminishing the redox status of cells (Hardeland *et al.,* 1993) and generating reactive oxygen species in tissues as well as stimulating the expression and function of antioxidant enzymes like catalase, glutathione peroxidase, and superoxide dismutase (Rodriguez *et al.,* 2004). Melatonin, once oxidized, cannot be reduced to its former state due to the formation of several stable end-products upon reacting with free radicals, thus, melatonin is considered as a terminal (or suicidal) antioxidant.

Melatonin also decreases electron leakage from the mitochondrial electron chain and modulates mitochondrial homeostasis through enhancing the production of glutathione. It has been found that melatonin has a well-established anti-inflammatory function through suppressing proinflammatory cytokines and their producing cells (leukocytes), as well as regulating the relevant transcription signaling pathways like nuclear factor (NF)-kB and PI3K/Akt (Mayo *et al.,* 2005; Maldonado *et al.,* 2010). The antioxidant and anti-inflammatory activities of this indoleamine are important in protecting neural cells against age-related neurodegeneration as well as oxidative damage pathologies. The production of inflammatory cytokines including TNF-α, IL-1β, or IL-6, subsides by melatonin in numerous experimental models of inflammation (Agil *et al.,* 2013). Various mechanisms have been reported for anti-inflammatory activity of melatonin including prevention of the activation of cyclooxygenase-2 and inducible isoform of nitric oxide synthase, as well as blocking of the transcriptional factors- that stimulates production of pro-inflammatory cytokines like NFk-B, HIF, *c*AMP, CREB, STAT, PPARs, and AP-19 (Korkmaz *et al.,* 2009).

Role in heavy metal toxicity

Experimental studies have shown that melatonin can reduce the lipid peroxidation and oxidative stress induced by certain heavy metals or their congeners in the central nervous system (CNS) (Rao and Purohit, 2011) or the vital organs elsewhere in the body (Uygur *et al.,* 2013). Further, in vitro studies using neuronal or other appropriate cell lines have explicitly shown that melatonin can (i) prevent the cobalt induced oxidative stress, cytotoxicity and amyloid-β (Aβ) release (Olivieri *et al.,* 2001), (ii) diminish sodium arsenite induced elevation of cyclooxygenase–II (COX2) and expression of inducible nitric oxide synthase (*i*NOS) (Lin *et al.,* 2007) and (iii) attenuates the toxin induced inflammatory stress and nitric oxide (NO) production (Song *et al.,* 2015).

In heavy metal toxicity melatonin pre-treatment significantly depresses the levels of *n*NOS, *e*NOS, *i*NOS, nitrotyrosine, nitric oxide production which in turn significantly reduce the 3-NT levels in brain tissue of rats and *caspase*-3 protein expression in hippocampus (Song *et al.,* 2015). Melatonin significantly reduces the production of the inflammatory mediators induced by heavy metals like arsenic, lead and titanium. Melatonin inhibits the activation of NF-kB pathway (prototypical pro-inflammatory signaling pathway) induced by TNF-α or ionizing radiation (Jain *et al.,* 2015).

Anticancer activity

Melatonin potentiates chemotherapeutic –induced apoptotic effects of anticancer agents that generate intracellular ROS. Melatonin causes significant depletion of glutathione (GSH), which in turn protects cells against oxidative hazard, causing a prooxidant mechanism related to the cytotoxic features of this indolamine. *Fas* ligand is a trans-membrane protein, subsequent to binding to its receptor plays a fundamental role in apoptosis induction. Oxidative stress induces the expression of *fas* receptor and fas ligand. Melatonin pro-oxidant properties trigger fas-induced cell death in human leukemic Jurkat cells (Wolfler *et al.,* 2001).

Significance in cardiovascular dysfunction

Melatonin plays a vital role in various coronary heart diseases and other cardiovascular diseases. Melatonin itself and also in combination with other cardio active drugs produces antihypertensive, anti-ischemic, and antianginal effects in patients (Zaslavskaia *et al.,* 2010). There are many biological factors such as hyper-cholesterolemia, diabetes, infectious agents and an excess of free radicals could cause cardiovascular disease. Most of the studies revealed that reactive oxygen species play a key role in pathogenesis of coronary atherosclerosis. ROS and subsequently oxidative stress leads to development and maintenance of hypertension.

Melatonin causes vasodilatation and reducing blood pressure by making availability of nitric oxide. Bioactive nitric oxide content will be reduced by accelerated generation of ROS by chemical inactivation of NO to form toxic peroxynitrite. As melatonin is a strong antioxidant agent which in part improve the endothelial function. Many studies have proven that melatonin have protective role against the oxidative stress and its consequent cardiac conditions and atherosclerosis (Eghbal *et al.,* 2016).

Several studies have investigated the antioxidant effect of melatonin on total cholesterol and VLDL (very low-density lipoprotein). Tengattini *et al.,* (2008) reported that plasma levels of total cholesterol and very low-density lipoprotein cholesterol as well as the low-density lipoprotein cholesterol sub-fraction was reduced by melatonin in hypercholesterolemic rats. Melatonin may demonstrate the effects by enhancing endogenous cholesterol clearance. Since, melatonin has a lipophilic property, it can enter the lipid phase of the LDL particles and inhibit lipid peroxidation.

To conclude, there exits ample scope to exploit melatonin as a therapeutic for several of its non-hormonal properties. However, studies involving long term exposure to melatonin are required before appreciating it potential application in various clinical circumstances.

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F-MNP-O1

IN SILICO APPROACHES TO UNVEIL THE HOTSPOT RESIDUES OF ANGIOGENIC CHEMOKINES AND ITS RECEPTORS IN CANCER CELL

K. Kanagarajadurai

Assistant Professor, Dept. of Veterinary Pharmacology, Veterinary College and Research Institute, Tirunelveli.

Email:kanagarajadurai.k@tanuvas.ac.in

Background: Chemokines and its receptors are found to have their role in various physiological and pathological functions, despite an established fact that they are chemotactic factors that attract leukocytes to the site of inflammation. ELR-CXC chemokines are potent angiogenic factors, which are involved in wound healing process as well as they found to have association with tumor growth and metastasis. One among the ELR-CXC chemokines is CXCL7, which is reported to have co-expressed in excess amount with its receptor, CXCR2 in advanced stage of canine transmissible venereal tumor (CTVT). ELR-CXC chemokines help tumor cells to metastasize. Earlier reports on CXCL7 reveal that they have the ability to stimulate tumor cell proliferation. Similarly, CXCR2 is reported to be expressed more during the inflammatory processes, tumor growth and metastasis conditions. Hence, canine CXCR2 might be a good target to inhibit the tumor growth in CTVT cells.

Objectives: The present study is to find hotspot residues of canine CXCR2, which may interacts with the inhibitor through *in silico* approaches. However, the study requires molecular structure of canine CXCR2. Due to lack of canine CXCR2 crystal structure, the objective of the current study is to model the molecular structure of canine CXCR2 through homology modeling and predict the hot-spot residues through *in silico* sequence and structure analysis of chemokines and its receptors.

Results: The present study revealed few hotspot residues, which are preserved for its structure and function. An *in silico* site directed mutagenesis revealed the importance of these residues in maintaining the structural stability of CXCR2.

Conclusion: The current study may throw a light on considering the CXCR2 as a target molecule for suppression of growth of various tumor or cancer cells. The identified hotspot residues may be proved experimentally. The constructed molecular structure of CXCR2 might be useful in designing a novel ligand as inhibitor.

F-MNP-O2

EFFECT OF MONOCROTOPHOS ON ELECTROSHOCK AND CHEMOSHOCK INDUCED CONVULSIONS IN ADULT SWISS ALBINO MALE MICE

Shree Lakshmi BN, Kumar V., Lohiya A.

Department of Veterinary Pharmacology and Toxicology, LalaLajpat Rai University of Veterinary and Animal Sciences, archana.lohiya@gmail.com

Monocrotophos (MCP), is widely and commonly used insecticide and acaricide for the control of variety of sucking, chewing and boring insects and pests.It is a direct acting organophosphorus insecticide and has been found most frequently associated with both accidental and intentional fatal pesticide poisonings. Study to assess its effects on experimentally induced convulsions was carried out in adult male Swiss albino mice at two dose levels, 0.8 and 1.6 mg/kg (20% and 40 % of MTD, respectively) by oral gavage. Effects were compared with respective control groups.Electroconvulsiometer was used to inducesupramaximal electroshock (MES) induced convulsions. Chemoshock convulsions were induced by using CNS convulsantdrugs such as pentylenetetrazol, strychnine, picrotoxin, tremorine and phenytoin + pentylenetetrazol. Observations were made at time of peak effect of monocrotophosi.e. 45 minutes after treatment of mice in case of electroshock convulsions and in chemoshock induced convulsions observations were made at that time when peak effect of MCP and respective chemoshock agent coincides. Different parameters such as time and duration of myoclonic jerks, clonic convulsions, tonic convulsions, tonic flexion, tonic extension, onset and peak time of tremors and death were recorded as per experiment.There was protective effect of MCP on MES induced convulsions in a dose dependent manner. There was also protective effect observed against tonic convulsions induced by PTZ and status epilepticus induced by phenytoin-PTZ at both doses of MCP. It potentiated the strychnine induced convulsions at both dose levels. Action of picrotoxin was also potentiated. Effect of tremorine was potentiated by MCP. The results can be useful for safety evaluation of MCP for its wide spread use.

F-MNP-O3

ANTICANCER EFFICACY OF COUMARINS WITH OR WITHOUT PIPERINE

Rajashekaraiah R[1*], N. Prakash[1], D. Rathnamma[1], Suguna Rao[1], A. Sahadev[1], C. R. Santhosh[1], U. Sunilchandra[1], Naveen Kumar S[1], Wilfred Ruban S[1], K.R. Anjan Kumar[1]

[1]Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar - 585401, Karnataka, India

rashmirvet7@gmail.com

Recently, phytocompounds have gained considerable attention of the scientific community and have been widely exploited for their anticancer potential. Among various phytochemicals, polyphenols have attracted considerable interest in the past few years due to their potential health benefits. Among polyphenols, coumarins have recently attracted much attention because of their broad pharmacological activities. In the present study, MTT[3-(4, 5- dimethylthiazolyl- 2)-2, 5- diphenyltetrazolium bromide] assay was conducted on MCF-7 and MDA-MB-231 cell lines at 48h incubation to determine percent cell viability. The IC_{50} values of umbelliferone, esculin and piperine for MCF-7 were 15.56, 20.35 and 17.83µM, respectively. Likewise, IC_{50} values of umbelliferone, esculin, and piperine for MDA-MB-231 were 10.31, 22.65 and 14.28 µM, respectively. A combination of umbelliferone (IC_{25}) and piperine (IC_{25}) on MDA-MB-231 and MCF-7 showed percent cell viability of 8.24±0.01and15.12±0.01, respectively. A combination of esculin (IC_{25}) and piperine (IC_{25}) on MCF-7 and MDA-MB-231 showed percent cell viability of 37.90±0.03 and 10.12±0.03, respectively. This study suggests that the cytotoxicity of umbelliferone, esculin, and piperinewas dose-dependent. The *in vitro* cytotoxicity potential in terms of percent cell viability, early apoptosis, G2M phase arrest and ROS indices of umbelliferone alone and in combination with piperine proved to be more effective ontriple-negative breast cancer cell lines.

F-MNP-O4

EFFECT OF PRAVASTATIN ON OXIDATIVE STRESS IN ISOPRENALINE-INDUCED CARDIAC FIBROSIS IN MOUSE MODEL

Abhinav Rana, Meemansha Sharma, Thakur Uttam Singh, Subhashree Parida, Madhu C Lingaraju, Kesavan M, Dinesh Kumar

Division of Pharmacology and Toxicology, ICAR-IVRI, Izatnagar-243122, Bareilly, Uttar Pradesh

The current investigation was done to observe the effect of pravastatin on oxidative stress parameters in isoprenaline-induced cardiac fibrosis in mouse model. Isoprenaline was administered for 14 days in mice to induce the cardiac fibrosis. Mice were divided into four groups as control, pravastatin alone, isoprenaline-administered and isoprenaline plus pravastatin. Pravastatin was co-administered orally to the mice; however, isoprenaline was administered via subcutaneous route. Mice were sacrificed by bleeding from vena cava under urethane anaesthesia after 24 hours of last dose of isoprenaline administration. Then, heart tissues were collected from all the groups. Different oxidative stress parameters such as lipid peroxidation, non-enzymatic antioxidant reduced glutathione (GSH), enzymatic antioxidants superoxide dismutase (SOD) and catalase were assessed in heart tissue homogenates. Pravastatin co-administration significantly reduced the MDA level in isoprenaline-administered myocardial injured mice. Further, non-enzymatic antioxidant reduced glutathione was also significantly increased in pravastatin treated cardiac injured mice. Nevertheless, enzymatic antioxidants such as catalase and superoxide dismutase were attenuated in isoprenaline administered mice and catalase was markedly increased in pravastatin treated mice but not significantly. Further, superoxide dismutase was not increased with the pravastatin treatment in cardiac injured mice. In conclusion, pravastatin showed attenuation in lipid peroxidation and increase in reduced glutathione activity, however, superoxide dismutase activity was not increased.

F-MNP-O5

LEPTIN INHIBITS LATE PREGNANT UTERINE CONTRACTION INDEPENDENT OF CANONICAL JAK-STAT PATHWAY

Srinivasan G, Subhashree Parida, Kesavan M, Pavithra S, Thakur Uttam Singh, Dinesh Kumar

Division of Pharmacology and Toxicology, IVRI, Izatnagar, Bareilly-243122

Leptin is predominantly secreted by adipose tissue and exerts an inhibitory effect on both spontaneous and oxytocin-induced contractions in myometrium. In the present study, we studied the uterine contraction characteristics in presence of leptin and the possible mechanisms of its effect in late pregnant mouse uterus. Isolated longitudinal strips (6–8 mm long by 2–3 mm cross section) from the mid-horn region of 19day pregnant uterus were mounted in a thermostatically-controlled isolated organ bath containing modified Krebs–Henseleit solution which was continuously bubbled with medical airmaintained at 37 ±1˚C under a constant passive tension of 1g throughout the experiment. Leptin exerted an inhibitory response (I_{max} 40.5±3.99%) on basal uterine contractions in late(19-day)pregnant mouse uterus. The extent of inhibition

was small but significant, and was less than that obtained with known uterine relaxants, salbutamol (I_{max}103±8.66%)and BRL-37344(I_{max} 84.79±8.12%). Leptin-induced uterine response was inhibited by leptin receptor antagonist SHLA(10 nM) but was not affected by JAK-STAT pathway inhibitor, AG-490 (10µM). The relaxant response was also found to be mediated by stimulation of NO and COX pathway. We detected leptin receptor mRNA and protein in late pregnant mouse uterus. The later was located in endometrial luminal epithelium and myometrial layers. In conclusion, the results of the present study suggest that leptin inhibits mouse uterine contraction by stimulating leptin receptors and activating NO and COX pathways in a JAK-STAT independent manner.

S-MNP-O1

IMMUNOMODULATORY EFFECT OF MALABARI GOAT LACTOFERRIN ON BOVINE PERIPHERAL BLOOD MONONUCLEAR CELLS

Deepak Chandran, Uma R., Jayavardhanan K. K. and Shynu M.

Department of Veterinary Biochemistry, CVAS, Mannuthy, Thrissur

Lactoferrin, well known as a minor whey protein, is an 80kDa iron-binding glycoprotein primarily present in milk. It displays a broad spectrum of biological activities including antioxidant, antimicrobial, anticancer, metal binding and immunomodulatory properties. The present study focussed on the isolation of lactoferrin (gLf) from the colostrum of Malabari goats and the assessment of its *in vitro* immunomodulatory potential on peripheral blood mononuclear cells (PBMCs). Varying concentrations of gLf was utilized to assess its cytoproliferative effect on PBMCs in the presence and absence of a known mitogen, phytohemagglutinin. Higher concentrations of lactoferrin were found to significantly inhibit the proliferation of PBMCs whereas lower concentrations brought about significantly active proliferation of the cells both in the presence and absence of mitogen. The proliferative responses induced by the mitogen were inhibited by gLf at higher concentrations. These results indicate that gLf possesses potent immunomodulatory effect with respect to the proliferation of lymphocytes.

S-MNP-O2

MODULATION OF ACETYLCHOLINE INDUCED INTESTINAL MOTILITY BY PIPERINE IN TYPE 2 DIABETIC RATS

Megha M.[1],Suraj S.V[1]., Nair S. N[1]., Nisha A.R[1]., Ajithkumar K.G[2]., Sanjay B.M[1].,Saranya C[1]., Mery, S. J[1]., Shijin M.S., Ranjith D[1]., and Juliet S[1]

[1]Department of Veterinary Pharmacology and Toxicology, [2]Department of Veterinary Parasitology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala- 673576.

Piperine is a piperidine-ring containing alkaloid and a major constituent of *Piper nigrum* Linn. And *Piper longum* Linn. species, belonging to the Piperaceae family. Piperine exhibits antidiarrheal and antispasmodic activities, mediated possibly through calcium channel blockade. Altered digestion and assimilation of nutrients have been a major concern in long term cases of diabetes mellitus. In hyperglycaemic state there may be changes in receptor structure and function in many tissues due to impaired glucose metabolism. The present study was undertaken to evaluate whether piperine will protect the acetylcholine induced intestinal activity in streptozotocin induced high fat diet (HFD) fed type 2 diabetic rats compared to normal rat. The study was conducted on six adult Sprague Dawley rats of either sex weighing 250-350 grams (g) body weight. Rats were fed with high fat diet for three months followed by streptozotocin injection to induce type 2 diabetes. The animals were humanely sacrificed on the day $10^{th}$and two to three centimetre long ileum was separated from a region five centimetre away from the ileo-caecal junction and was transferred to physiological saline solution (Tyrode solution),kept at 37.2^{o}C. The contractile activity of rat ileum will be measured using isolated organ bath containing 20 mL Tyrode solution and tied to an isometric force transducer with a resting tension of 0.5- 1 g. The tissue was equilibrated for 60 minutes and the tension was recorded using a polygraph digital data acquisition system linked to isometric transducer connected to a recorder. A dose response curve was made by adding cumulative concentrations of 100 µL and 300 µL of acetylcholine ranging from 10^{-7} M to 10^{-2}M and median effective concentration (EC_{50}) was calculated for the tissue. Piperine was added at concentrations of 10^{-2} to 10^{-7} alone and in presence of acetylcholine. The contractile response was also studied in presence of inhibitors of contractile activity. The EC50 value of acetylcholine in diabetic rat was in range of $4.481\times10^{-7}$and that of normal rat was2.877×10^{-6}. The result suggested that acetylcholine significantly increased the contractile response in diabetic rats and the acetylcholine induced intestinal motility was modulated by piperine.

ISVPT – 2019

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RAPPORTEUR : DR. RAMESH KUMAR NIRALA

College of Veterinary and Animal Sciences, Mannuthy

Pharmacogenomics in the development and validation of indigenous drugs
XIX ISVPT
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LP-IAI-O1

USE OF PROBIOTIC SUPPLEMENT (FEED UP YEAST) IN IMPROVING PERFORMANCE OF COMMERCIALBROILER FARMS

C.K. Steephen

Managing Director, Kevinjo Pharmaceuticals Private Limited, Tnrwa 50, Tagore Nagar, Kadavanthara, Kochi-682020

Abstract: Indiscriminate use of antibiotics to treat infections in human and animals has resulted in enormous antimicrobial pressure on pathogens, leading to the development of antimicrobial resistance. The present study was to find out whether probiotics can be used to reduce infections thereby reduce use of antibiotics in Poultry and enhance performance in poultry farms.

PROBIOTIC USED: *Saccharomyces cerevisiae* (Brand: Feed Up Yeast contains *Saccharomyces cerevisiae* 1000 CFU/100gm)

Present study was conducted at a commercial broiler farm in Mannarkkad, Palakkad in a batch of 7500 broiler batch and a control batch of 2000 birds. Supplemented feed Up Yeast 50gm/1000 chicks for the first 3 days and then alternative days as per the manufacturer's recommendation through drinking water. Whereas control batch was supplemented with Vitamin ADEB12 liquid from 8^{th} to 10^{th} day from 24^{th} day onwards Liver tonic for 3 alternated days and cephalexin powder as prophylaxis in chicks for first 4 days. Feed, Chicks, Vaccination were same with both batches.

Results: Sold the birds of the Feed Up yeast supplemented batch from 36^{th}-40 day with average FCR: 1.59whereas the FCR of control batch was 1.75.there was a substantial decrease of 0.19 FCR in the Feed Up yeast supplemented batch compared to the control batch. Following positive results found in the batch supplemented with Feed Up yeast

1. There was no wet dropping 2.Substancial reduction in the bad smell in the cage. 3. Dry litter compared to control batch. 4. Water and feed intake was high. 5. Substantial reduction in back infection due to dry droppings 6.Lower fat deposition observed in the batch.

Conclusion

It is the age old practice in the commercial broiler farms to use antibiotics as a prophylaxis during first week ever since the industry started to prevent early chick mortality resulting from to suppressed immunity due to transportation stress. Detailed study is required to find out the benefits of probiotics to improve FCR and performance in the commercial broilersector. A dedicated campaign is required by the stakeholder to stop using antibiotics as prophylaxis and use probiotics in addition to maintaining biosecurity, vaccination etc.

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Pharmacogenomics in the development and validation of indigenous drugs
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F-PG-P1

IN VITRO SYNERGISTIC ACTIVITY OF PIPERINE WITH CIPROFLOXIN IN *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS ISOLATES* FROM BOVINE MASTITIS

Vanishree, Nisha A.R., Sanis Juliet,Naicy Thomas, Suja Rani S.

Department of Veterinary Pharmacology and Toxicology, CVAS, Pookode, Kerala

Mastitis as the multifactorial, endemic and economical disease of the mammary gland. Extensive and indiscriminate use of antibiotics in the treatment and control of mastitis may lead to emergence of antibiotic resistant bacteria and transfer of resistant genes. Among various pathogens *Escherichia coli* and *Staphylococcus aureus* contributes to major occurrence of mastitis in dairy animals and also posses antimicrobial resistance. Combination of phytochemicals with antimicrobial agents shown effective measure to overcome antimicrobial resistance. Piperine is one such phytochemical used in this study in combination with ciprofloxacin in *Escherichia coli* and *Staphylococcus* isolates from mastitis. In the present study disc diffusion antimcrobial assay is inducted in *Escherichia coli* and *Staphylococcus aureus* isolates from mastitis with Ciprofloxacin and Piperine combination at different doses and Piperine increased the zone of inhibition for Ciprofloxacin in both *Escherichia coli* and *Staphylococcus aureus* isolates. This property of Piperine can be further exploited to overcome antimicrobial resistance.

F-PK-P1

MILK PHARMACOKINETICS OF CEFTIZOXIME IN SHEEP AFTER SINGLE DOSE INTRAMUSCULAR ROUTE

Patel H.A., Mody S.K., Patel H.B. and Singh R.D.

Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry

Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat- 385506

patelharshad49@gmail.com

Ceftizoxime is a third generation cephalosporin having high bactericidal activity against a wide range of Gram-positive and Gram-negative microorganisms including Streptococci, Staphylococci, Proteus, Bacillus, Klebsiella, Clostridium, Salmonella and Shigella spp. It is commonly used for the treatment of the infections of respiratory tract, mastitis, urogenital tract, skin, soft tissues, bones and joints. Ceftizoxime has certain pharmacological and clinical advantages over other cephalosporin. It has better activity against anaerobes, broader spectrum of activity against Gram negative bacteria, penetrates the cerebrospinal fluid in

sufficient concentration due to greater lipid solubility, and is resistant to hydrolysis by β-lactamase. Looking to these facts, the present study was conducted in milk to investigate single dose intramuscular (IM) pharmacokinetics of ceftizoxime in six Patanwadi sheep aged between 2 to 4 years and weighing between 25 – 35 kg, at the dose rate of 10 mg kg^{-1}. The milk samples were collected before (0 min), and after single dose intramuscular administration of ceftizoxime at 10 min, 30 min and 1h, 2h, 6h, 12 h, 24 h, 36 h, 48 h, 72 h and 96 h after drug administration. Milk concentration of ceftizoxime was analysed using UHPLC with UV detector. The pharmacokinetic parameters were calculated using PK solver software. Following single dose IM administration, the average value of C_{max} of ceftizoxime (48.13 ± 0.30 µg ml^{-1}) was obtained at 6 h. The milk concentration of 0.33 ± 0.01 µg ml^{-1} detected above MIC level for up to 72 hr. The mean values of pharmacokinetic parameters *viz.* elimination rate constant, elimination half-life, Mean resident time and area under curve were found as 0.11 h^{-1}, 6.19 h, 20.09 h and 996.23 µg h ml^{-1}, respectively. The average value of total milk clearance (Cl_M) was obtained to be 0.01 L h^{-1} kg^{-1}. Therapeutically high concentration of ceftizoxime ≥0.2 µg ml^{-1} was maintained for 72 h after single dose intramuscular administration. So the single intramuscular dose of ceftizoxime (10 mg kg^{-1}) effectively concentration of ceftizoxime in sheep and can be used for the treatment to combat infectious disorders caused by susceptible bacteria.

F-PK-P2

PHARMACOKINETIC INTERFACE OF ROXITHROMYCIN AND CIPROFLOXACIN IN BROILER BIRDS

Singh R. D., Mody S. K., Patel H. B., Sarvaiya V. N. and Patel B. R.

Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry

Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat- 385506

ratn1709@yahoo.com

The combined use of roxithromycin (macrolide) and ciprofloxacin (fluoroquinolone) is promising antimicrobial therapy to treat complicated CRD (Chronic Respiratory Disease) in broiler birds. This proposed combination can be put forward only after having the knowledge of interaction pharmacokinetics of two antimicrobials when used in combination.The present study was undertaken to study the influence on pharmacokinetics of roxithromycin and ciprofloxacin due to each other when concomitantly administered in broiler chickens (n=8). The plasma concentrations were assayed by two validated UHPLC methods using UV detector. Following concomitant administration of single oral doses of roxithromycin (20 mg/kg body

weight) and ciprofloxacin (10 mg/kg body weight) in broiler chickens, mean plasma maximal concentrations of roxithromycin and ciprofloxacin were 3.70 and 1.51 µg/ml, respectively. The pharmacokinetic parameters of roxithromycin and ciprofloxacin showed no significant effect on values of either drug when given in combination as compared to their alone oral administrations. Thus, there was a lack of pharmacokinetic interaction between the two antimicrobials.

F-PK-P3

ESTIMATION OF *IN-VITRO* PLASMA PROTEIN BINDING OF AMPICILLIN IN HORSES AND BUFFALO CALVES BASED ON SPECTROPHOTOMETRIC METHOD

K. Kasturi Devi, Rajdeep Kaur, M.K. Lonare, V.K. Dumka and S.P.S. Saini

Department Veterinary Pharmacology & Toxicology

Guru Angad Dev Veterinary And Animal Science University, Ludhiana

Estimation of plasma protein binding (PPB) is of paramount importance in the pharmacokinetics characterization of drugs, as it can cause significant change in volume of distribution, clearance and drug half-life. Ampicillin (α-amino benzyl penicillin) is most commonly used penicillin drug in veterinary practice. This study was conducted to determine the extent of PPB nature of ampicillin in healthy horses and buffalo animals (n=6).A spectrophotometric method was applied for the determination of ampicillin at 320nm, based on acid degradation product of penicillin at 75^0C in the presence of citrate buffer (pH 5.2) and a trace of copper salt. In the study, it was observed that this method permits the detection of ampicillin to a level above 0.5µg/ml. The plasma samples and buffer were also quantified for the drug levels by using HPLC, and the results were in agreement with the spectrophotometric method. Various concentrations of ampicillin (3.125, 6.25, 12.5, 25, 50, 100 µg/ml) were prepared in triplicate in pooled plasma collected from healthy animals. *In vitro* binding of ampicillin to plasma proteins was determined by employing the equilibrium dialysis technique. The study revealed that the percent of ampicillin binding to plasma protein was very less in horses *i.e.* to the extent of 7.25±0.19% whereas in buffalo calves it was 18.5±0.78%. Binding capacity of ampicillin to plasma protein (β_i) and dissociation rate constant of protein-drug complex (K_β) in the present study were $2.035x10^{-7}$ mol.gm^{-1} and $0.0557\ x10^{-8}$ mol, respectively in horses. In buffalo calves, (β_i) it was $2.54x10^{-7}$ mol.gm^{-1} and (K_β) $1.068x10^{-8}$ mol. Low plasma protein binding aspect of ampicillin indicates that as an unbound drug it can easily diffuse into the extra cellular fluid through the

capillary pores and attain an almost equal concentration to that in plasma; which can influence its antimicrobial efficacy.

F-PK-P4

INTERACTIVE EFFECT OF CARBENDAZIM AND IMIDACLOPRID ON MESENCHYMAL STEM CELLS DERIVED FROM BUFFALO BONE MARROW

H. Singh, M.K. Lonare, M. Sharma, S. Singla and S.P.S. Saini

Department Veterinary Pharmacology & Toxicology

Guru Angad Dev Veterinary And Animal Science University, Ludhiana

Present investigation was planned to investigate effect of carbendazim (CBZ) and imidacloprid (IMI) alone and in combination on bone marrow-derived mesenschymal stem cells (bMSCs) of buffalo origin. bMSCs were isolated and cultured in Dulbecco's Modified Eagle's Medium supplemented with 15% fetal bovine serum. bMSCs were exposed to the concentrations 8.98 µM, 4.49 µM, 2.25 µM for CBZ and 3.22 mM, 1.61 mM, 0.81 mM for IMI alone and in combinations and observations were made: alterations in cell morphology, viability, biochemicals (CK-MB, APL, LDH and GGT), antioxidant markers (GSH, GPx, SOD, CAT and, GST), oxidative stress markers (LPO, O^{2-} radical, ROS and $\Delta\Psi m$), apoptotic index and cell senescence. bMSCs were characterized for stem cell surface markers and found to be positive for AP, CD73 and OCT4. Results revealed significant reduction ($p \leq 0.05$) in % cell viability and anti-oxidant markers (GPx, SOD, CAT and, GST) whereas, significant ($p \leq 0.05$) increase in LPO, O^{2-} radical and biochemical parameters (ALP and, CK-MB). ROS positive cells, cells with loss of $\Delta\Psi m$, % apoptotic index and senescent cells were significantly increased in CBZ and IMI treated groups. All changes were observed in dose dependant manner in CBZ and IMI alone treated groups, while lower doses combinational group showed elevated effect similar to higher dose groups when compared with them. Present findings suggest that CBZ and IMI induced cytotoxicity in bMSCs mediated via LPO, ROS production, altered $\Delta\Psi m$ and lower antioxidant status which further responsible for cellular damage, apoptosis and senescence process. This study suggested that co-existence of CBZ and IMI in a medium has additive or synergistic effect on buffalo bMSCs even at lower doses.

F-PK-P5

COMPARATIVE PHARMACOKINETICS OF COMMERCIAL PREPARATIONS OF ENROFLOXACIN FOLLOWING INTRAVENOUS ADMINISTRATION IN GOATS

V.K.Gond [1], R. K. Nirala 1, Kumari Anjana[2], C.Jayachandran[3]

[1] Animal Production Research Institute,R.P.C.A.U.,Bihar, Pusa, Samastipur- 848125

1, 2, 3 Department of Veterinary Pharmacology & Toxicology,

Bihar Veterinary College, Bihar Animal Sciences University Patna-14

Antimicrobial therapy constitutes a major component of modern medical and veterinary practices. Enrofloxacin has been developed exclusively for veterinary use. It is a potent inhibitor of DNA-gyrase enzyme and is highly effective against many organisms that are resistant to β-lactamases, aminoglycosides, macrolides, tetracyclines, folic acid antagonist *etc* (Bauditz, 1987; Elmas *et al*, 2000). Because of high prevalence of enrofloxacin sensitive bacterial infections and high cost of the pioneer product, there has been tremendous increase in the use of other brands of enrofloxacin with increase availability use of generic enrofloxacin product from different pharmaceutical companies, practitioners are faced with dilemma of therapeutic failure and side effects following the use of some of these array of multisource product in the market. Since these clinical conditions result in great economic losses to farmer and the pioneer formulations and few brands have severally proven effective. Keeping in view of above facts, the present study was undertaken and compared with each other with the respect of pharmacokinetics parameters. In the present investigation, five clinically healthy female goats of non-descript breed were used .Three commercial preparations of enrofloxacin (10%) were used @5mg/kg body weight.wt intravenously (IV) .The samples of plasma were collected at different time interval *i.e.,* 0.042, 0.083, 0.125, 0.333, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 h after IV administration of drugs. The drug was present significantly at lower concentration in brand II (8.26 ± 0.66) as compared to brand I (17.08 ± 1.92 $\mu g.ml^{-1}$) and brand III (14.11 ± 2.91) at 0.042 h. Similarly, brand II shows lower concentrations up to 0.333 h and at 3 & 24 h. The drug maintained its therapeutic concentration (≥ 0.125 $\mu g.ml^{-1}$) up to 12 h in brand I and II while it was present up to 24 h in brand III.

S-PK-P1

DISPOSITION OF ISONIAZID IN BOVINE CALVES BY ORAL VERSES INTRAVENOUS ROUTE

Khiratkar Krutika[1] , Sole Sushant[2] and Bagal Niranjan[3]

[1] Postgraduate Research Scholar, Department of Pharmacology and toxicology, Mumbai Veterinary College, Mumbai krutikakhiratkar04@gmail.com, [2]Sectional In-charge,Department of Pharmacology and toxicology, Mumbai Veterinary College, Mumbai, [3] Research Scholar, Department of Pharmacology and toxicology, Mumbai Veterinary College, Mumbai

Isoniazid is an integral part of conventional tuberculosis therapy in human as well as animal population. In view of growing concern of zoonotic diseases like tuberculosis, the effective management of animal tuberculosis is of atmost importance. The use of isoniazid in the therapy of animal tuberculosis was in place since long time, however very limited reports are available on pharmacokinetics of isoniazid in bovine calves. The research finding in the same laboratory has given a baseline data of isoniazid in bovine calves. The present study is being undertaken for detailed analysis of pharmacokinetics of isoniazid at different doses so that the comparative assessment can be done.

S-PK-P2

PHARMACOKINETIC STUDY OF ISONIAZID IN COW (CALF)

Bagal Niranjan[1], Sole Sushant[2] and Khiratkar Krutika[3]

[1,3] Research Scholar, Department of Pharmacology and toxicology, Mumbai Veterinary College, Mumbai, krutikakhiratkar04@gmail.com [2]Sectional In-charge, Department of Pharmacology and toxicology, Mumbai Veterinary College, Mumbai

Isoniazid also known as isonicotinylhydrazide (INH) is an integral part of conventional tuberculosis therapy in human as well as animal population. In view of growing concern of zoonotic diseases like tuberculosis, the effective management of animal tuberculosis is of atmost importance. The use of isoniazid in the therapy of animal tuberculosis was in place since long time, however very limited reports are available on pharmacokinetics of isoniazid in bovine calves. This research finding gave a baseline data of isoniazid in bovine calves. The present study was undertaken in bovine calves in which three calves received an oral dose of INH @25mg/kg b.wt and other three calves received an intravenous dose of INH @10mg/kg b.wt, after 15 days crossover of drug administration was repeated i.e calves who received an oral INH drug were subjected to intravenous INH administration and vice-versa. Blood collection was done at various time intervals i.e. 15,30 minutes,1,2,4,6,8,12,24 and 48 hours and pharmacokinetic effect was analysed by high pressure liquid chromatography (HPLC).INH showed a retention time of 5.8 minutes on using isocratic

mobile phase made up of buffer(Sodium Dihydrogen orthophosphate pH-6 and acetonitrile (96:4 ratio),flow rate of 1ml/min and C-18 reverse phase column as used to INH at wavelength 265nm after injecting 20µl of samples with Pyraziamide (PZA) as internal standard. The results conclude that the oral route of INH given was more convenient than intravenous route of INH. Oral route of INH shows a bacteriostatically effective blood level for considerable length of time and has high half life ($t^{1/2}$) and Mean residence time (MRT).

F-EP-P1

EVALUATION OF BIOCHEMICAL PARAMETERS OF RATS FED WITH HIGH FAT DIET TREATED WITH *AVERRHOA BILIMBI* FRUIT POWDER

Lisha V[1]*, Preethy John[2], Sujith S[3], Usha PTA[4]

Department of Veterinary Pharmacology and Toxicology,

College of Veterinary and Animal Sciences, Mannuthy- 680 651, Thrissur, Kerala

E-mail: lisha49@gmail.com

The fruits of the *A.bilimbi* were used folklorically to control obesity in some villages in India.The effects of *Averrhoa bilimbi* fruit powder in hyperlipidemic rats on biochemical parameters of liver and kidney were evaluated. Group I constitutes normal control and received standard diet. For all groups except normal control, hyperlipidemia was induced by giving the fat rich diet for 15 days. Group II received high fat diet and remained hyperlipidemic throughout the experiment. Groups III, IV and V was fed with 125, 250 and 500 mg/kg body weight of *A. bilimbi* fruit powder orally from 16th day along with high fat diet for 30 days. Standard drug rosuvastatin was given orally to Group VI at a dose rate of 10mg/kg body weight from 16th day along with high fat diet. Biochemical parameters like alanine amino transferase (ALT), blood urea nitrogen (BUN) and creatinine were assessed to assess the effect of fruit powder on liver and kidney. After the induction of hyperlipidemia, the serum ALT and creatinine were found to be elevated in all groups except normal control. However, the ALT and BUN levels were found to be increased with *A. bilimbi* given at the dose of 500 mg/kg of body weight as well as with rosuastatin group at the end of the experiment. The fruit powder did not significantly ($P < .05$) alter the creatinine level in the serum of rats. The results suggests that *A.bilimbi* fruit powder is capable of normalizing the biochemical abnormalities associated with the pathophysiology of hyperlipidmia.

F-EP-P2

PHYTOCHEMICAL ANALYSIS AND ASSESSMENT OF ANTIOXIDANT ACTIVITY OF HYDROETHANOLIC EXTRACT OF *PLATYCLADUS ORIENTALIS* IN RATS

Saklani, S. and Singh, S.P.

Department of Veterinary Pharmacology & Toxicology,

CVASc, Pantnagar-263145.

This study was conducted with the aim to conduct phytochemical analysis and evaluate antioxidant potential of hydroethanolic extract of (HEPO) *Platycladus orientalis* following oral administration @ 200mg/kg and 400mg/kg b.wt. in paracetamol @500mg/kg b.wt for 21 days in rats. standard and widely accepted methodology was used for phytochemical nalysis and to determine of various antioxidant parameters. The protocol for experimentation was approved by IAEC.The phytochemical analysis of leaf extract of hydroethanolic extract of *Platycladus orientalis* revealed 11% yield and the presence of alkaloids, flavonoids, phenolic compounds, sugars, glycosides, proteins and saponins. Thirty rats were divided equally and randomly into 5 groups with 6 rats in each group. Group I served as control, group II rats were administered with PCM, group III with PCM @ 500mg/ kg b. wt. plus silymarin @100 mg/kg, group IV with PCM @ 500mg/ kg b. wt. plus HEPO @ 200mg/kg and group V with PCM @ 500mg/ kg b wt. plus HEPO @ 400mg/kg b. wt. for 21 days . A significant ($P<0.05$) decline in RBC and tissue GSH, SOD and catalase activity and a significant ($P<0.05$) increase in LPO were observed in paracetamol treated group as compared to control. HEPO showed amelioration in dose dependent manner indicating potent antioxidant effect of HEPO following 21 days oral administration in rats. This was also supported by *in vitro* DPPH and ABST free radical scavenging activity of HEPO.

Thus, it is concluded from the present study that hydroethanolic extracts of *P. orientalis* (HEPO) revealed presence alkaloids, flavonoids, phenolic compounds, sugars, glycosides, proteins and saponins and its daily oral dose @ 200 & 400 mg/ kg b. wt. produced antioxidant potential against paracetamol daily oral dose @ 500 mg/kg b. wt. induced oxidative stress after 21 days treatment in rats.

F-EP-P3

HISTOPATHOLOGICAL CHANGES FOLLOWING TREATMENT WITH *PLATYCLADUS ORIENTALIS* IN PARACETAMOL TREATED RATS

Saklani, S., Singh, S.P. and Batra, M.[1]

*Prof &Head, Department of Veterinary Pharmacology

& Toxicology, 1Dept.of Veterinary Pathology, CVASc, Pantnagar-263145.

The aim of this study was to examine histopathological changes induced by paracetamol (@ 500 mg/kg b. wt., oral) and their amelioration by hydroethanolic extract of (HEPO) *Platycladus orientalis* following oral administration @ 200mg/kg and 400mg/kg b.wt. after 21 days treatment in rats. Standard and widely accepted procedure was used for histopathological examination of visceral organs such as liver, spleen, kidney and heart. The protocol for experimentation was approved by IAEC.Histopathological changes in paracetamol treated groups were characterized by severe degree of congestion of central vein and sinusoids, severe degeneration and necrosis of hepatocytes. In spleen, severe lymphoid depletion and loss of lymphocytes in area of pariarteriolar lymphoid sheath (PALS) was evident. Histopathological changes in liver from paracetamol treated groups were characterized by severe degree of congestion of central vein and sinusoids, severe degeneration and necrosis of hepatocytes which were of low and mild intensity in HEPO treated groups. In heart, fragmentation of muscles fiber bundle and necrosis of cardiomyocytes and in kidney, severe congestion of blood vessel, haemorrhage and fragmentation and complete loss of glomeruli, necrosis of renal tubular epithelial cells were observed in paracetamol treated group which were ameliorated by treatment with HEPO in a dose dependent manner after 21 days in rats.Thus, it is concluded from the present study that hydroethanolic extracts of *P. orientalis* (HEPO) at daily oral dose @ 200 & 400 mg/ kg b. wt. ameliorated histopahological changes in visceral organs in paracetamol (@ 500 mg/kg b. wt.,oral) induced toxicity after 21 days treatment in rats.

F-EP-P4

EVALUATION OF *IN VIVO* ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF *LABLAB PURPUREUS* LEAF EXTRACT IN RATS

Prakash, V.S[1]., Shridhar, N.B[1]., Prakash, N[2]., Prashantkumar Waghe[3]., Kavitha Rani, B[4]., Sunilchandra, U[1] ., Revanasiddappa, Y[1]., Punith, K. G[1] and Manikanth, C. L[1]

[1] Department of Veterinary Pharmacology and Toxicology, Veterinary College Shivamogga., [2] Dean, Veterinary College, Shivamogga., [3] Department of Veterinary Pharmacology and Toxicology, Veterinary College, Bidar., [4] Department of Veterinary Pathology, Veterinary College, Shivamogga.

Email: prakashvs1126@gmail.com

Lablab purpureus, commonly referred to as field bean or hyacinth bean, is a legume widespread throughout the tropics. In India, it is an important legume used as a pulse and vegetable for human consumption and forage. It is a versatile multipurpose plant that offers food, fodder, green manure and some traditional home remedies. Different parts of the plants such as leaves, seeds, stem, roots and pods are traditionally used for curing arthritis, inflammation, skin diseases, and colic and as an anti-helminthic in India. Hence, the present study was undertaken to evaluate the *in vivo* anti-inflammatory and analgesic activity of the plant *Lablab purpureus* leaf methanolic extract using carrageenan induced paw oedema and acetic acid induced writhings, respectively, in Wistar rats (Either of the sex). The leaf extract at the doses of 400 and 600 mg/kg is having significant anti-inflammatory as well as analgesic property *in vivo*, compared with control group rats. Based on the present study it can be inferred that the methanolic extract of *L. purpureus* leaves might contain some phytochemicals against the inflammation and pain mediators and this property of the same leaves can be utilized to explore the therapeutic efficacy.

F-EP-P5

EVALUATION OF ANTI-OSTEOPOROTIC POTENTIAL OF *MORINGA OLIFERA* IN RATS

Rawat, P., Ahmad, A.H., Pant, D., Sharma, S., Pathak, A., Verma, M.K.

Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand

Email-ahahmad1@yahoo.com

Moringa oliefera is a middle-sized multipurpose tree, cultivated throughout the country and used as a vegetable, spice, cooking, cosmetic oil and as a medicinal plant. It has many medicinal properties, viz., anti-inflammatory, antioxidant, antimicrobial, antihyperlipidaemic, anti fertility and anticancer activities. The aim

of this study was to explore anti-osteoporotic activity of aqueous extract of *Moringa oleifera* (MOAE) in rats which was evaluated by bone histomorphometry, X-ray radiography of femur of rats and estimation of osteoporotic biomarkers such as alkaline phosphatase (ALP) and serum calcium. Adult Albino rats were randomly divided into 6 groups. Group I served as a control, group II and group III were administered aqueous extract of *Moringa oleifera*(MOAE) was administered @ 200mg/kg b. wt and 500mg/kg b.wt orally, repectively. In group IV and V aqueous extract of *Moringa oleifera*(MOAE) @ 200mg/kg b. wt and 500mg/kg b.wt orally with prednisolone @ 1mg/kg b wt. intra peritoneal and in group VI prednisolone was administered @ 1mg/kg b wt. intra peritoneal daily for 28 days. Our study indicated that MOAE significantly ($P<0.05$) increased ALP and serum calcium levels. Histomorphometry, X-ray radiography of femur confirms the anti-osteoporotic activity. It can be concluded that MOAE has the potential to prevent osteoporosis in rats.

F-EP-P6

IMMUNOSTIMULANT POTENTIAL OF *CUMINUM CYMINUM* IN COMBATING CYCLOPHOSPHAMIDE INDUCED IMMUNOSUPPRESSIONS IN RATS

Patwal P.C., Ahmad A.H., Pant D., Aswathy, P.U., Arya, N., Verma, M.K.

Department of Pharmacology and Toxicology, College of Veterinary and Animal Sciences, G. B. Pant University of Agriculture and Technology, Pantnagar – 263145

Email-ahahmad1@yahoo.com

Cuminum cyminum commonly known as Jeera is a fast growing annual plant cultivated throughout the country. The objective of this study was to analyze immunostimulant potential of hydroethanolic extract of *Cuminum cyminum* (CCHE) in combating cyclophosphamide induced immunosuppression in rats. This activity was evaluated by estimating humoral antibody titre, delayed type hypersensitivity, neutrophil adhesion, phagocytic index and total immunoglobulin level. Healthy Sprague Dawley rats were divided into eight groups. Group I served as a control, group II rats were administered with cyclophosphamide @ 100 mg/kg b.wt. on 9^{th} and 16^{th} day of study orally, group III rats were administered with levamisole @ 50 mg/kg b.wt. subcutaneously whereas rats of group IV were administered with cyclophosphamide (on 9^{th} and 16^{th} day) and levamisole for a period of 28 days, group V and VI were administered with CCHE @ 200 and 400 mg/kg b. wt. orally respectively and group VII and VIII were administered with CCHE @ 200 and 400 mg/kg b.wt. orally along with cyclophosphamide @ 100 mg/kg b.wt. on 9^{th} and 16^{th} day of study for a period of 28 days. A significant ($p<0.05$) decrease in humoral antibody titre, DTH response, total immunoglobin level, phagocytic index and neutrophil adhesion was recorded in cyclophosphamide treated group as compared to control, whereas, treatment with CCHE alone restored these parameters towards normal in a

dose dependent manner. Thus, it can be concluded that *Cuminum cyminum* is a promising candidate as an immunomodulatory agent against cyclophosphamide induced immunosuppression which acts by stimulating both humoral and cellular immune system.

F-EP-P7

EFFECT OF *MADHUCA LONGIFOLIA* SEED OIL TO MANAGE CANINE MANGE.

Ravindra Pratap Singh[1], Rachna Verma[2], Rishi Kant [3]

[1]MVSc Scholar,Department of veterinary Pharmacology & Toxicology, College of Veterinary Science & Animal Husbandry, N.D.U.A.T., Kumarganj Ayodhya 224229(UP), [2]Professor Department of veterinary Pharmacology & Toxicology, College of Veterinary Science & Animal Husbandry SVBPUAT Meerut(UP),[3]Assistant Professor Department of veterinary Pharmacology & Toxicology, College of Veterinary Science & Animal Husbandry, N.D.U.A.T. Kumarganj Ayodhya 224229(UP)

The study was designed to observe the effect of *Madhuca longifolia* oil in mange infested dogs. The oil was extracted from seeds of *Madhuca longifolia*, and yield of extract was 33% . Thirty animals (dogs) were divided into five groups. First group was treated with standard drug i.e. Benzyl benzoate (25%), in II group 100% oil was used, in group third 80% oil with 20% DMSO, in group IV 50% oil with 50% DMSO and in group V 20% oil with 80% DMSO was used topically. Various clinical findings, hematological and biochemical parameters were recorded after of 0, 14, 28 and 42 days of treatment.On zero day in total score of clinical signs and lesions in mange infested dogs varied from (20.44 ± 0.12 to 22.36 ± 0.15). After treatment with various therapeutic preparations a gradual recovery was seen in all the groups but most significant results were appeared in group III followed by group I. Significant changes were found in Hb, PCV, TEC, TLC and DLC. Highest increase in Hb and PCV was observed in group III followed by group I then group II, IV and V at 14, 28 and 42 days post treatment. A significant increase in TEC was observed in group I followed by group II and group III after 14, 28 and 42 days of treatment with various therapeutic preparations. Though TLC value lies in the normal range before and after treatment but group III showed maximum reduction in TLC in comparison to all other treated groups. Lymphocyte count was below normal in all the mange infested dogs. After treatment with various therapeutic preparations the lymphocyte count was increased significantly in all the groups, but the maximum response was recorded in group I followed by group III, II, IV and group V at day 14. Similar results were found on day 28 and 42 but the increase in lymphocyte count was of greater extent as compare to day 14. Percentage of monocyte count was towards lower range but after treatment monocyte level was increased in all the groups, though the monocyte count was in normal range before and after treatment with various therapeutic preparations. Neutrophil values were

also increased slightly which became normal after treatment with various concentrations of oil. There was no significant difference in levels of eosinophils and basophils. Biochemical profile was also recorded. At zero days SGOT level was higher than normal level in all groups. At 14 days the SGOT level was start reducing. Maximum reduction in SGOT level was found in standard drug group i.e. group I, followed by group III and group II where as in group IV and group V little increase in SGOT level. At 28 days SGOT level was continue to decrease and highest reduction was found in group III followed by group I, II and group IV. In group V SGOT level was continue to rise. After 14 days of treatment SGPT level was reduced in all the groups. Highest reduction was observed in group I where standard drug was used followed by group III, group II and group IV where as in group V slight increase in SGPT level was observed. On 28 days maximum response was observed in group I followed by group III, group II, group IV and group V. On 42 days SGPT level was continued to decrease in the same pattern as it was on 28 days i.e. group I followed by group III, II, IV and group V. Other biochemical parameters ALP, LDH, serum protein, albumin and creatinine were not changed significantly.Present study suggests that *Madhuca longifolia* seed oil has moderate protective action on haematological and biochemical parameters altered by mange infestation in dogs.

F-EP-P8

EVALUATION OF ANTIMICROBIAL ACTIVITY OF HERBAL EXTRACTS AGAINST CLINICAL ISOLATES OF *STAPHYLOCOCCUS AUREUS*

Devi, S.S[1]. Surya S[3], Linju L[3] and Sujith, S[2].

Department of Veterinary Pathology[1]Department of Veterinary Pharmacology and Toxicology[2], Department of Veterinary Microbiology[3], College of Veterinary and Animal Sciences, Mannuthy

Evolution of new pathogens and re-emergence of eradicated pathogens along with the development of resistance to pharmaceuticals necessitate the development of newer drug moieties with different mechanisms of action. The use of drugs and dietary supplements derived from plants has accelerated recently and a wide variety of secondary metabolites in plants like tannins, terpenoids, alkaloids and flavonoids have been found to have many antimicrobial properties. In this study, extracts of three commonly available plants namely Tulsi (*Ocimum sanctum*), Neem (*Azadirachta indica)* and Red Kamla (*Mallotus philippensis*) were tested *in vitro* for their anti-bacterial activity against *Staphylococcus aureus* isolated from bovine mastitis cases in and around Thrissur district. The plant leaves were dried, pulverized and extracted using methanol in a soxhlet extraction apparatus. The extracts were dried using a rotary vacuum evaporator and qualitatively analysed for various phytochemical constituents as per standard procedure. The extracts were diluted using

10% DMSO/ tween 80 solutions to get serial dilutions of 500, 250, 100, 50, 25 mg/ml of the solution. Antimicrobial assay was done in Muller- Hinton agar plates. Microbial cultures were prepared as suspensions in nutrient broth. Inhibition zones were measured and the mean diameter (mm) of complete growth inhibition was recorded. The tests were done in triplicates. The inhibition zone diameters were compared with that produced by standard antibiotics. Considerable antibacterial activity was noticed for all the three plants at higher concentrations and lack of activity in lower concentrations may be due to the increased resistance of field pathogens as against laboratory strains. Detailed studies on phytochemical constituents, and further testing are required to identify the potent antimicrobial molecules present in the extract. The study recommends the use of methanolic extracts of above herbs against Staphylococcal infections.

F-EP-P9

PHYTOCHEMICAL CHARECTERISATION OF *Ficus hispida* L.f.

Sumena K.B., Sujith, S. and Usha P.T.A.

College of Veterinary and Animal Sciences, Mannuthy, KVASU, Pookode

sumena@kvasu.ac.in

In the present study, the plant was collected from the agricultural land of Thottipal, Thrissur and prepared a herbarium. This was authenticated as *Ficus hispida* L.f. in the Department of Botany, St. Thomas College, Thrissur. Collected leaves of *Ficus hispida* L.f. were dried in shade and pulverized. This was used to prepare the extract using 95% ethanol and concentrated in a Rotary vacuum evaporator (Equitron, Germany) under reduced pressure and temperature (55^{o}C) and kept under refrigeration for further use. The ethanolic extract was used for phytochemical analysis. Leaves were plucked, cut into small pieces; shade dried and using an electrical pulveriser. In present study, the phytochemical screening of ethanolic extract of dried leaves of *Ficus hispida* L.f. revealed the presence of steroids, alkaloids, glycosides and flavonoids. The presence of the glycosides, saponins, ditripenes, triterpenoids and flavonoids in the leaves of *Ficus hispida* L.f. attributed the hepatoprotective property of *Ficus hispida* L.f.. In TLC, Hexane: Ethylene acetate (8.5:1.5) solution is the best one among the solvent system used in this study. Good separations of the different compounds were obtained with a total of five clear bands were obtained including one band in UV range. When Hexane: Acetic acid (9:1) was used as solvent a fair separation with five bands in visible light was observed. But only two bands were obtained in Hexane: Ethyl acetate (7:3) was the solvent for TLC.No separation was noticed for Benzene: Ethanol (9:1); Dichloromethane: Benzene: Ethyl acetate (8:1:1); Benzene: Ethanol (1:1); Chloroform: Ethanol (96:4) solvent systems. Thus, it could be concluded that the phytochemical screening of ethanolic extract of dried leaves of *Ficus hispida* L.f. revealed the presence of

steroids, alkaloids, glycosides, saponins, tannins, phenolic compounds, triterpenoids, diterpenoids and flavonoids.

F-EP-P10

AMELIORATING EFFECT OF QUERCETIN AND CURCUMIN AGAINST CADMIUM INDUCED TESTICULAR DAMAGE

Makwana C. N., Patel U. D., Shreesha S Rao, Patel H. B., Modi C.M.

Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and AH, Junagadh Agricultural University, Junagadh-362001, Gujarat, India. Email: urvesh1981@yahoo.com

The present experiment was carried out to evaluate the ameliorating effect of quercetin and curcumin against cadmium induced testicular damage. The study was conducted on 36 male rats which were randomly divided into six groups. Rats of group C1 were kept as normal control. Rats of toxic control group (C2), vehicle group (C3), quercetin treatment group (T1), curcumin treatment group (T2) and, quercetin and curcumin in combination treatment group were administered with cadmium in drinking water (100 ppm) for 28 days. Rats of vehicle group (C3) were administered with corn oil (vehicle). Rats of group T1, T2 and T3 were orally administered with quercetin (50 mg/kg, P.O.), curcumin (100 mg/kg, P.O.) and both quercetin and curcumin in combination, respectively for 28 days. In testes, cadmium exposure to animals caused slight increase in SOD activity, unaltered catalase activity and level of GSH as compared to normal control animals. However, the lipid peroxidation in testes was higher. Quercetin treatment was able to increase CAT activity which resulted in low level of MDA. Curcumin treatment did not improve oxidative stress parameters. However, combined treatment of quercetin and curcumin resulted in nearly normal activity of SOD, higher activity of CAT with lowest level of MDA amongst all groups. Mean values of total epididymal sperm count, epididymal sperm motility and total epididymal live sperm count were significantly altered in cadmium-exposed rats which were not significantly lower in rats which received the treatment of quercetin, curcumin alone as well as in combination. As compare to individual treatment of quercetin and curcumin alone, the combination of both agents produced more ameliorating effect against cadmium-induced histopathological changes.

F-EP-P11

EVALUATION OF *IN-VITRO* ANTI-INFLAMMATORY, ANTIOXIDANT AND ANTI-DIABETIC ACTIVITIES OF SELECTED MEDICINAL PLANTS OF SAURASHTRA REGION (GUJARAT)

Bhatt P. R.; Patel U. D.; Patel H. B.; Modi C.M. and Pandya K.B.

Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and AH, Junagadh Agricultural University, Junagadh-362001, Gujarat, India. Email: harshadvet@gmail.com

The present study was planned to evaluate phytochemical constituents and *in-vitro* anti-inflammatory, antioxidant and anti-diabetic activities of aqueous, methanolic and hydroalcoholic extracts of different medicinal plants viz. *Luffa echinata* (fruit), *Operculina turpethum* (leaf), *Sphaeranthus indicus* (fruit), *Cressa cretica* (leaf), *Corchorus depressus* (root), *Cassia absus* (seed)**.** An *in-vitro* anti-inflammatory activity was evaluated by albumin denaturation and protease inhibition assays, antioxidant by DPPH (2, 2-diphenyl-1- pricrylhydrazyl) and nitric oxide free radical scavenging activity and anti-diabetic by α-amylase and α-glucosidase inhibition method. Aqueous extract of *Operculina turpethum* (500 µg/mL) showed significant ($p < 0.05$) albumin denaturation inhibitory activity of 84.10 ± 0.34 %, and hydro alcoholic extract of *Sphaeranthus indicus* has shown significant ($p < 0.05$) protease inhibitory activity of 80.97 ± 0.05 % which were comparable to the standard drug aspirin (84.79 ± 0.44 %). Aqueous, methanolic and hydro alcoholic extracts of *Cressa cretica* (200 µg/mL) have shown significant ($p < 0.05$) nitric oxide free radical scavenging activities of 81.45 ± 0.30, 80.79 ± 0.34 and 74.80 ± 0.23 %, respectively which were comparable to standard drug ascorbic acid (80.14 ± 0.31 %). Aqueous extract *Cressa cretica* and hydro alcoholic extract of *Luffa echinata* (1000 µg/mL) have shown significantly higher ($p < 0.05$) α- glucosidase inhibitory activities of 80.60 ± 0.35 and 78.70 ± 0.25 %, respectively as compared to the standard drug acarbose (76.91 ± 0.47 %). Phytochemical analysis revealed the presence of glycosides, flavonoids, alkaloids, tannins and steroids in different extracts of medicinal plants which might have responsible for significant *in-vitro* anti-inflammatory, antioxidant and anti-diabetic activities. Identification and isolation of active principles and their *in-vivo* evaluation will be helpful for further validation of pharmacological activities of these medicinal plants.

S-EP-P1

EVALUATION OF PHYTOCHEMICAL, ANTIOXIDANT AND FIBRINOLYTIC ACTIVITY OF *BOSWELLIA OVALIFOLIATA, ANACARDIUM OCCIDENTALE* AND MARINE ALGAE *GRACILARIA TENUISTIPITATA*

Y. Kavya, T. Divyasri, Afroz Jahan, K. Bharavi, P. Anand Kumar[1], J. Shyma Dayal[2], G.S. Rao

Department of Veterinary Pharmacology and Toxicology

[1]Department of Veterinary Microbiology, NTR College of Veterinary Science, Gannavaram

[2]Principle Scientist, ICAR, CIBA, Chennai

E-mail: kavyayerrapureddy@gmail.com

Cardiovascular diseases (CVS) involving deep vein thrombosis, stroke, heart attack and hypertension are the main causes of mortality throughout the World. Worldwide number of patients suffering from CVS is ever increasing yearly at an alarming rate. Thrombolytic agents such as tissue plasminogen activator,

urokinase, streptokinase etc., are used for the treatment but their use is associated with risk of haemorrhage, anaphylactic reaction and lacks specificity. In the recent past, many phyto-phenolics have been reported with antiplatelet, anticoagulant, antithrombotic and thrombolytic activity. Hence, the present study was carried out to evaluate preliminary phytochemical analysis, antioxidant and fibrinolytic activity of acetone extract of *Boswelia ovalifoliata* resin (Indian frankincense), nut peel of *Anacardium occidentale* (cashew) and marine algae *Gracilaria tenuistipitata.* The phytochemical analysis of *B. ovalifoliata* revealed mainly the presence of Carbohydrates, saponins, triterpenes, phenols, proteins, glycosides whereas *A. occidentale* revealed presence of reducing sugars, carbohydrates, saponins, triterpenes, phenols whereas marine algae showed presence of carbohydrates and flavonoids. *B. ovalifoliata*, *A. occidenetale* and marine algae showed antioxidant activity equivalent to 170µg/ml, 237µg/ml and 24.8µg/ml of Ascorbic acid respectively. Fibrinolytic experiment was carried out in Sheep blood collected in 3.8% sodium citrate solution, the difference in weight before and after clot lysis was expressed as per cent clot lysis. The per cent clot lysis observed with urokinase, *B. ovalifoliata* resin, nut peel of *A. occidentale* (cashew) and marine algae *Gracilaria tenuistipitata* were 75.91, 22.29, 11.65 and 3.72%, respectively. From the results, it was evident that *B. ovalifoliata* resin, marine algae *Gracilaria tenuistipitata and A. occidentale* extract showed clot lysis equivalent to 29.3, 15.3 and 4.9% urokinase respectively.

S-EP-P2

AMELIORATIVE POTENTIAL OF *TRIANTHEMA PORTULACASTRUM* L. IN CYCLOPHOSPHAMIDE INDUCED HEPATOTOXICITY AND NEPHROTOXICITY IN RATS

Aswathy P.U., Ahmad A.H., Pant D., Patwal P.C., Saklani. S., Dhayananth B. and Narayan S. R.

Department of Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Pantnagar- 263145

asharies999gmail.com

Trianthema portulacastrum L. (family: Aizoaceae) commonly known as Horse purslane or Biskhapra is a weed distributed throughout India. It has been used in traditional medicine used for the treatment of ascites, inflammation and rheumatism. This study was undertaken to explore the hepatoprotective and nephroprotective efficacy of hydroethanolic extract of *Trianthema portulacastrum* (TPHE) in rats administered with cyclophosphamide. It was assessed by estimation of the various enzymes involved in the normal functioning of these organs using kits. In this study adult Albino Sprague Dawley rats were randomly divided into six groups. Group I served as control, group II rats were administered with cyclophosphamide at dose rate 100 mg/kg bwt (body weight) p. o on 9^{th} and 16^{th} day of study; groups III and V were administered with hydroethanolic extract of *Trianthema portulacastrum* (TPHE) at dose rates 200

mg/kg and 400 mg/kg p. o respectively, daily and groups IV and VI were given cyclophosphamide at dose rate 100 mg/kg p. o (on 9th and 16th day) along with hydroethanolic extract of *Trianthema portulacastrum* TPHE at 200 mg/ kg and 400 mg/ kg respectively, for 28 days. A significant ($p<0.05$) increase in important hepatoprotective and nephroprotective enzymes concentration was observed in group II as compared to that of control, in contrast, treatment with TPHE restored these parameters towards normal in the extract groups in a dose dependent manner. From the results, it was showed that the hydroethanolic extract of *Trianthema portulacastrum* has the potential to ameliorate hepatotoxicity and nephrotoxicity induced by cyclophosphamide in rats.

S-EP-P3

EVALUATION OF ANTI-INFLAMMATORY POTENTIAL OF ASHWAGANDHA ESSENTIAL OIL, EMU OIL AND OIL OF WINTERGREEN ON CARRAGEENAN-INDUCED PAW EDEMA IN RATS

Dumka V.K.,Vemu Bhaskar , Dattaray Debolina , Sagar Rashmi, Sharma S.K. , Lonare M. K. Lonare

Department of Veterinary Pharmacology and Toxicology

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana

From time immemorial, plants have been widely used as medicinal agents for curing a variety of ailments. Emu oil has been used to relieve minor aches, faster wound healing and protecting the skin from UV rays. Ashwagandha oil provides excellent nourishment to bones and muscles and Oil of wintergreen has been used as a folk remedy for joint problems, heart diseases and rheumatism. Keeping this viewpoint,the present study was undertaken to evaluate *in-vivo* anti-inflammatory activities of Ashwagandha oil, Emu oil and oil of wintergreen individually and in combinations. For the study, Wistar rats(180-200 gm weight) were divided into 8 groups containing 3 animals each and maintained under ideal laboratory conditions. Group I served as control; Group II, III,IV were given Emu oil at 20%, 50% and 100% concentrations respectively. Group V,VI,VII and VIII were administered the combinations of Emu oil (50%)+Ashwagandha oil(10%), Emu oil (20%)+Ashwagandha oil(10%), Emu oil (50%)+Oil of Wintergreen(10%), Emu oil (20%)+Oil of Wintergreen(10%) respectively.The anti-inflammatory potential was assessed by carrageenan-induced paw oedema method in which percent change in paw volume was noted at time intervals of 1h, 2h, 3h, 4h, 6h, 8h, 10h, 12h and 24h. It was observed that all the groups II-VIII showed anti-inflammatory activity with maximum activity in group VII [Emu oil (50%)+Oil of Wintergreen(10%)] in which inflammation reached its peak at 1h and started to subside afterwards reaching the normal around 12 hrs. Also in group VIII [Emu oil (20%)+Oil of Wintergreen(10%)], maximum inflammation was recorded at 2h and then started to decrease beyond 2h thereby reaching the normal values around 10h. So it can be concluded from the present

investigation that at the above mentioned concentrations; Emu oil, Ashwagandha oil and oil of wintergreen possess good anti-inflammatory potential.

S-EP-P4

ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC LEAF EXTRACTS OF *VITEX NIGUNDU, MURRAYA KOENIGII* AND *CYMBOPOGON CITRATUS* AND EMU OIL ON CARRAGEENAN-INDUCED INFLAMMATION IN RATS

Dhumka V. K, Vemu Bhaskar, Kumawat Sunita, Sagar Rashmi, Saini S. P. S. , Singla Saloni

Department of Veterinary Pharmacology and Toxicology

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana

Email.id- sunitakumawat60970@gmail.com

With the growing menace of adverse effects of synthetic non-steroidal anti-inflammatory drugs, the focus is shifting towards the development of drugs from plant sources. In folklore medicine, *Vitex nigundu*, commonly known as Chinese chastetree,has been used as antiseptic, astringent and antipyretic agent whereas Emu oil is very effective in relieving joint pains, faster wound healing and protecting the skin from UV rays.*Murraya koenigii*, also called as Curry tree is very effective in curing hepatic dysfunction, dysentery and for management of diabetes.*Cymbopogon citratus*, commonly known as lemon grasshas been used traditionally as anxiolytic, antioxidant, analgesic and antibacterial agent. Keeping this viewpoint,the present study was undertaken to evaluate *in-vivo* anti-inflammatory activities of Emu oil and ethanolic leaf extracts of *Vitex nigundu*, *Murraya koenigii* and *Cymbopogon citratus* individually and in combinations. For the study, Wistar rats(180-200 gmweight) were divided into 9groups containing 3 animals eachand maintained under ideal laboratory conditions. Group I served as control; Group II, III, IV were given Emu oil at 10%, 20% and 50% concentrations respectively. Group V,VI,VII,VIII and IX were administered the combinations of Emu oil (50%)+ethanolic extracts of*Vitex nigundu*(10%), Emu oil (20%)+ ethanolic extracts of*Vitex nigundu*(10%), Emu oil (50%)+ ethanolic extracts of *Murraya koenigii* (10%), Emu oil (20%)+ ethanolic extracts of *Murraya koenigii*(10%) and Emu oil (50%)+ ethanolic extracts of *Cymbopogon citratus*(10%) respectively. The anti-inflammatory potential was assessed by carrageenan-induced paw oedema method in which percent change in paw volume was noted at time intervals of 0h, 1h, 2h, 3h, 4h, 6h, 8h, 10h, 12h and 24h. It was observed that except for group I (control group) and group II (Emu oil 10%), all the groups III-IX showed anti-inflammatory activity with maximum activity in group VI [Emu oil (50%)+ ethanolic extracts of*Murraya koenigii*(10%)] in which inflammation was maximum at 2h and started to subside afterwards

reaching the normal around 12 hrs.However in group II (Emu oil 10%), there was constant increase in paw volume till 6 h thereby revealing least anti-inflammatory potential at this concentration. So it can be concluded from the present investigation thatEmu oil (20%,50%) and ethanolic leaf extracts of *Vitex nigundu*, *Murrayakoenigii* and *Cymbopogon citratus*, alone and in combinations, have good anti-inflammatory potential.

S-EP-P5

ANTIINFLAMMATORY POTENTIAL OF ESSENTIAL OILS AND ETHANOLIC EXTRACTS OF SOME MEDICINAL PLANTS ON CARRAGEENAN-INDUCED PAW EDEMA IN RATS

Dumka V. K., Vemu Bhaskar , Sagar Rashmi, Kumar Sumeet, Kaur Rajdeep, Lonare Snehal

Department of Veterinary Pharmacology and Toxicology

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana

Email.id - dr.sumeet50@gmail.com

Since foregone times, many diseases have been cured by administration of plant extracts. Interest in ethnopharmacology as a source of bioactive compounds has increased worldwide, particularly in the search for anti-inflammatory drugs. Essential oils like cinnamon oil, palmrose oil and citronella oil are known to have many health benefits. Cinnamon oil has been used in relieving aches, joint stiffness and also in treating digestive disorders. Palmrose oil has got hydrating properties and Citronella oil, obtained from leaves and stems of *Cymbopogon citratus* (lemongrass) has excellent analgesic and antifungal properties.Taking this background, our present investigation was planned to demonstrate the effect of these essential oils and the ethanolic extracts of *Dalbergia sisso*, Ashoka, *Cymbopogon citratus* on carrageenan-induced inflammation in rats. For the study, Wistar rats (180-200 gm weight) were divided into 7 groups containing 3 animals each and maintained under ideal laboratory conditions.Group I served as control; Group II was given Cinnamon oil, Group III Palmose oil, Group IV Citronella oil, Group V ethanolic extracts of *Dalbergia sisso*, Group VI ethanolic extracts of Ashoka, and Group VII was given ethanolic extracts of *Cymbopogon citratus*.The anti-inflammatory potential was assessed by carrageenan-induced paw oedema method in which percent change in paw volume was noted at time intervals of 0h, 1h, 2h, 3h, 4h, 6h, 8h, 10h, 12h and 24h. It was observed that all the essential oils viz. cinnamon oil, palmrose oil and citronella oil showed anti-inflammatory activity with maximum activity in Citronella oil in which peak inflammation started to reduce after 3h. In the groups treated with ethanolic extracts of different plants, maximum activity was demonstrated by Group VI in which inflammation reached its peak at 2h and started to subside afterwards. From the present study it can be

concluded that all the essential oils; cinnamon oil, palmrose oil, citronella oil and ethanolic extracts of Ashoka possessed good anti-inflammatory potential.

S-EP-P6

TREATMENT OF CONTAGIOUS ECTHYMA (ORF) LESIONS IN GOATS USING ETHNOMEDICINE

Sunitha Thomas, Roshin M. Reji and R.Thirupathy Venkatachalapathy

College of Veterinary and Animal Sciences

Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, India

Email ID: sunithathottan@gmail.com

Contagious ecthyma (ORF) was observed in 15 Malabari goats at University Goat and Sheep Farm, Mannuthy functioning under Kerala Veterinary and Animal Sciences University. The animals developed pyrexia with restricted milk and kid starter intake, appeared dull, depressed with lesions on nostrils, lips and inside the mouth. Later, similar lesions developed on the entire ear of the affected kids. In the beginning, viscous exudates oozed out which eventually hardened into thick brown scabby lesions. The morbidity was 100 percentage, but there was no mortality. The animals were isolated and lesions were wiped with cotton dipped in potassium permanganate solution. An ethno veterinary medicine comprising of equal amount of crushed neem leaves (*Azadirachta Indica*) turmeric (*Curcuma longa*) a few drops of lemon juice (*Citrus limon*) and a pinch of salt was applied over the lesions twice daily. There was complete recovery within two weeks.

S-EP-P7

IN VITRO ANTI-DERMATOPHYTIC ACTIVITY OF ESSENTIAL OIL OF *ARTEMISIA JAPONICA THUNB* LEAVES

Akhil G C, Usha N Pillai and N Madhavan Unny

[1]Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of Veterinary and Animal Sciences, Mannuthy, Thrissur. 680651

Corresponding author: ushanp@kvasu.ac.in

Dermatophytes are the group of filamentous fungi which are known to cause superficial fungal infections in humans as well as in animals. The common dermatophytes included *Trichophyton*, *Microsporum* and *Epidermophyton*. The pathogenecity of dermatophytes and their mechanistic basis of action are mainly involved through infecting the keratinous tissues of the host by invading through the skin, hair and nails. In order to manage the same, various topical and systemic drugs such as Griseofulvin (Grifulvin V, Gris-PEG), Terbinafine, Itraconazole (Onmel, Sporanox) and Fluconazole (Diflucan) have

been routinely used. Continuous use of antifungal drugs can lead to the emergence of resistance and environmental toxicity, hence the eco- friendly and cost effective drugs are highly demanding. Here comes the importance of natural compounds with the potential for anti-dermatophytic activity. *Artemisia japonica* Thunb of *Asteraceae* family is widely distributed in India and used as a traditional medicine to treat various diseases. However, the leaves of medicinal plants contain bioactive metabolites and these metabolites are highly rich in essential oils of leaves. Hence the present study aims to evaluate the *in vitro* anti-dermatophytic activity of essential oil (EO) extracted from *Artemisia japonica* leaves on *Trichophyton mentagrophytes* and *Microsporum canis*. The results revealed that the essential oil has remarkable anti-dermatophytic activity against *Trichophyton mentagrophytes* and *Microsporum canis* with the MIC values of 1.534±0.201 mg/mL and 0.578±0.0311 mg/mL respectively. This indicated the ability of the EO to cure dermatophytic infections. The details will be discussed.

F-AMR-P1

MULTIDRUG RESISTANCE (MDR) IN COAGULASE NEGATIVE STAPHYLOCOCCI (CONS) ISOLATED FROM BOVINE MASTITIS

Solanki K. M.,Singh R.D.*, Prajapati B.I. and Patel A. C.

* Department of Pharmacology and Toxicology, Department of Veterinary Public Health, College of Veterinary Science and Animal Husbandry, SardarkrushinagarDantiwada Agricultural University, Sardarkrushinagar, Gujarat- 385506, ratn1709@yahoo.com

Bovine mastitis is a devastating economic menace to dairy industry affecting livestock farmers across the world. Appraising the emerging multidrug resistant strains of pathogens is one of the most serious causes forfailure of antimicrobial therapy of mastitis.Generally, when a bacterium is resistant to three or more antibiotics of different classes, it is regarded to be multidrug-resistant, andphenomenon mainly occursdue to presence of different resistance genes in single bacterium.Coagulase negative *Staphylococcus aureus*(CoNS) is the emerging bacterial pathogen responsible for the bovine mastitis. The present investigation was carried out to know extent of multidrug resistant CoNS in the North Gujarat region. Antimicrobial sensitivity test against fifteen antimicrobial drugs were performed after molecular confirmation of 33 CoNS isolated from 185 clinical bovine mastitis cases. Multidrug resistance was reported in 84.85 % (28 out of 33) of the CoNS isolates showing resistance to at least three antibacterials, representing three different classes, out of fifteen used in the present study.

F-AMR-P2

ANTIBACTERIAL ACTIVITYOF LEAVES EXTRACT OF *EUCALYPTUS GLOBULUS* AND *TAMARINDUS INDICA* AGAINST *R. EQUI*

Kumar, L.; Sankhala, L.N.; Dedar, R.K.; Kant, L.; Badsiwal, D.K.; Mehta, S.C. andTripathi, B.N.

College of Veterinary and Animal Science, Bikaner, Rajasthan-334001, lalitcvas11@gmail.com

The present study was carried out toevaluate the in vitro antibacterial activity of extracts of leaves of *Eucalyptus globulus* and *Tamarindus indica* against Vap A and Vap C positive *Rhodococcus equi*. Ethanolic extracts of these plants leaves showed good in vitro antibacterial activity against *R. equi* using disc diffusion method. These plants leaves were further fractionated using chloroform and water sequential extraction to separate non-polar and polar compounds. Non-polar fraction of *E. globulus* and *T. indica* leaves did not show antibacterial activity against *R. equi* using disc diffusion method whereas polar fraction showed good antibacterial activity against *R. equi* using disc diffusion method and agar well diffusion method. Further, solvent based fractionation of sequentially extracted aqueous (SEWE) fraction of *E. globulus* and *T. indica* L. leaves in ethanol, methanol and water showed good antibacterial activity against *R. equi*. On phytochemical analysis, most active WSF fraction of SEWE of *E. globulus* leaves was found positive for catechol tannins, phenolic compounds, saponins and carbohydrates whereas most active ESF fraction of SEWE of *T. indica* L. leaves was found positive for catechol tannins, phenolic compounds, flavones and carbohyrates. On comparison with currently used antibioitcs (azithromycin and rifampicin), required concentration of the leaves extract of these plants was too high for their possibilities of in vivo use. However abundant availability of *T. indica* and *E. globulus* and their activity against *R. equi* suggests their potential for use as disinfectant against *R. equi*.

F-AMR-P3

CATHEPSIN D DEGRADABLE DENDRIMER-MPEG-HISTONE 3-ENROFLOXACIN CONJUGATE NANOVEHICLE FOR TARGET SPECIFIC BOVINE MASTITIS THERAPY

Senthil Kumar,P.[a], Sambandam Anandan[b] and Subramanian,N[c]

Veterinary College and Research Institute, Orathanadu-614625, Tamil Nadu, India, [a]Veterinary College and Research Institute, Orathanadu-614625, Tamil Nadu, India, [b]National Institute of Technology, Tamil Nadu [c]Bharathidasan Institute of Technology, Anna University,Tamil Nadu

p.senthilvet@gmail.com

Mammary gland overexpresses cathepsin D aspartic enzymes during mastitis. Dendrimer (dend)-methoxy poly(ethylene glycol) (MPEG)-enrofloxacin (enro) conjugate nanoparticles were formulated for targeting mammary gland using cathepsin D and cathepsin D cleavable histone 3 peptide. Histone 3 peptide was conjugated with the carboxylic acid end groups of a dendrimer, which was then conjugated with MPEG amine. The antibacterial agent, enrofloxacin was conjugated with dendh3-MPEG conjugates. Dend-MPEG-enro conjugates without histone 3 peptide linkage was also synthesized for comparison. These conjugates were converted into nanoparticles using a dialysis procedure. Particle size and surface morphology of the developed nanoparticles were measured using photon correlation spectroscopy and transmission electron microscope. In vitro drug release study of dend-h3-MPEG-enro conjugate nanoparticles and dend-MPEG-enro conjugate nanoparticles was performed by dialysis bag diffusion technique over a period of 48 h. Conjugation of enrofloxacin within dend-h3-MPEG-enro conjugates nanoparticles were assessed using UV–Vis spectrophotometer. The mean (± SD) particle size of the dend-h3- MPEG-enro conjugate nanoparticles were 69.4 ± 43 nm and were spherical and circular in shape. The dend-h3-MPEG-enro conjugates had an absorption peak at 273.8 nm and it confirmed successful conjugation of enrofloxacin. Enrofloxacin was released from the dend-h3-MPEG-enro conjugate nanoparticles via biodegradation of the histone 3 peptide upon exposure to cathepsin D. From this study, it can be suggested that dend-h3-MPEG-enro conjugate nanoparticles could be used to deliver antibacterial drug selectively to the mammary gland by exploiting the over expression of cathepsin D during mastitis.

F-AMR-P4

EVALUATION OF EFFECT OF HALQUINOL ON INTESTINAL MOTILITY IN RATS

Pavithra B.H., Prakash Nadoor, Santhosh C .R*, Leena G., Rashmi R., Rakshith.K., Shivaprasad G.R., Nithyashree N and Rudresh Gowda B.

Department of Pharmacology & Toxicology, Veterinary College, Bengaluru

Halquinol, a quinoline derivative having antibacterial, antifungal and antiprotozoal activity was introduced across the world to overcome common challenges of modern poultry and swine farming, like pathogenic microbial infections and growth promotion aspects.In Asian countries including India, halquinol is being extensively used as growth promoter in poultry and to control intestinal infections.The present study was undertaken with an aim to determine the effect of halquinol on motility of intestine in Wistar rats as there is paucity of scientific data in this regard. Effect of halquinol on intestinal motility was determined by carrying out charcoal meal test. Experimental animals were divided into five groups; each group included

sixWistar rats. Group I served as control and received plain vehicle that is tragacanth suspension @ 1mL/100 g b.wt. P.O, group II served as standard and received loperamide @ 5 mg/kgP.O. and animals of groups III, IV and V, the treatment groupsreceivedhalquinol suspended in tragacanth @ 200, 400 and 1000 mg/kg b. wt., P.O. Animals of groups III, IV and V were pre-treated with halquinol suspended in tragacanth twice a day at 12 hrs interval for six days @ 200, 400 and 1000 mg/kg b. wt., P.O. On seventh day charcoal meal test was carried out. The distance of the small intestine travelled by charcoal meal in group V rats was44.5±8.22cm which is less compared to control group (61.33±6.39cm) which reflects decrease in intestinal motility and Peristalsis index of rats in group V is 0.50±0.09 that iscomparable to that of the standard whose peristalsis index is 0.37±0.03.Charcoal meal test in experimental Wistar rats indicated that halquinol possess antimotility effect upon its pre-treatment with halquinol @ 1g/kg b.wt.

F-AMR-P5

ANTIBIOGRAM OF BACTERIAL ISOLATES FROM BOVINE DERMATITIS

Tresamol P.V and Saseendranath M.R

Department of Veterinary Epidemiology and Preventive Medicine

College of Veterinary and Animal Sciences, Mannuthy, tresamol@kvasu.ac.in

Bovine dermatitis is caused by multiple etiological factors and bacterial pathogens forms one of the most important cause for the condition. Identification of bacterial agents and their antibiogram is very important for the prompt and effective treatment and control of the problem. The present study was planned for identification of the bacterial agents associated with dermatitis in cattle and to study the antimicrobial susceptibility of the isolates. Skin swabs and skin scrapings were collected from 100 cattle with dermatitis and the samples were subjected to cultural isolation. The isolates obtained were identified based on colony morphology, Gram's staining and biochemical tests. A total of 104 isolates were obtained out of which 89 were Gram positive and 15 were Gram negative.The Gram positive isolates were identified as *Dermatophilus congolensis* (75), *Staphylococcus aureus* (5), *S.hyicus* (8) and *S.xylosus* (1). Gram negative isolates included *Bacillus* spp.(3),*Proteus* spp.(7) and *Klebsiella* spp.(5). The most sensitive antibiotics found were ciprofloxacin (99.04%) and enrofloxacin (99.04%) followed by gentamycin (98.07%). The sensitivity percentage of other antibiotics included tetracycline (83.65%), chloramphenicol (81.73%), ceftriaxone (78.85%), streptomycin (75%), amoxyclav (73.08%), ampicillin (72.12%) and amoxicillin (72.12%).Only 63 isolates (60.58%) were sensitive to Cotrimoxazole and 41 (39.42) were sensitive to Penicillin. The knowledge on the type of bacteria associated with dermatitis in cattle and sensitivity and resistance pattern of

the isolates will help in selecting the most suitable treatment protocol for the effective management of bovine dermatitis.

F-AMR-P6

BETA LACTAM ANTIBIOTICS RESIDUES IN RAW MILK FROM THRISSUR, KERALA

Binsy Mathew, Latha C., Sunil B.,SethulekshmiC., VrindaMenon, K. and Deepa Jolly

Assistant Professor, Department of VPH, CVAS, Mannuthy, binsymathew@kvasu.ac.in

Milk is one of the most consumed foods in the world. Antibacterial drug residues are often reported from milk of developing countries. The overuse of antimicrobials results in detectable traces of residues in milk.Numerous studies indicate that improper use ofdrugs in the control of mastitis is the major source of antimicrobialresidues in milk. Beta lactam antibiotics are widely used in therapy of cattle, particularly for the treatment of mastitis.The public health significance of beta lactam residues includes development of antimicrobial drug resistance and hypersensitivity reactions. The present study was envisaged to assess the presence of beta-lactam antibiotic residues in milk samples collected from milk cooperative societies and to compare the same between societies at rural and semi urban areas. Twenty milk samples each were collected from five different milk cooperative societies in and around Thrissur, Kerala which included two milk societies from semiurban and threesocieties from rural areas.The samples of raw milk were collected from the farmers as they brought it to the milk cooperative society and were analysed for the presence of beta lactam antibiotic residues above Maximum residue limit (MRL) level using Rapid One Step Charm Assay. Of the 100 samples tested,17 per cent of the samples were found to have beta lactam residues above MRL level. Out of the 40 samples collected from semi urban areas, 12.5 per cent was above MRL and out of the 60 samples collected from rural areas, 20 per cent samples were found to be above MRL limits. There was no significant difference ($p \leq 0.05$) in the presence of residues above MRL level between samples from rural and semi urban areas. Sincere efforts such as awareness, observance of the withdrawal period, effective surveillance and control on the use of veterinary drugs needs to be adopted to prevent this menace of drug residues in milk.

F-AMR-P7

ELUCIDATION OF DRUG RESISTANCE IN CANCER

Sajitha I. S[1]., T.R. Santhoshkumar[2] and M.Radhakrishna Pillai[3]

[1]Assistant Professor, Department of Veterinary Pathology, CVAS, Mannuthy, Scientist G, [3]Director, Rajiv Gandhi Centre for Biotechnology, Trivandrum

sajitha@kvasu.ac.in

Multidrug resistance in cancer implies the insensitivity of the cancer cells to the cytostatic/ cytotoxic actions of structurally and functionally unrelated chemotherapeutics. As per the cancer stem cell hypothesis, cancer stem cells or the tumour initiating cells are a sub population of cells which has the capacity to self-renew, are tumourigenic and have multilineage differentiation capacity. They are protected by specific resistance mechanisms, such as quiescence, self-renewal ability, multi drug resistance-pump activity, low level of reactive oxygen species (ROS) and evasion of apoptosis, so are not eliminated by conventional radio and chemotherapy, while the bulk of the cancer cells are killed. Such surviving cells will repopulate the original tumor causing recurrence and also lead to distant metastasis and these cells will be resistant to multiple drugs. The present study was formulated to develop drug resistant cells *in vitro* by subjecting different human cancer cell lines to various chemotherapeutic drugs and characterization of their multi drug resistant potential was done. The cells used were tagged with luciferase or td-tomato fluorescent probe to enable noninvasive bioluminescence/ fluorescence imaging. The parental and drug resistant cells so developed were injected into severe combined immune deficient (SCID) mice to study the difference in biology of the tumours induced. Bioimagingwas done at specific time periods to know the disease progression. Animals were sacrificed at the end of the experiment period and gross and histopathology of the primary tumour, affected lymph nodes and organs with or without metastasis was done which demonstrated that drug resistant cancer cells induced highly aggressive and metastatic tumours compared to the parental cells. Proteomics revealed that Unfolded Protein Response (UPR), an evolutionarily conserved protein quality control pathway, was mainly involved in the emergence of drug resistant cancer cells. Targeting UPR could be utilized to combat cancer drug resistance, recurrence and metastasis.

F-AMR-P8

DETERMINATION OF THE CURRENT MINIMUM INHIBITORY CONCENTRATION (MIC) OF HALQUINOL

Pavithra B.H., Prakash Nadoor, Santhosh C.R*., Leena G. ,Rashmi R., Shivaprasad G.R., Nithyashree N and Rudresh Gowda B.

Dept. of Pharmacology & Toxicology, Veterinary College, Bengaluru

Halquinol, a quinoline derivative having antibacterial, antifungal and antiprotozoal activity was introduced across the world to overcome common challenges of modern poultry and swine farming, like pathogenic microbial infections and growth promotion aspects. As MIC of microbial pathogens changes with place and time, the present study was conducted to determine the current MIC of halquinol for local

pathogenic bacterial isolates from poultry. In the present study two individual isolates from poultry *viz.,Salmonella gallinarum* and *Escherichia coli* were used as test bacteria. The minimum inhibitory concentration (MIC) of Halquinol was determined by employing broth dilution technique following standard protocol. The test was performed by preparing two-fold dilutions of halquinol in series of tubes containing nutrient broth. The macro-dilution method was followed using 5 mL tubes. This provided halquinol concentrations of 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.12, 0.06, 0.03 and 0.01µg/mL in the first to fifteenth tube respectively. Each tube was inoculated with actively growing pure culture suspension of the test bacterium that contained between 10^4 to10^5 bacteria/mL. The required concentration of bacteria was achieved by adjusting the bacterial cell suspension turbidity matching to 0.5 on McFarland scale. The inoculated tubes of broth were incubated at 35-37 º C for 24 hrs and then observed for visible growth in the form of turbidity in the tubes. The MIC was determined as the lowest concentration of halquinol that completely inhibited the growth of bacteria (showing no visible growth/no turbidity in the tube).The current minimum inhibitory concentration (MIC) of halquinol against two local pathogenic isolates from poultry *viz: Escherichia coli* and *Salmonella gallinarum* was 4 µg/mL, and the values were within the reported global MIC values of halquinol.

S-AMR-P1

EVALUATION OF PHYTOCHEMICAL, ANTIOXIDANT AND ANTIBACTERIAL SYNERGISM WITH ENROFLOXACIN OF *BOSWELLIA OVALIFOLIATA* AND *ANACARDIUM OCCIDENTALE* AND MARINE ALGAE *GRACILARIA TENUISTIPITATA*

T. Divyasri*, Y. Kavya, AfrozJahan, K. Bharavi, P. Anand Kumar[1],J. Shyma Dayal[2] , G. S. Rao

Department of Veterinary Pharmacology and Toxicology,[1]Department of Veterinary Microbiology

[2]Principle scientist, ICAR, CIBA Chennai, NTR College of Veterinary Science, Gannavarm

divyasritappitla@gmail.com

Antimicrobial drug resistance is currently one of the major worldwide health problem. Infections caused by Multi-Drug resistance (MDR) strains are hard to treat and often turn out to be fatal. It is reported that phyto-chemicals posses synergistic antibacterial action along along with antimicrobial agents against many bacterial pathogens. Hence the present study was carried out to evaluate the Acetone extracts of *Boswellia ovalifoliata* resin (Indian frankincense), nut peel of *Anacardium occidentale* (cashew) and marine algae *Gracilaria tenuistipitata* were subjected to preliminary phytochemical analysis, Antioxidant activity and antimicrobial property alone and in combination with enrofloxacin. The phytochemical analysis of

B.ovalifoliata revealed mainly the presence of carbohydrates, saponins, triterpenes, phenols, proteins, glycosides and *A.occidentale* revealed presence of reducing sugars, carbohydrates, saponins, triterpenes, phenols whereas marine algae showed the presence of carbohydrates and flavanoids. Acetone extracts of *B.ovalifoliata*, *A.occidenetale* and marine algae showed antioxidant activity equivalent to 170µg/ml, 237µg/ml and 24.8 µg/ml of Ascorbic acid respectively. MIC and Agar well diffusion method of antimicrobial assays revealed *B.ovalifoliata* and marine algae *Gracilaria tenuistipitata* has no antibacterial activity alone whereas *A. occidentale* showed inhibition of bacterial growth; however *B.ovalifoliata* and *A.occidenetale* showed synergistic activity along with Enrofloxacin against *E.coli* organism. In addition to antioxidant potential, a synergistic antibacterial activity was observed when Boswellia resin and Cashew nut peel were used in combination with enrofloxacin whereas marine algae does not have any antibacterial activity alone and no synergistic activity with enrofloxacin.

S-AMR-P2

ANTIBACTERIAL POTENTIAL OF GREEN SYNTHESISED SILVER NANOPARTICLES USING *Oxalis corniculata* LEAF EXTRACT

Sreya K. Radhakrishnan, Uma R., K. K. Jayavardhanan and Preethy John

Nanoparticles which have a wide range of applications can be synthesised using a variety of physical, chemical and biological methods.The present study focused onthe green synthesis of silver nanoparticles (AgNPs) using *Oxalis corniculata* leaf extract and evaluation of their activityagainst representative bacterial pathogens.The leaves of *O. corniculata*were collected manually, identified and authenticated. Aqueous extract of the leaves was prepared by decoction method and the phytochemicalscontained in it were analysed which revealed the presence of alkaloids, glycosides, flavonoids and diterpenes.Theextract was used for the synthesis of AgNPs using1mM, 3mM and 5mM silver nitrate ($AgNO_3$)solutions.The colour change of the solution from yellow to dark brown owing to the surface plasmon resonance (SPR) of AgNPsupon exposure to sunlight for 5-10 minutes and further incubation in darkness overnight confirmed the formation of AgNPs. The nanoparticles formed were characterised by UV-visible spectroscopy (UV-Vis), Fourier transform-infrared spectroscopy (FTIR), X-ray diffraction (XRD), andfield emission scanning electron microscopy (FESEM). The UV-VIS showed the SPR peaksfor the nanoparticlesat 438, 440 and 443nm. The FTIR analysis revealed the presence of different functional groups inthe biomolecules responsible for the reduction of AgNPs. The XRD pattern clearly indicated that the nanoparticles formed were of crystalline nature with Face Centered Cubic structure.The FESEM displayed the nanoparticles which were spherical in shape with the diameter between 23-45nm.The synthesised AgNPs were found to have excellent antibacterial activity

against Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli*. The nanoparticles formed of 1mM $AgNO_3$ were found to be more effective against Gram negative bacteria while those made of 3 mM $AgNO_3$ were more active against Gram positive bacteria.It could be observed that the minimum inhibitory concentration and minimum bactericidal concentration of the nanoparticles were 20µg/Ml against both *E. coli* and *S. aureus*.

F-CR-P1

LECTIN HISTOCHEMISTRY OF EPIDERMIS IN DEER, GOAT AND SHEEP

S. Maya, N. Ashok, K.M.Lucy, V.R. Indu, A.R. Sreeranjini, N.S. Sunilkumar and K.B. Sumena

Department of Veterinary Anatomy and Histology, College of Veterinary and Animal Sciences, Mannuthy.

Lectin histochemistry of epidermis in deer, goat and sheep was studied using skin samples collected from spotted deer brought for post mortem at College of Veterinary and Animal Sciences, from Thrissur zoo and forest department; and from goat and sheep freshly slaughtered in Meat Technology Unit, Mannuthy. Samples of $1cm^3$ were collected from 27 regions of skin, *viz.* muzzle, infraorbital, horn glands, dorsal face, lateral face, ventral face, pinna ear, dorsal neck, lateral neck, ventral neck, dorsal abdomen, lateral abdomen, ventral abdomen, dorsal forelimb, palmar, dorsal hindlimb, plantar, interdigital fore limb, interdigital hind limb, foot pad forelimb, foot pad hindlimb, inguinal, preputial (from male), scrotal (from male), dorsal thorax, perineum and dorsal nasal. Lectin histochemistry was done using Fluorescein iso-thiocyanate (FITC) conjugated lectin from *Ulex europaeus* (UEA1) and examined under Fluorescence (Leica DM 2000 LED) microscope with green filter. The lectin Concanavalin A (ConA) was also used for the study. Stratum granulosum of epidermis exhibited positive reaction as green fluorescence to FITC conjugated lectin from UEA1. Intensity of reaction was more in sheep among the three species studied. The pattern of lectin binding to routinely processed sections of normal skin is related to cellular maturation. All adult epidermal cells bind UEA1 to the upper layers. Epidermis exhibited positive response to the lectin ConA in stratum granulosum and diffused moderate reaction in its other layers.Mild to moderate response was seen in its cellular cytoplasm to ConA.The diagnostic use of lectin histochemistry is restricted to the identification of abnormal storage products in cells of normal and neoplastic endothelial cells and of fungi. In research, lectins are invaluable for the study of cell-surface interactions and may provide a more reproducible method for the grading of malignancy for many tumors than simple morphological examination. Nevertheless, it is essential that lectin-binding patterns are correlated with careful morphological evaluation of the cells to ensure that neoplastic rather than reactive cells are being studied.

F-CR-P2

ANTIBACTERIAL ENHANCING EFFECT OF METHANOLIC EXTRACT FROM THE LEAVES OF *OROXYLUM INDICUM* WITH ANTIBIOTICS IN BACTERIAL ISOLATES FROM BOVINE MASTITIS

Sreeranjini, A.R[1].,Nisha, A.R[2]., Arya Mohan[2], Keerthika[2], V., Preethy John[2] and Maya, S[1].

[1]Department of Veterinary Anatomyand Histology, [2] Department of Veterinary Pharmacology and Toxicology

College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala.

The present study was conducted to analyse the antibacterial properties of methanolic extract from the leaves of *Oroxylum indicum*against bacterial isolates from mastitis samples. Phytochemical analysis of the methanolic extract revealed the presence of alkaloids, flavonoids, tannins and saponins. For assessing the antibacterial activity, Kirby Bauer assay and minimum inhibitory concentration by microdilution were carried out in isolates from mastitis samples. Biofilm assay was performed by Congo red method. The bacterial isolates used were *Escherichia coli, Staphylococcus aureus and Klebsiella sp.* The whole leaf extract alone did not show any significant antibacterial activity, but when used in combination with various antibiotics, methanolic extract from the leaves of *Oroxylumindicum* showed potent antibacterial effect against the bacteria used.

F-CR-P3

EFFICACY OF ENROFLOXACIN THERAPY FOR ACUTE PROSTATITIS IN FIVE DOGS

Dr.Sindhu K. Rajan and Dr.Usha Narayana Pillai

Assistant Professor, Department of Veterinary Clinical Medicine, Ethics & Jurisprudence, CVAS, Mannuthy,

Professor and Head, Department of Veterinary Clinical Medicine, Ethics & Jurisprudence, CVAS, Mannuthy

sindhu.rajan@kvasu.ac.in

Prostatic diseases are one of the commonly encountering disease in old age dogs. The dogs presented to the teaching veterinary clinical complex, Mannuthy with the complaint of dyschezia and mild anorexia were screened for the presence of prostatitis. A total of five dogs affected with prostatitis were selected. The average age of dogs affected with prostatitis was 5.3 years. Anorexia, purulent urethral discharge and dyschezia were the most common presenting signs. Four dogs had vomiting. Ultrasonographic examination of all the dogs with prostatitis revealed diffused increase in echogenisty of prostatic parenchyma and multifocal hyperechoic areas in two dogs.Prostatic fluid cytology revealed large numbers of bacteria- laden

neutrophils. Cultural examination of prostatic fluid of five dogs revealed *E. coli* that was sensitive to enrofloxacin and tetracycline. The mean value of serum creatinine was 4.42 mg/dL. All the animals were treated with antibiotics enrofloxacin @ 10 mg/kg IV for one week and other symptomatic therapy as the condition warranted. Complete recovery could be observed by 14^{th} day of treatment in three cases. The other two cases did not respond to treatment. Further details will be presented.

F-TOX-P1

HEPATOPROTECTIVE POTENTIAL OF CYNARASCOLYMUS IN CISPLATIN INDUCED HEPATOTOXICITY IN RATS

Priyanka Sharma,Harpreet Kour, Pawan K. Verma, N K Pankaj, N. Nashiruddullah, R. Raina

Division of Veterinary Pharmacology and Toxicology,Faculty of Veterinary Science and Animal Husbandry,R S Pura

drpawankv@yahoo.co.in

The present investigation was aimed to determine the alterations in antioxidant, biochemical and histopathological parameters in cisplatin (cDDP) induced hepatotoxicity and its protection by treatment with hydro-alcoholic extract of *C. scolymus*. The experiment was conducted on seven groups of rats with six rats in each group. Normal untreated rats (Group I) served as normal control and received only distilled water. Group II received a single intra-peritoneal dose of cisplatin (12 mg/kg BW). Group III and IV received hydro-alcoholic extract at doses 150 and 300 mg/kg BW orally whereas group V and VI received plant extracts at the dose rate of 150 and 300 mg/kg BW, before 1h and after 24h and 48h of cDDP administration respectively. In Group VII, single intra-peritoneal dose of quercetin (50 mg/kg BW) was given at least 6h before cDDP administration.Daily oral administration of the extract for 3 days in rats significantly attenuated altered hepatic biomarkers (ALT, AST and ALP) and anti-oxidant biomarkers (TTH, MDA, CAT, SOD, GP_X, GST and GR). Pre and post treatment with plant extract at the rate of 150 and 300 mg/kg attenuated the altered levels of various enzymatic and oxidative parameters in blood and hepatic tissue but extract at higher dose levels (300 mg/kg) was more effective in restoring these parameters. These findings corroborated with reduced degenerative and necrotic changes of hepatic tissue as indicated in histopathological studies. Reduced hepatic, oxidative biomarkers and histopathological alterations indicate a good hepatoprotective potential of *C. scolymus*extract in cDDP induced hepatotoxicity in rats.

F-TOX-P2

DIFFERENT METHODS FOR ASSESSING DECELLULARISATION EFFICIENCY OF EXTRACELLULAR MATRIX-BASED BIOSCAFFOLDS FOR TRANSLATIONAL RESEARCH

Dhanush Krishna B, N. Divakaran Nair, Syam K.Venugopal, N. Vijayan, Sisilamma George, Mammen J Abraham and T.V Anilkumar

College of Veterinary and Animal Sciences, KVASU, Mannuthy, Kerala-680 651, Division of Experimental Pathology, Biomedical Technology Wing, SreeChitraTirunal Institute for Medical Sciences and Technology, Poojappura, Trivandrum 695012

Biomaterials have major applications in delivery of drugs, cancer therapy, developing artificial ligaments, tendons and bone plates, ophthalmic fields, wound healing and vaccine development. Despite promising *in vitro* results, most biomaterials have failed to translate into the clinic due to significant *in vivo* toxicity. During scaffold preparation, decellularisation was carried out to remove the intracellular components from a tissue while preserving the native extracellular components to reduce *in vivo* toxicity. However, macroscopic appearance alone is insufficient to determine the extent of decellularisation. Here, histological analysis, 4′, 6-diamidino-2-phenylindole (DAPI) staining and scanning electron microscopy (SEM) have been employed to assess the efficiency of decellularisation. The extracellular matrix-based scaffolds used were decellularised porcine-derived cholecyst (d-PC), small intestinal submucosa (d-PSIS) and a reference bioscaffold graft, Surgisis (SSS). The d-PC and d-PSIS scaffolds were subjected to decellularisation*via* non-detergent and non-enzymatic method. The normal histology of d-PC scaffold displayed absence of cellular remnants. In contrast, d-PSIS bioscaffold showed presence of many residual cells whereas only a few residual cells were present in the Surgisis graft. The SEM revealed that the abluminal surface of d-PC bioscaffold had only extracellular matrix and bundles of crimped collagen fibres. The d-PSIS bioscaffold showed exposed extracellular matrix and residual cells on the abluminal surface while extracellular matrix and bundles of crimped collagen fibres with fewer cell remnants were seen associated with Surgisis graft. On DAPI staining, the d-PC showed very minimal blue fluorescence. The d-PSIS exhibited moderate blue fluorescence whereas SSS showed mild fluorescence. Our results suggest that d-PC is a better bioscaffold for clinical applications as well as the present study provides different methods for evaluating *in vivo* toxicity of biomaterials to reduce toxicity for eventual clinical translation. Inadequate decellularisation of tissue leads to adverse reactions upon *in vivo* implantation, including a pro-inflammatory response with recruitment of M1 macrophages and subsequent fibrosis.

F-TOX-P3

EFFICACY OF *ANTHOCEPHALUS CADAMBA* ON HEMATOLOGICAL ALTERATIONS CAUSED BY SUB-ACUTE FIPRONIL TOXICITY IN RATS

Singh Devendra, Anand Shweta, Sharma Pratistha, Swarnkar Rahul, ChoudharyAbhishek, Boghia Sunil

College of Veterinary and Animal Science, Navania, Udaipur, RAJUVAS, Bikaner, Rajasthan

dr.devenchoudhary@gmail.com

The present study was undertaken to evaluate the ameliorative effect of *Anthocephaluscadamba* (kadamba) on hematological alterations induced by sub-acute exposure of fipronil in rats. Rats weighing 100-250g were divided in four groups having six rats in each group. Group I served as vehicle control (corn oil). In Group II fipronil @ 10 mg per kg body weight was administered and in Group III aqueous extract of *Anthocephaluscadamba* leaves @ 300 mg per kg body weight along with fipronil @ 10 mg per kg body weight were administered for 28 days. Group IV was administered with aqueous extract of*Anthocephaluscadamba* leaves @ 300 mg per kg body weight. TEC, Hb, PCV, MCV and MCH values were significantly decreased in Group II compared to control and group IV while in group III the parameters were significantly increased compared to group II. In group II, TLC level was significantly decreased compared to control and group IV, while in group III there was significant increase in TLC values in comparison to group II. DLC percentage showed significant alteration in group II compared to control but there was significant improvement in group III in comparison to group II. The results suggest that subacute exposure of fipronilcaused significant alteration in haematological profile which was restored by aqueous extract*Anthocephaluscadamba* (kadamba) @ 300mg/kg body weight orally.

F-TOX-P4

ASSESSMENT OF ANILOFOS INDUCED HEMATOTOXICITY IN BONE MARROW OF RAT

Bagri P., Kumar V., Singh P., Gauri A. Chandratre[1] and Joshi V.G.[2]

Department of Veterinary Pharmacology and Toxicology,[1]Department of Veterinary Pathology, [2]Department of Animal Biotechnology, LalaLajpatRai University of Veterinary and Animal Sciences, Hisar, Haryana

Anilofos is an organophosphate compound and is widely used as a pre-emergence and early post-emergence herbicide for the control of annual grasses, sedges and some broad-leaved weeds in transplanted and direct-seeded rice crops. The present study was designed to evaluate the anilofos induced sub-acute hematotoxicity in bone marrow of rats. A combined approach employing colony forming unit assay and

histopathology of bone marrow and spleen was utilized to assess the hematotoxicity of anilofos in rats. The hematotoxic potential of anilofos were studied at two dose levels of 2.5% and 5% of maximum tolerated dose (750 mg/kg b.wt.) i.e. 18.75 and 37.5 mg/kg b.wt., respectively, administered orally daily for 28 days. The effect of anilofos on bone marrow progenitor cells was studied by colony forming unit assay. In this assay, colonies of three types of cells viz. granulocyte (G), macrophages (M) and mixture of granulocytes and macrophages (GM) were observed. The number of their colonies was counted and compared with control. There was no significant difference observed in number of these colonies in treated group as compared to control. Histopathology of bone marrow revealed no significant changes in control and treated group. Histopathology of spleen showed adequate population of lymphocytes in the periarteriolar lymphoid sheath (PALS) in white pulp area and normal histological features in red pulp area in control group whereas treated group showed multifocal areas of hemosiderosis in red pulp and mild thinning of lymphocytes in PALS area of white pulp in spleen. On the basis of colony forming unit assay and histopathology of bone marrow it can be concluded that anilofos is nontoxic to bone marrow tissues when administered at dose of 18.75 and 37.5 mg/kg b.wt. orally for 28 days.

F-TOX-P5

ARSENIC DECREASES ANTINOCICEPTIVE ACTIVITY OF PARACETAMOL: POSSIBLE INVOLVEMENT OF SEROTONERGIC AND ENDOCANNABINOID RECEPTORS

Vijayakaran K[1], Kesavan M[2], Kannan K[1], Sankar P[3], Tandan SK[2], Sarkar SN[2], Ranganathan V[1]

[1]Department of Veterinary Pharmacology and Toxicology, VCRI, Orathanadu, Thanjavur, TN, [2]Division of Pharmacology and Toxicology, IVRI, Bareilly, UP.[3]Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute Namakkal, Tamil Nadu.

The study was conducted to assess the effect of repeated arsenic exposure on paracetamol-mediated antinociception by modulating serotonergic and endocannabinoid pathways. Rats were preexposed to elemental arsenic (4ppm) as sodium arsenite through drinking water for 28 days. Next day paracetamol's (400mg/kg, oral) antinociceptive activity was assessed through formalin-induced nociception. Serotonin content and gene expression of 5-HT_{1A}, 5-HT_{2A} and CB_1 receptors were evaluated in brainstem and frontal cortex by spectroflourimeter and qPCR studies. Arsenic decreased paracetamol-mediated analgesia. Paracetamol, but not arsenic, increased serotonin content in these regions. Arsenic attenuated paracetamol-mediated increase in serotonin level. Paracetamol did not alter 5-HT_{1A} expression, but caused down-regulation of 5-HT_{2A} and up-regulation of CB_1 receptors. Arsenic down-regulated these receptors. However,

paracetamol-mediated down-regulation of 5-HT_{2A} was more pronounced. Arsenic did not modify paracetamol's effect on 5-HT_{1A} expression, but reduced paracetamol-mediated down-regulation of 5-HT_{2A} and reversed up-regulation of CB_1 receptors. From the study, it can be concluded that arsenic reduced paracetamol-induced analgesia possibly by interfering with pronociceptive 5-HT_{2A} and antinociceptive CB_1 receptors.

F-TOX-P6

MONITORING OF RESIDUES OF TETRACYCLINES, SULFONAMIDES AND FLUOROQUINOLONES IN MILK OF CATTLE AND BUFFALOES IN SAMASTIPUR, BHAGALPUR AND ROHTAS DISTRICTS OF BIHAR

Nirbhay Kumar*and R.K. Nirala

Department of Veterinary Pharmacology & Toxicology,Bihar Veterinary College, Bihar Animal Sciences University, Patna-800014, Bihar, drnkvet76@rediffmail.com

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 tetracyclines and fluoroquinolones was done in three districts (*viz.* Samastipur, Bhagalpur and Rohtas) of Bihar with the objectives to estimate the residues of these antibiotics in raw milk samples. Sample survey was done in raw milk samples from cattle and buffaloes. The samples were stored in deep freeze till analysis. Samples were processed for high performance liquid chromatography (HPLC) analysis as per standard analytical procedures. Analytical methods for estimation of residues of tetracyclinesand fluoroquinolones were standardized. The antimicrobial residues in milk were estimated above MRL values [MRL values in respect of tetracyclines and fluoroquinolones is 0.1 µg/ml].A total of 300 milk samples (150 cow milk and 150 buffalo milk) were collected from three districts of Bihar *viz.* Samastipur, Bhagalpur and Rohtas districts. The area of sampling was rural, semi-urban and urban areas of selected districts. Tetracyclines were detected in 6 (4.00%) cow milk and 05 (3.33%) buffalo milk samples out of which 01 (0.66%) cow milk sample contained residues above MRL levels as prescribed by Codex Alimentarius and European Commission standards. Fluoroquinolones could not be detected in any sample above MRL level. Overall, cow milk was showing more contamination of drug residues compared to buffalo milk. District-wise, among the surveyed districts, Bhagalpur district was having highest contamination of

drug residues while Rohtas was least and moderate in Samastipur district. Among the rural, semi-urban and urban areas samples, urban areas were showing maximum contamination.Back tracing the history and analysis of feed concentrate samples confirmed the presence of drug residues in feed.

F-TOX-P7

PATHOLOGY OF HAEMOTOXIC SNAKE ENVENOMATION IN A DOG

Divya C, Navya Elizabeth Sajeev, Dhanush Krishna B, Devi S S, Sajitha I S

Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy , divyac@kvasu.ac.in

Venomous snake bites are responsible for more than 1 lakh animal deaths in the world annually. Snake bite attains considerable importance in tropical and sub-tropical countries like India and dog is the species most frequently affected. The present study details the necropsy findings in an 11 month old Labrador dog brought for post-mortem examination to the Department of Veterinary Pathology, CVAS, Mannuthy with a history of sudden death. The animal was reported to exhibit limping and unilateral forelimb oedema a few hours before death. On enquiry, it was understood that the animal exhibited limping following its usual walk on a bushy ground in the owner's premises, which presumably had the possibility of inhabitation by venomous snakes. The carcass of the dog was presented with congested mucous membranes and severe swelling on the left forelimb. Upon necropsy, severe bruising with haemorrhages, blood clots and oedema was noticed throughout the length of left forelimb. Distinct fang marks were evident in the subcutis of dorsal aspect of metacarpal region of the oedematous limb. Severe haemorrhages were observed in all the visceral organs of the body. Pulmonary and subpleural haemorrhages with pulmonary oedema were discernible in the lungs. Splenomegaly was noticed with splenic congestion. A segmental area of haemorrhage arranged in hexagonal pattern was noticed on the surface of liver coupled with hepatic necrosis. Multifocal spots of petechiae could be seen on the capsular surface of renal cortex. Severe haemorrhagic gastroenteritis could be observed with blood mixed ingesta from oesophagus to the terminal portion of large intestine. The urinary bladder was filled with blood tinged urine with multifocal petechiation on mucosa. The histopathologic lesions observed were also consistent with the gross findings. The gross and histopathologic findings coupled with circumstantial evidences suggested the cause of death as haemotoxic snake envenomation. Acute cardiopulmonary failure due to hypotensive crisis caused by severe haemorrhage and haemolysis from the haemotoxic action of venom injected into the body following bite from a snake belonging to the family Viperidae appeared to be the cause of death of the dog.

F-TOX-P8

ASSESSMENT OF PESTICIDE RESIDUES IN BLOOD SAMPLES OF CATTLE OF PALAKKAD DISTRICT

Dhanya.V.R[1], Usha.P.T.A[2], Nisha.A.R[3] , and Ajaykumar.V.J[4]

[1]District Epidemiologist, Animal Disease Control Project, Thrissur, [2]Professor and Head ,Deparment of Veterinary Pharmacology &Toxicology,COVAS, Mannuthy, [3]Assistant Professor, Deparment of Veterinary Pharmacology &Toxicology,COVAS, Mannuthy,[4] Professor, Department of Veterinary Public Health and Epidemiology, RIVER,Puducherry.

Organochlorine pesticides are ubiquitous and persistent organic pollutants used widely throughout the world. The organochlorines are more hazardous due to their prolonged persistence, lipophilic nature and tendency to accumulate in animal and plant tissues. The present study was therefore undertaken to assess the level of endosulfan residues in blood samples collected from cattle of Pudussery and Perumattypanchayaths of Palakkad district, Kerala. .A total of 20 blood samples were collected from the study area and serum was separated. Clean up techniques were used to remove the impurities and for the extraction of pesticide residues from the collected samples before injecting it to the gas liquid chromatograph. The mean alpha endosulfan, beta endosulfan, endosulfan sulphate and totalendosulfan (ppm) levels were $1.477 \times 10^{-3} \pm 5.148 \times 10^{-4}$, $7.1452 \times 10^{-3} \pm 2.916 \times 10^{-3}$, $1.137 \times 10^{-2} \pm 5.756 \times 10^{-3}$ and $1.995 \times 10^{-2} \pm 8.448 \times 10^{-3}$ respectively. In the present study the residues present in the serum samples were low and were within the safe level.

S-TOX-P1

OESTROGENIC EFFECT OF CHEMICALS IN PLASTIC PRODUCTS

Dabholkar Akshay Rajkumar, Bibu John Kariyil, Aathira K.K., Akhil G H, Dhanusha G, Haima J.S.

Department of Veterinary Pharmacology and Toxicology, CVAS, Mannuthy, KVASU

Plastics are used widely everywhere in our life and without plastic, modern civilization would indeed look very diverse. Many chemical additives that give plastic products desirable performance properties also have negative environmental and human health effects. It includes direct toxicity, carcinogenicity and

endocrine disruption. In this study, the oestrogenic effect of various chemicals like polypropylene, polyethylene, polyethylene terephthalate, cyclic olefin copolymer, polystyrene, polycarbonate, bisphenol A, Triphenyl phosphate and Fluorene-9- bisphenol used in the manufacturing of plastics were analysed using molecular docking studies. The results of the present study revealed that all the chemicals except polyethylene showed a good binding affinity for oestrogen receptor. The maximum binding affinity was shown by Fluorene-9- bisphenol (-9.7 Kcal/mol), which has been introduced to produce the so called bisphenol A (BPA)-free plastics. Hence the present study revealed that the chemicals used in the manufacture of plastic/plastic products may cause adverse reproductive effects. The study suggests that the chemicals used in the manufacture of plastics should be tested for endocrine disruption effect and further studies are required to prove the toxicological effects of such chemicals on human/animal health.

F-AWA-P1

IN VITRO CYTOTOXIC POTENTIAL OF BAICALEIN IN DALTONS LYMPHOMA ASCITES (DLA) CELLS

Preethy John[1], Nisha A R[1], Sujith S[1], Dhanusha G[2], Usha P T A[3]

[1]Assistant Professor, [2]MVSc scholar, [3]Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences,Mannuthy,Thrissur-680651, preethy@kvasu.ac.in

Flavonoids have the potential of modulating many biological events in cancer such as apoptosis, vascularization, cell differentiation and cell proliferation. Baicalein (5, 6, 7-trihydroxyflavone) is a flavone, a type of flavonoid, originally isolated from the roots of *Scutellaria baicalensis* and *Scutellaria lateriflora*. It is also reported in *Oroxylum indicum* (Indian trumpet flower) and *Thyme*. The present study was carried out to evaluate the *in vitro* cytotoxic activity of baicalein in DLA cells. DLA cells were maintained intraperitoneally in mice. The cells were harvested and seeded to 96 well plate at a concentration of 1×10^5 cells/mL in RPMI media containing 10 per cent serum, 1 per cent antibiotic, antimycotic solution after treating with 80, 40, 20, 10, 5, 2.5, 1.25 µg/mL of the compound and incubated for 24 hours at 37˚C with 5 per cent CO_2. The viability was assessed using MTT Assay and there was a dose dependent decrease in viability of cells exposed to baicalein. Baicalein@ 80 µg/mL caused an inhibition of 85 per cent of cells after 24 hours of incubation and the IC_{50} was found to be 27.04µg/mL.

S-AWA-P1

CYTOTOXIC POTENTIAL OF METHANOLIC EXTRACT OF *CRATAEVA NURVALA* IN TRIPLE NEGATIVE BREAST CANCER CELL LINE

Aathira K.K., Bibu John Kariyil, Sujith S., Dhanusha G, Haima J.S., Dabholkar Akshay Rajkumar,

Department of Veterinary Pharmacology and Toxicology,College of Veterinary and Animal Sciences, Mannuthy, KVASU

Crataeva nurvala is a high value medicinal tree, known for its cytotoxic, antiproliferative, hepatoprotective and cardioprotective effects. The present study was carried out to evaluate the cytotoxic activity of methanolic extract of *C. nurvala* in MDA-MB-231 cells. The qualitatative phytochemical analysis of the extract was done. Cytotoxicity of the extract in MDA-MB-231 cells was evaluated using 4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Literature review suggested that triterpenoids like lupeol obtained from various plants like *C. nurvala* were capable of inhibiting NF-κB through different mechanisms. Hence *in silico* binding studies were carried out to find out the binding affinity of the phytochemicals to target protein, NF-κB. The results of the present study revealed the presence of steroids, alkaloids, phytosterols and triterpenes. The cells treated with methanol extract of *C. nurvala* at various concentrations of 640, 320, 160, 80, 40, 20, 10 and 5 µg/mL for 24 hours showed concentration dependent decrease in viability. The *in silico* binding studies with various phytochemicals from *C. nurvala* showed a binding affinity towards NF-κB with binding score ranging from -7.1 to-8.5 Kcal/mol. Hence the present study revealed that methanolic extract could evolve as a potent anticancer agent against triple negative breast cancer.

S-AWA-P2

A COMPARITIVE STUDY ON OCCURANCE OF INTESTINAL COCCIDOSIS IN CHICKEN UNDER FREE RANGE AND INTENSIVE SYSTEM OF REARING

Devavratha S., C.S.Suja, Geo Joseph

College of Veterinary and Animal Sciences, Mannuthy

mplyprasad@gmail.com

In the present study, a group of fifty birds reared under deep litter system and free ranging conditions were utilized. The native breed of chicken was selected which were fourteen weeks of age during the period

of study. Coccidiosis is a protozoan disease and caecalcoccidiosis is the severe form. The disease has a rapid onset and results in mortalities in most of the cases. Intestinal coccidiosis is most likely to occur between six and sixteen weeks of age. This form is more difficult to detect and is usually more insidious. The affected birds showed typical symptoms like depression, anorexia, standing with eyes closed, ruffled feathers along with passage of blood stained droppings. Birds had staggery gait and weight loss. The condition was diagnosed by conducting postmortem of dead birds and observation of typical lesions. The fecal sample examination of affected live birds demonstrated presence of coccidialoocysts. On postmortem examination, blood stained fluid contents with extensive hemorrhage of intestinal mucosa could be observed as characteristic lesions. The affected live birds were treated with anticoccidial drugs like sulfaquinoxaline and amprolium which increased the survival rate. On comparing the mortality rate due to coccidiosis under free range conditions to intensive conditions, the rate was found to be eight percent in free range and twelve percent in deep litter system. It is supposed that the stress and immunosuppression can pave path for coccidiosisor act as predisposition factors. The birds kept under free range conditions suffer less discomfort and stress than birds reared under intensive system. The welfare and natural behaviour is better expressed under free range. This may be the reason for decreased incidence of coccidiosis mortality under free range conditions.

S-MNP-P1

EXOGENOUS MELATONIN OVERCOME LEAD ACETATE INDUCED ALTERATIONS IN REDOX HOMEOSTASIS AND CERTAIN NEUROPHARMACOLOGICAL ACTIVITIES IN LABORATORY MOUSE

Sahithya C.P., Prakash.Nadoor.,Ravikumar C., Ravikumar P., Sharada R.,Suresh B.N and Pavankumar K.N.

Department of Pharmacology and Toxicology, Veterinary College, Hassan, Karnataka-573202

Lead (Pb) is an important toxic heavy metal which affects the central nervous system in addition to variety of toxicopathological lesions elsewhere. An experimental study was conducted to characterize alterations in neurobiochemistry and its impact on neuropharmacological activities in Swiss albino mouse following sub-chronic exposure and to determine the potential role of exogenous melatonin (MLT) as an antioxidant. Male Swiss albino mice were randomly divided in to four groups of six each. Group-I served as untreated control, while group-II received Lead (II) acetate [$Pb(CH_3COO)_2$] @ 25 $mg.kg^{-1}$b.wt. (*p.o*) on alternate days x 60 days. Mice in group-III received treatment similar to group-II but in addition received MLT @ 10 $mg.kg^{-1}$b.wt. (*p.o*) on alternate days for a period of 60 days, while group-IV received MLT alone

for a similar period. Sub-chronic exposure to lead acetate (group-II) significantly (p<0.05) reduced duration of immobility in forced swim test (FST) and decreased voluntary locomotorand forced motor activities. Exogenous source of MLT (group-III) showed a significant (p<0.05) improvement in FST and motor activities, when subjected to test on day '30' or '60'. Further, mice exposed to lead acetate showed a significant (p<0.05) reduction in pain latency when assessed at term, while MLT supplementation (group-III) showed a significant (p<0.05) increase in pain latency. Sub-chronic administration of 'Pb' induced (group-II) significant (p<0.05) increase in the levels of thiobarbituric acid reactive substance (TBARS) called malondialdehyde (MDA) in the brain tissue and their levels were significantly (p<0.05) reduced by MLT supplementation. Further, MLT supplementation prevented the histopathological lesions in hippocampus in experimental mice. Thus, exogenous melatonin can overcome lead induced oxidative stress in brain and restores disturbed neuropharmacological activities in Swiss albino mouse.

S-MNP-P2

MUSCARINIC RECEPTORS OF SMALL INTESTINE OF JAPANESE QUAIL (*COTURNIX COTURNIX JAPONICA*)

Sanjay B.M[1], Megha M[1]., Suraj S V[1], Suresh N Nair[4], Sanis Juliet[1], Muhsin Asaf[2], Ajithkumar K. G[3]. and Nisha A. R[4].

Dept of Pharmacology and Toxicology, Dept of Animal Genetics and Breeding, Dept of Veterinary Parasitology, CoVAS, Pookode, Dept of Pharmacology and Toxicology,CVAS, Mannuthy

Muscarinic receptors of intestine are essential for the digestion and assimilation of food. A study was conducted for identifying the presence of muscarinic receptor subtypes and its functional assessment, for better pharmacological management of dysfunctions of the small intestine. Eight healthy quails of either sex were raised under uniform management conditions and two to three centimetres length ileum was separated and transferred to tyrode solution at 37.2 ℃ with 1 g tension in an isolated organ bath unit. The contractile responses to the ACh alone, agonist in presence of specific and nonspecific antagonists and relaxant effect of muscarinic receptor antagonists with submaximal contraction of ACh were recorded with isometric transducer connected to a recorder. The median effective concentration 50 (EC50), median inhibitory concentration 50 (IC50) and pD2 values were determined. From the results, it is evident that muscarinic acetylcholine receptors are present in the small intestine of Japanese quail. The EC50 values of acetylcholine alone in ileum of Japanese quail varied from 1.235 X 10^{-7} M to 2.344 X 10^{-7} M with mean value of 1.701 X 10^{-7} M and pD2 value of 6.769. The EC50 of ACh in presence of atropine varied from 4.19 X 10^{-7} M to 8.36

X 10^{-7} M with a mean value of 5.92 X 10^{-7} M and pD2 value of 6.23. In presence of pirenzepine, mean EC50 of 4.51 X 10^{-7} M with a range of 3.17 X 10^{-7} M to 6.42 X 10^{-7} M and pD2 values of 6.35 and in presence of solifenacin EC50 value varied from 2.05 X10^{-7} M to 3.44 X 10^{-7} M with a mean value of 2.65 X 10^{-7} M and pD2 values of 6.58. Muscarinic receptor antagonists mainly atropine, pirenzepine and solifenacin completely relaxed the contraction induced by submaximal dose of ACh. The IC50 of atropine varied from 5.21 X 10^{-8} M to 8.64 X 10^{-8} M with a mean value of 6.71 X 10^{-8} M and pD2 value of 7.173, the IC50 of pirenzepine varied from 1.573 X 10^{-6} M to 1.926 X 10^{-6} M with a mean value of 1.74 X 10^{-6} M and pD2 value of 5.759. For solifenacin, the IC50 value varied from 6.25 X 10^{-7} M to 8.20 X 10^{-7} M with a mean value of 7.16 X 10^{-7} M and pD2 value of 6.145. There was a significant increase in the EC50 values of ACh in presence of atropine and pirenzepine compared to EC50 value of ACh alone. Also, there was a significant increase in the EC50 value of ACh in presence of atropine when compared to EC50 of ACh in presence of solifenacin. This indicates that the muscarinic receptor subtypes responsible for contraction of small intestine in Japanese quail is contributed by both M_2 and M_3 muscarinic receptor subtypes since M_1 receptor is absent in them just like other avian species as evidenced by a negative result in PCR. It was also being found that EC50 of ACh is increased 3.48 times in presence of atropine, 2.65 times in presence of pirenzepine and 1.56 times in presence of solifenacin. This indicates that the muscarinic receptor subtypes responsible for contraction of small intestine in Japanese quail is contributed by both M_2 and M_3 muscarinic receptor subtypes and molecular studies have revealed the absence of M_1 receptor gene in Japanese quail.

S-MNP-P3

EFFECT OF PROPOFOL ANAESTHESIA ON KIDNEY FUNCTION TEST VALUES IN POST PAN-OVARIOHYSTERECTOMIZED DOGS WITH PYOMETRA

[1]Rekha A, [2]Anoop R, [3]Niyas E, [4]Vishnu Dev R S

[1]MVSc Scholar, Dept of Livestock Production Management, CVAS, Mannuthy *, [2]Veterinary Surgeon, Multi Speciality Veterinary Hispital, Kudappanakunnu, [3]PhD Scholar, Dept of Animal Reproduction, Gynaecology and Obstetrics, [4]MVSc Scholar, Dept of Livestock Production Management, CVAS, Mannuthy.

rekhakunja@gmail.com

Pyometra is now a very common pathological condition affecting old, intact, mainly unbred female dogs. Although many medical treatments have been found to be effective in controlling pyometra, a complete cure for this is attained only through a surgical removal of both the ovaries and the affected uterus

(Pan-ovariohysterectomy). In veterinary practice injectable anesthetics are more frequently used as compared to the inhalant. Propofol is one such injectable, emulsion based anesthetic which is routinely used in dogs especially in geriatric patients. It has long been considered as one of the safest among its group. The present study is a report on evaluation of kidney function values of 6 female dogs belonging to age group of 7 to 10 years, which were operated up on for correction of pyometra with propofol (as 1%w/v, 10mg/ml solution) as anesthesia for both induction (4mg/kg body weight) and extension (1mg/1kg body weight) of general anesthesia, with atropine sulphate, dexamethasone and xyalzine premedication and with routine antibiotic, analgesic and antihistamines as post operative medication. All these cases shown an increase in the Kidney Function Test (KFT) values, 1 days post surgically, with a mean urea increase of 71.62 and creatinine increase of 3.26. Even minor postoperative increases in urea and creatinine levels are associated with adverse outcomes.Even though propofol is considered as one of the safe anesthetic, these results emphasize that there is chance of kidney affections while using propofol as injectable anesthetic in post pan-ovariohysterectomized dogs with pyometra.