Research Article

TOPICALLY APPLIED NANOQUERCETIN ENHANCED HEALING OF OPEN EXCISION WOUND IN RATS

ANU GOPAL[1*], VINAY KANT[2], ANU GOPALAKRISHNAN[1], S.P. SINGH[3], SURENDRA K. TANDAN[1] AND DINESH KUMAR[1]

[1]Division of Pharmacology and Toxicology, Izatnagar-243 122 (U.P.)
[2]Department of Pharmacology and Toxicology, L.U.V.A.S., Hisar (Haryana)
[3]Professor and Head, Department of Pharmacology and Toxicology , G.B.P.U.A.T., Pantnagar, 263 145
*Corresponding author: anugopal04vet@gmail.com

ABSTRACT

Accelerating wound healing is one of the challenging endeavours of all times which can increase the time as well as cost of the treatment. Quercetin is a member of flavonoid group which has proven to be useful in the treatment of hypertrophic scars, keliods and ulcers. Our present study was aimed to evaluate the wound healing potential of nano formulation of quercetin in excision wound model in rats. Nanoquercetin treated rats showed faster wound contraction, more hydroxyproline and glucosamine content, better epithelialization and collagen deposition than control rats suggesting its ability to enhance wound healing.

Key words: wound healing, nanoquercetin, flavonoid, collagen

INTRODUCTION

Wound healing remains a challenging clinical problem. It leads to early and/or late complications causing morbidity and mortality. Understanding the physiology of wound healing and its care with an emphasis on new therapeutic approaches constitutes an important aspect in rehabilitation medicine. The healing process starts with the formation of granulation tissue and ends with the re-epithalialization of the wound. Synthesis, remodelling and deposition of structural extracellular matrix (ECM) molecules are indispensable for initiating repair and progression into the healing state. In most of the cases, the complication in wound healing is due to formation of inadequate granulation, severe inflammatory reactions, excess scarring and improper extra cellular matrix deposition. Quercetin (3,3',4',5,7-penthydroxyflavone) is a member of flavonoid group having multiple effects like, anti-inflammatory, anti-cancer, anti-ulcer, anti-allergy, antimicrobial etc. It is widely distributed in the plant kingdom and is one of the most abundant flavonoid molecules. Presently, it is used as a therapeutic agent in hypertrophic scars and keloids (Goweini and Nour El Din, 2005). Topical application of quercetin containing formulations has been shown to prevent ultraviolet (UV) rays-induced skin damage (Casagrande *et al.*, 2006). Many studies have reported the ability of quercetin for better intercellular matrix regeneration and enhancement of healing of experimental cutaneous wounds in rats (Gomathi *et al.*, 2003; Calvo *et al.*, 2007). Recent advances in material sciences and nanotechnology have yielded improved therapeutic approaches for tissue repair and wound management. Quercetin nanoparticles can interact at molecular and cellular level, due to which it shows better cytoprotective activity and reduce time span for wound healing. However, there is lack of research work on nano particle formulations of quercetin in the area of healing and regeneration. So, the present study was conducted to evaluate the wound healing potential of nanoquercetin ointment on excision wound model in rats.

MATERIALS AND METHODS

Animals

Healthy adult male Wistar rats (120-140g) were procured from Laboratory Animal Resource Section, Indian Veterinary Research Institute, Izatnagar (U.P.), India. The animals, housed in polypropylene cages had free access to fresh water and balanced feed in divisional animal house. The experimental protocols involved in this study were approved by the Institutional Animal Ethics Committee, Indian Veterinary Research Institute, Izatnagar and conforms to the guidelines for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996).

Preparation of nanoquercetin

Quercetin was purchased from Sigma Aldrich, USA Nanoquercetin was prepared by dissolving the quercetin in ethanol followed by homogenization for 10-15 minutes. Then the solution was kept for lyophilisation and the lyophilized product was powdered carefully to get the nanoquercetin.

Preparation of nanoquercetin ointment (0.03%)

Nanoquercetin ointment (0.03%) was prepared by mixing nanoquercetin thoroughly in white petroleum jelly on an ointment slab with the help of a mixing spatula.

Wound creation

The rats were anesthetized with intraperitoneal

(i.p.) injection of pentobarbitone sodium (40 mg/kg). The dorsal skin of the animals was shaved and cleaned with 70% ethanol and a full-thickness open excision type skin wound (≈400 mm^2) was created. The animals were equally and randomly divided into following two groups of five each.

Group I *(control):* Simple ointment base (petroleum jelly) was applied topically once daily for 14 days

Group II (nanoquercetin–treated): 0.03% nanoquercetin ointment was applied topically once daily for 14 days

Photography of wounds and measurement of percentage wound contraction

Photograph of each wound was taken on days 0, 3, 7, 11 and 14. Margins of the wounds were traced on a transparent paper by a fine tip permanent marker. The area (in square millimeters) within the boundaries of each tracing was determined planimetrically. The results of wound measurements on various days were expressed as per cent wound contraction. The values were expressed as per cent values of the 0 day measurements and were calculated by Wilson's formula as follows:

$$\% \text{ wound contraction} = \frac{\text{0-day wound area} - \text{unhealed wound area}}{\text{0-day wound area}} \times 100$$

Collection of tissue and estimation of hydroxyproline and glucosamine

On the 14th day animals were sacrificed with an overdose of diethyl ether and the granulation/healing tissue was carefully collected. It was divided into two parts. One part was immediately preserved in 10% neutral buffer formalin for histopathological observations such as, hematoxylin and eosin staining and picrosirius red staining for collagen and second part was used for estimation of hydroxyproline (Woessner, 1961) and glucosamine (Rondle and Morgan, 1955).

Histological study by H & E staining

Granulation/healing samples which was fixed in 10% neutral buffer formalin, embedded in paraffin were subjected to sectioning using microtome. 5 μm thick tissue sections obtained were stained with hematoxylin and eosin and visualized for histological changes under light microscope and photographs were taken.

Picrosirius red staining for collagen

To evaluate collagen in healing wound, sections were stained with picrosirius red (direct red 80 from Sigma Aldrich, USA) by modified picrosirius procedure (Dayan *et al.*, 1989). Collagen types were differentiated based on birefringence as thick and denser collagen showing orange to red colour and thinner collagen fibres showing yellow to green (Vodovotz *et al.*, 1993; Rizzoni *et al.*, 2005)

Statistical analysis

Results are expressed as Mean ± SEM. with n equal to number of animals. Data were analyzed applying unpaired't' test using the GraphPad Prism v4.03 software program (San Diego, CA, USA), and the differences were considered statistically significant at * $P<0.05$ or lower (Snedecor and Cochran, 1989).

RESULTS

Gross examination of wounds

On visual observation, scab formation and shedding was faster in nanoquercetin-treated wounds than the control. The representative photographs of the wound area on days 0, 3, 7, 11 and 14 of both the groups of rats are shown in Fig. 1. The photographs reveal the time-dependent reduction in the wound area in both the groups. Reduction was rapid in nanoquercetin-treated group compared to control. The wound was almost covered by epithelial layer in nanoquercetin-treated group on day 14.

Effect on wound area in rats

The reduction in the wound area (mm^2) was observed in nanoquercetin-treated as well as control groups in time dependent manner (Fig. 2). However significant ($P<0.05$) decrease in wound area was started from 7th day onwards in the nanoquercetin-treated group (178±25.57), compared to the control (198.4±4.64). This reduction in wound area in quercetin-treated group remained significantly lower till 14th day post-wounding (18.50±7.18), as compared to control (41±1.29).

Effect on wound closure (wound contraction) in rats

Wound contraction was markedly greater in nanoquercetin-treated wounds, as compared to control on different days of post-wounding (Fig. 2). The pronounced effect of nanoquercetin treatment on wound contraction was observed after day 7 of application. The significant difference ($P<0.01$) in wound contraction was observed on day 7 (58.66±2.25), 11 (90.21±1.55) and 14 (95.53±1.694) in nanoquercetin-treated group, as compared to day 7 (50.65±2.92), 11 (69.39±1.43) and day 14 (88.99±.1.29) of control.

Effect on the hydroxyproline and glucosamine content of the granulation tissue of excision wounds in rats

On day 14 nanoquercetin-treated groups showed significantly higher content of hydroxyproline (16.42±1.33) as compared to the control group (9.48±0.68). The glucosamine content was also significantly higher in nanoquercetin-treated groups (6.27±0.30), as compared to the control group (4.73±0.19) on day 14 (Fig. 3)

Effect of on maturity of granulation/healing tissue of excision wound in rats (H & E staining)

On 14th day, sections of both groups showed well formed granulation tissue with collagen deposition and superficial epithelialization (Fig. 4). However, nanoquercetin-treated group showed better granulation, more compact and regular collagen deposition and complete superficial epithelialisation, as compared to control group.

Effect of on collagen deposition in granulation/

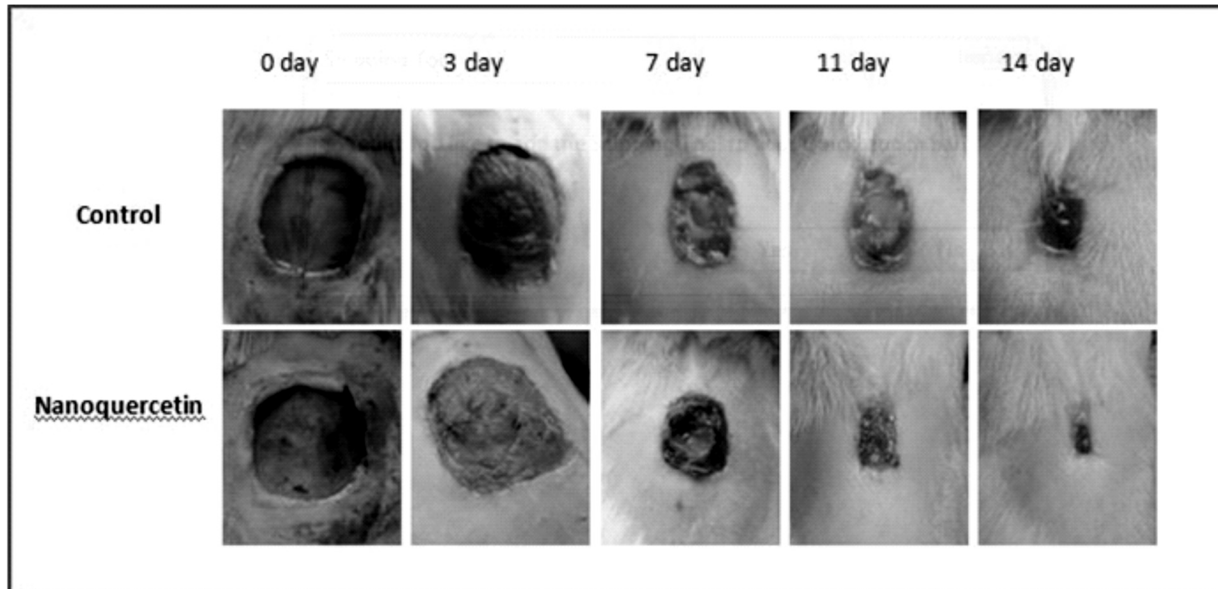


Fig. 1:
Photographs of wound in control and nanoquercetin-treated groups on 0, 3, 7, 11 and 14 days post- wounding.

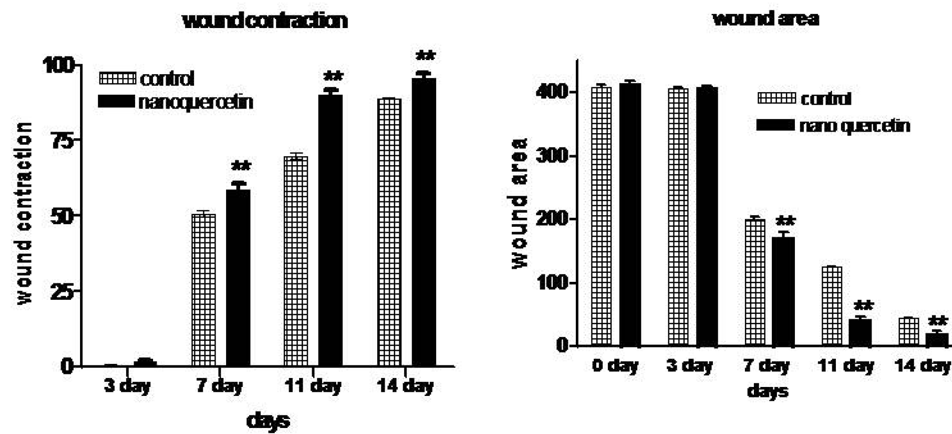


Fig. 2:
Effect of topical application of nanoquercetin on wound contraction and wound area in rats (percent values)

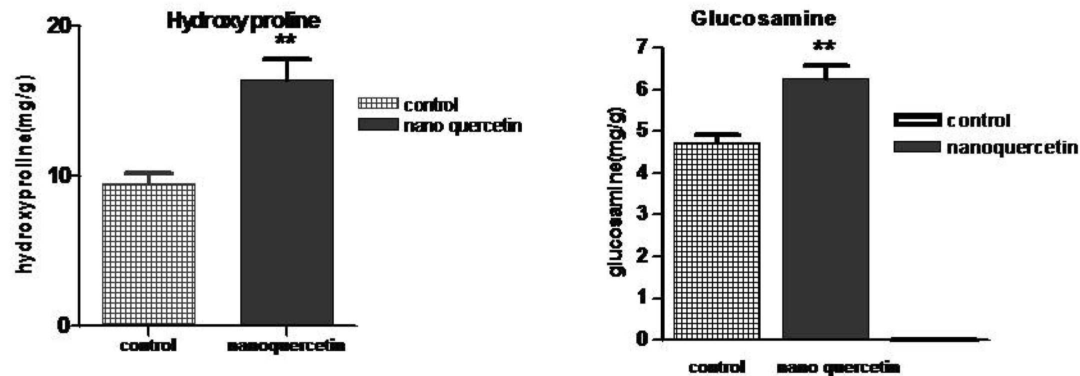


Fig. 3:
Effect of topical application of nanoquercetin on hydroxyproline and glucosamine contents of the granulation tissue of excision wounds in rats

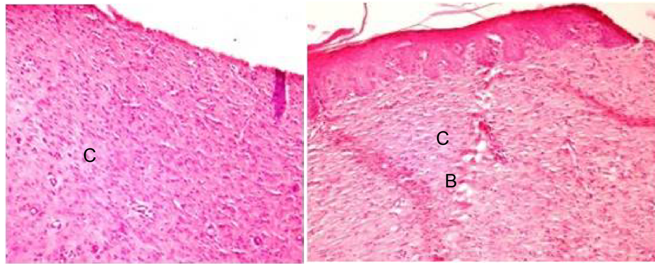


Fig. 4:
Histological sections of healing wound (H & E stained) of control and nanoquercetin treated groups on day 14 (X400); C: Collagen; E: epithelium; B: blood vessels

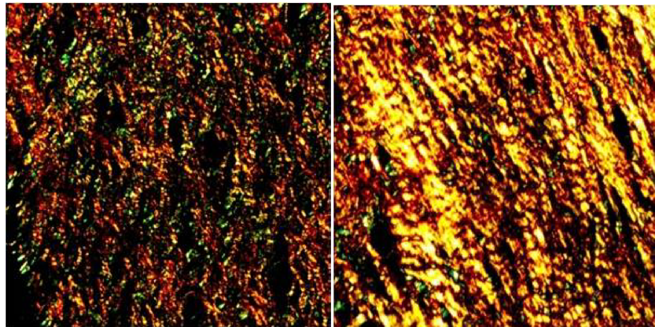


Fig. 5:
Picrosirius red stained histological sections of cutaneous wound of control and nanoquercetin-treated groups showing collagen fibers under polarized light on day 14

healing tissue of excision wound in rats

On day 14, both the groups showed presence of thick (yellow-red) collagen fibers. The arrangement of the thick fibers in control was irregular (Fig. 5). However, the parallel arrangement and more compactness of the thick mature fibers were evident in the nanoquercetin-treated group.

DISCUSSION

Wound contraction begins soon after wounding and it helps in significant reduction of wound area. It is initiated by the myofibroblasts which are stimulated in the presence of various cytokines and growth factors involved in healing. Cytoprotective effects and healing potential of quercetin in gastric and oral ulcers has already been reported by various studies (Suzuki *et al.*, 1998; Calvo *et al.*, 2007). It is also in use as a therapeutic agent for hypertrophic scars and keloids (Goweini and Nour El Din, 2005). In this study, nanoquercetin-treated wounds showed significantly higher contraction than that of control from 7th day onwards which is suggestive of increased fibroblast and myofibrablast activity. It was already reported that quercetin can accelerate healing of experimental cutaneous wounds in rats (Calvo *et al.*, 2007). The nano formulation of quercetin might have enhanced its healing potential, because it will help in a better molecular level interation with the cellular constituents involved in healing process. Thus, the findings of our study imply beneficial effect of nanoquercetin in improving cutaneous wound contraction and healing.

Ability of quercetin to accelerate extracellular matrix regeneration in rats has already been reported by Calvo *et al (*2007). Hydroxyproline constitutes major portion of the collagen protein while glucosamine is an essential moiety of proteoglycans. So, the hydroxyproline and glycosamine content directly indicate the quality and quantity of the extra cellular matrix deposited by fibroblasts during healing process. Our study reveals that the hydroxyproline and glycosamine contents are significantly higher in nanoquercetin treated wounds which would have helped in the proper deposition of collagen and extracellular matrix.

Histological evaluation helps in the morphometric evaluation of repithelialization, wound contraction, maturity of granulation tissue, angiogenesis and inflammation. The nanoquercetin treated group revealed efficient granulation and proper epithelialisation compared to control on histological evaluation which supports its wound healing ability.

Collagen is a major protein of the extracellular matrix and contributes to the wound strength (Singer and Clark, 1999). It plays a pivotal role in maintaining skin structure and therefore, essential for healthy and firm skin (Oikarinen, 2006). Picrosirius staining is one of the method to identify collagen types based on the birefringence pattern it gives under plain polarized light. Based on the colour, collagen types were differentiated as the thick and denser collagen (mature collagen) showing orange to red colour , and thin collagen fibers (immature collagen) showing yellow to green colour (Vodovotz *et al.*,1993; Rizzoni *et al.,* 2005; Kant *et al.,* 2014). In the present study, picrosirius staining revealed increased presence of thick collagen (as evident by the yellowish to red colour) on the nanoquercetin-treated group on 14th day which indicate the presence of more mature ECM. The control group revealed less thick and more immature thin collagen fibers. Previously in 2010 Cho *et al.* demonstrated that quercetin can induce matrix metalloproteinase-1 (MMP-1) expression in hairless mouse skin. MMPs are enzymes secreted by epidermal keratinocytes and dermal fibroblasts in response to various stimuli, including UVR, oxidative stress, and inflammatory cytokines. A balance between MMP-1 (collagenase) and tissue inhibitor matrix metalloproteinase-1 (TIMP-1) enzymatic activity affects the amount of extracellular matrix (including collagen) formed at the wound site. It helps in the replacement of excess immature collagen with thick stronger mature collagen with less scarring of the wounds. As evident from our present study, nanoquercetin appears to influence balanced extracellular

matrix deposition with minimum hypertrophic scarring.

ACKNOWLEDGEMENTS

The authors are thankful to the Director, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India, for providing necessary facilities and support for conducting present study.

REFERENCES

Calvo, T.R., Lima, Z.P., Silva, J.S., Ballesteros, K.V., Pellizzon, C.H., Hiruma-Lima, C.A., Tamashiro, J., Brito, A.R., Takahira, R.K. and Vilegas, W. (2007). Constituents and antiulcer effect of Alchornea glandulosa: activation of cell proliferation in gastric mucosa during the healing process. *Biol. Pharm. Bull.* **30:** 451-59.

Casagrande, R., Georgetti, R.S., Waldiceu, A., Verri, Jr., Dorta, J.D., Dos Santos, C.A. and Fonseca, V.J.M. (2006). Protective effect of topical formulations containing quercetin against UVB-induced oxidative stress in hairless mice. *J. Photochem. Photobiol*. **84**: 21–27.

Cho, J.W., Cho, S.Y., Lee, S.R. and Lee, K.S.(2010). Onion extract and quercetin induce matrix metalloproteinase-1 in vitro and in vivo. *Int. J. Mol. Med.* **25**:347-352.

Dayan, D., Hiss, Y., Hirshberg, A., Bubis, J.J. and Wolman, M. (1989). Are the polarization colors of picrosirius red-stained collagen determined only by the diameter of the fibers?. *Histochem.* **93**: 27-29.

Gomathi, K., Gopinath, D., Ahmed, R. M. and Jayakumar, R. (2003). Quercetin incorporated collagen matrices for dermal wound healing processes in rat. *Biomaterials*. **24**: 2767-72.

Goweini El, F. M. and Nour El Din, M. N. (2005). Effect of quercetin on excessive dermal scarring. *Eqyptian. Dermatol. Online J.* **1**: 6-10.

Kant, V., Gopal, A., Kumar, D., Gopalkrishnan, A., Pathak, N.N., Kurade, P.N., Tandan, S.K. and Kumar, D. (2014). Topical pluronic F-127 gel application enhances cutaneous wound healing in rats. *Acta Histochem.* 116:5–13.

Oikarinen, A. (1994). Aging of the skin connective tissue: how to measure the biochemical and mechanical properties of aging dermis. *Photodermatol. Photoimmunol. Photomed*. **10:** 47-52.

Rizzoni, D., Porteri, E., Ciuceis, C., Sleiman, I., Rodella, L., Rezzani, R., Paiardi, S., Bianchi, R., Ruggeri, G., Boari, G.E., Muiesan, M.L., Salvetti, M., Zani, F., Miclini, M. and Rosei, E.A.(2005). Effect of treatment with candesartan or enalapril on subcutaneous small artery structure in hypertensive patients with noninsulin-dependent diabetes mellitus. *Hypertension*. **45**: 659-665.

Rondle, C.J.M. and Morgan, W.I.J. (1955). The determination of glucosamine and galactosamine. *Biochem. J.* **61**: 586–589.

Singer, A.J. and Clark, R.A.F. (1999). Cutaneous wound healing. *N. Engl. J. Med*. **341**:738-746.

Snedecor, G.W. and Cochran, W.G. (1989). Statistical Methods. 8th ed. Oxford and IBH Publishing Company, New Delhi.

Suzuki, Y., Ishihara, M., Segami, T., and Ito, M. (1998). Anti-ulcer effects of antioxidants, quercetin, alpha-tocopherol, nifedipine and tetracycline in rats. *Jpn. J. Pharmacol*. **78**:435-41.

Vodovotz, Y., Bogdan, C., Paik, J., Xie, Q. and Nathan, C. (1993). Mechanism of suppression of macrophage nitric oxide release by transforming growth factor beta. *J. Exp. Med*. **178**:605-613.

Woessner Jr, J.F.(1961). The determination of hydroxyproline in tissue and protein sample containing small proportions of this imino acid. *Arch. Biochem. Biophys*. **93**: 440–447.

Received on: 05.11.2013
Accepted on: 07.12.2013

Research Article

PHARMACOKINETICS OF CIPROFLOXACIN FOLLOWING INTRAVENOUS, INTRAMUSCULAR AND SUBCUTANEOUS ADMINISTRATION IN CATTLE

S.K. BHAVSAR[1] AND M.P. VERMA[2]

Prof. & Head,Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Navsari Agricultural University,Navsari 396450
[2]Retd. Prof. & Head, Anand Agricultural University, Anand 388001, Gujarat, INDIA
[1]Corresponding author: E-mail- skbhavsar@yahoo.com; Cell : 09825768508

ABSTRACT

Disposition kinetics of Ciprofloxacin (CIP) was studied after single dose Intravenous (I.V.), Intramuscular (I.M.) and Subcutaneous (S.C.) administration (5 mg/kg) in cow calves. Serum CIP concentration was measured by validated HPLC assay. The pharmacokinetics of the drug was best described by two-compartment open model following the single dose I.V. administration of ciprofloxacin. The drug was widely distributed (Vd area; 2.13±0.09 L/kg) and rapidly eliminated ($t_{½}\beta$; 1.78±0.15 h) following I.V. administration. Following I.M. and S.C administration, drug disposition follows one compartment model. Following I.M. and S.C administration, peak serum concentrations of 2.03±0.04 μg / mL at 0.53 h and 1.09±0.11 μg /mL at 1 hour were observed. The elimination half-life following I.M. and S.C Injection was 2.64±0.13 and 3.35±0.14 h. The drug shows wide distribution following I.M. (1.84±0.14 L/kg) and S.C (1.82±0.07 L/kg) administration. The bioavailability of ciprofloxacin was higher following I.M. (0.83±0.07) than S.C. administration (0.54±0.07).

Key words: Pharmacokinetics, Ciprofloxacin,Intravenous, Intramuscular, Subcutaneous, Cow calves

INTRODUCTION

Ciprofloxacin (CIP) is a second generation fluoroquinolone, which has been extensively used for the treatment of bacterial infections in men and now finds increasing use in Veterinary medicine. CIP has excellent activity against enterobacteriaceae, fastidious gram negative bacteria and Pseudomonas aeruginosa. It has good to moderate activity against staphylococci, mycobacteria, chlamydia and mycoplasma and is poorly effective against streptococci, enterococci and anaerobic bacteria (Prescott and Yielding 1990). It has good tissue penetration, larger volume of distribution and relatively short elimination half-life. CIP seems to be extremely useful in variety of infections including those of urinary tract, respiratory tract, soft tissues and bones/joints (Brown, 1996). Pharmacokinetics of CIP has been studied in cattle (Nouws *et al.*,1988; Jaykumar et al, 2000, Idowu *et al.*, 2010) and Buffalo calves (Raina *et al.*, 2000;), sheep (Munoz,1996), goat (Pu-ShiJin *et al.*, 1999; Gracia-Ovando *et al.*, 2000) and horses (Dowling *et al.*,1995). Studies on pharmacokinetics of CIP following I.M and S.C. administration in cow calves are limited. The present study was therefore undertaken to study pharmacokinetics of single dose I.V., I.M. and S.C. administration of CIP (5 mg/kg of body weight) in cow calves.

MATERIALS AND METHODS

The experiment was conducted on six healthy young (4-6 months of age) calves weighing 42 - 84 kg. The animals were examined clinically to establish health of cow calves. Each calf was housed in a separate pen and provided standard ration. Water was provided ad libitum. CIP hydrochloride injection (200 mg / 100 mL, Torrent Pharmaceutical Ltd., Ahmedabad) was used for I.V. administration (5 mg/kg) through jugular vein. A 1 per cent solution of CIP hydrochloride was prepared and used for I.M. and S.C. administration. Intramuscular injection was given in the lateral deeper neck muscles (just anterior to leading edge of scapula through the Trapezius and Serratus ventrailis) using a 20 G × 25 mm needle. Subcutaneous administration was made into loose skin folds of neck region. The animals were randomly allotted to receive either I.V., I.M. and S.C. Inj. of CIP by cross-over design. An interval of 21 day was observed between to successive injections. Blood samples (5 ml) from calves were collected in clean sterilized test tubes with the help of an intravenous catheter fixed into jugular vein. Following I.V.administration blood samples were collected at 0 time (before drug administration) and at 0.033 (2 minutes), 0.066 (4 minutes), 0.133 (8 minutes), 0.266 (16 minutes), 0.533 (32 minutes), 1, 2, 4, 8, 12 and 24 hour after drug administration. Following I.M. and S.C. administration blood samples were collected at time schedule as mentioned following I.V. administration and additionally the blood samples were collected at 36 and 48 h after drug administration. Blood was allowed to clot at room temperature and serum was collected in sterile storage vials. Serum samples were stored at -20^{0}C and assayed for CIP concentration within 24 hour.

CIP concentration in serum samples was determined by reverse-phase High Performance Liquid Chromatography (HPLC) (Jim *et al.*, 1992). Two hundred microlitre of serum was taken in a centrifuge tube. Acetonitrile (400 μl) was added in order to precipitate proteins. The mixture was shaken on a vortex mixer for 1 minute and centrifuged for 10 minute at 4000 revolutions /

TABLE 1:
Pharmacokinetic parameters of ciprofloxacin in calves after a single dose intravenous, intramuscular and subcutaneous administration (5 mg/kg of body weight).

Parameters	Unit	Intravenous (Mean ± S.E., n = 6)	Intramuscular (Mean ± S.E., n = 6)	Subcutaneous (Mean ± S.E., n = 6)
$t_{½}K(a)$	h	-	0.18 ± 0.03	0.30 ± 0.02
$t_{½}\alpha$	h	0.14 ± 0.02	-	-
$t_{½}\beta$	h	1.78 ± 0.15	2.64 ± 0.13	3.35 ± 0.14
AUC	μg • h/ml	5.99 ± 0.23	8.58 ± 0.28	7.09 ± 0.55
AUMC	μg • h2/ml	19.03±0.73	32.75 ± 2.11	38.14 ± 3.59
Vd (area)	L/kg	2.13 ± 0.09	1.84 ± 0.14	1.82 ± 0.07
ClB	ml/min/kg	13.70±0.63	8.14 ± 0.79	6.32 ± 0.37
MRT	h	3.11 ± 0.03	3.80 ± 0.13	5.37 ± 0.27
MAT	h	-		2.22 ± 0.26
Cmax	μg / mL	-		
Tmax	h	-		
F	%	-	0.83 ± 0.07	0.54 ± 0.07

$t_{½}k(a)$: absorption half life; $t_{½}\alpha$: distribution half life ; $t_{½}\beta$: elimination half life; AUC: total area under serum drug concentration-time curve; AUMC: area under first moment of curve; Vd(area): apparent volume of distribution; ClB: total body clearance; MRT: mean residence time; MAT : mean absorption time Cmax: maximum drug concentration; Tmax: time of maximum observed concentration in serum; F: systemic bioavailability.

minute. The supernatant was decanted in glass vials and was evaporated to dryness at 45^0 C in a water bath and under a stream of dry nitrogen. The dried material was reconstituted in 200 μl of mobile phase and 20 μl aliquot was injected into HPLC injector.Assembly consist of isocratic solvent delivery pump (model LC 10 AD), fluorescent detector (model RF 10A), rheodyne manual injector and integrator. Chromatographic separation was performed using reverse phase C 18 column (Zorbax, ODS ; 25cm X 46 mm ID) at room temperature. The mobile phase consisted of a mixture of acetonitrile and 0.1 M sodium dihydrogen phosphate (20:80 v/v) adjusted to pH 3.0 with phosphoric acid. The mobile phase was pumped isocratically at a flow rate of 1.0 ml/minute at ambient temperature. The effluent was monitored at excitation and emission wavelength of 280 and 455 nm, respectively. Calibration curve was prepared by adding known amount of CIP to blank unfortified serum for the expected range of concentrations from 0.01 to 600 μg/ml. Quantification was done by reference to the resultant calibration curve. The calibration curve were prepared daily and not accepted unless it had a R2 value > 0.99. The lower limit of quantitation (LLOQ) was 0.01 μg/ml. The assay was sensitive, reproducible and linearity was observed from 0.1 to 600 μg/ml with a mean correlation coefficient of 0.9985. Precision and accuracy were determined with quality control (QC) samples at concentrations of 0.1 , 100 and 600 μg/ml (five replicates each per day). The retention time of CIP was 5.0 minutes. Various pharmacokinetic parameters were calculated from serum concentration of CIP using software PK solution (version 2.0). The bioavailability (F) was calculated using following formula:

$$F = \frac{t\,½\,\beta\,(IV) \times AUC\,(IM / SC)}{t\,½\,\beta\,(IM / SC) \times AUC\,(IV)} \quad \text{(for IM / SC study)}$$

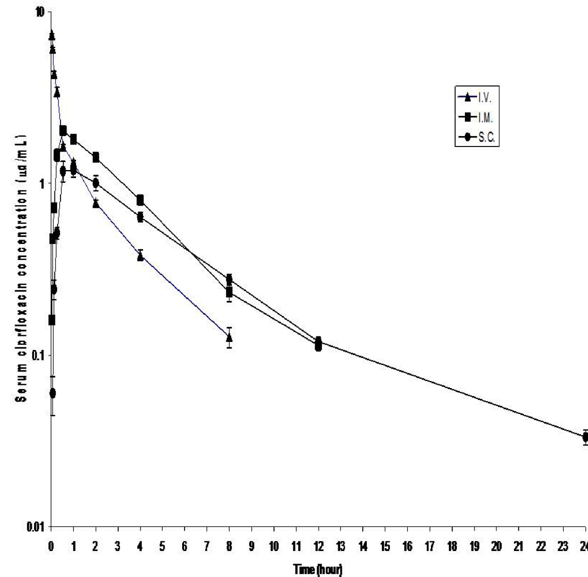
Cefpirome serum concentration and pharmacokinetic parameters of different treatment groups were compared by students' "t" test using SPSS software (version 12.0.1).

RESULTS AND DISCUSSION

The disposition of CIP following single dose I.V., I.M. and S.C. administration in cow calves was plotted on semi logarithmic scale as shown in figure 1. Various pharmacokinetic parameters calculated from serum concentration of CIP after I.V., I.M. and S.C. administrations are summarized in table 1. The semi-logarithmic plot of the serum drug concentration versus time following I.V. administration exhibited biexponential decline and could be best fitted to a two-compartment open model. The semi-logarithmic plot following I.M. and S.C. administration of CIP followed one-compartment open model. The elimination half-life of CIP after I.V. administration observed in the present study is shorter than that of 3.54 ± 0.3, 3.31 ± 0.38 and 2.96 ± 0.30 hours reported in calves (Raina *et al.*,2000) and cows (Jayakumar *et al.*,2000; Idowu *et al.*, 2010), respectively. The disappearance of ciprofloxacin from serum of calves following intravenous administration is faster than that reported in pigs (Nouws *et al.*,1988) and horses (Dowling *et al.*,1995). The elimination half-life observed in the present study following I.M. administration is in line with that of 2.51 ± 0.76 hours reported in goats (Pu-ShiJin *et al.*,1999) but shorter than that of 3.08 ± 0.33 hours reported in sheep (Munoz *et al.*, 1996). Variation in the half-life of enrofloxacin, a fluoroquinolone and precursor of ciprofloxacin, has also been observed. Enrofloxacin has an elimination half-life of 7.3, 1.4, 1.2, 2.1 and 3.3 hours in turkey, calf, dog and horse, respectively (Babish *et al.*, 1990). Inter-species variations are likely to occur in half-lives of lipid soluble

Fig.1:

Semilogarithmic plot of ciprofloxacin concentrations in serum versus time following single dose Intravenous, Intramuscular and Subcutaneous administration (5 mg/kg of body weight) in calves. Each point represents the mean ± S.E. of six animals.



drugs eliminated by biotransformation when the metabolic process is mediated by hepatic microsomal enzyme system (Baggot, 1977).

Following I.M. and S.C. Injection of drug, peak serum CIP concentration of 2.03 ± 0.04 µg/mL at 0.533 hour and 1.09 ± 0.11 µg/mL at 1 hour was observed. The Cmax observed following I.M. administration in the present study is higher than that observed in sheep (Munoz,1996) and goats (Gracia-Ovando *et al.*, 2000). The elimination half-life, total body clearance and apparent volume of distribution of CIP after I.M. and S.C. administration indicate slower elimination and moderate distribution of the drug as compared to I.V. administration in calves. The bioavailability of CIP following I.M. and S.C. Injection was 0.83 ± 0.07 and 0.54 ± 0.07. On the contarary, lower bioavailability of 0.49 and 0.69 has been reported for CIP following IM administration in sheep (Munoz,1996) and goats (Pu-ShiJin *et al.*,1999), respectively. Following oral administration of CIP lower bioavalability has been reported in calves (0.53) and pigs (0.37) (Nouws *et al.*,1988). Based on the observed serum drug concentration and pharmacokinetic variables it may be prudent to administer CIP by I.M.route in calves at dosage of 5 mg/kg repeated at 12 hour interval.

REFERENCES

Babish J., Wilder, J. and Davidson, J. (1990). The comparative pharmacokinetics of a new quinolone enrofloxacin in dogs, horses, calves, chickens and turkeys. *J. Vet. Pharmacol. Therap.* **13**: 472-478

Baggot, J. D. (1977). Principles of drug disposition in domestic animals. In : The basis of veterinary clinical pharmacology. W.B. Saunders Co., Philadelphia, U.S.A. pp.144-189.

Brown, S. A. (1996). Fluroquinolones in animal health. J. Vet. Pharmacol. *Therap.*19:1-14.

Dowling, P. M., Wilson, R. C., Tyler, J.W. and Duran, S. H. (1995). Pharmacokinetics of ciprofloxacin in ponies. J. Vet. Pharmacol. *Therap.* **18**:7-12.

Gracia-Ovando, H., Gorla, N., Poloni, G., Trolti, N., Prieto, G. and Erracalde, C. (2000). Intravenous pharmacokinetics of ciprofloxacin in goats. *Int. J. Antimicrob. Agents.* **15**: 77-79.

Idowu, O. R., Peggins, J. O., Cullison, R. and Bredow, J. V. (2010). Comparitive pharmacokinetics of Enrofloxacin and Ciprofloxacinin lactating dairy cows and beef steers following intravenous administration of enrofloxacin. *Res Vet Sci.* **89**: 230-35

Jayakumar C., Honnegowda and Narayan K. 2000. Pharmacokinetics of ciprofloxacin in lactating cows. *Indian Vet. J.* **77**: 765-767.

Jim, L. K., El-Sayed, N. and Al-khamis, K. I. (1992). A simple high performance liquid chromatographic assay for ciprofloxacin in human serum. *J. Clin. Pharm. Ther.* **17**: 111-115.

Munoz, M. J., Lloveria, P., Santoz, M. P., Abaida, A. R. and Aramayona, J. J. (1996). Pharmacokinetics of ciprofloxacin in sheep after single intravenous or intramuscular administration. *Vet Quart.* **18**: 45-48.

Nouws, J. F. M., Mevius, D. J., Vree, T. B., Baars, A. M. and Laurensen, J. (1988). Pharmacokinetics, renal clearance and metabolism of ciprofloxacin following intravenous and oral administration to calves and pigs. *Vet Quart.* **10**:156-163.

Prescott, J. F. and Yielding, K. M. 1990. In vitro susceptibility of selected veterinary bacterial pathogens to ciprofloxacin, enrofloxacin and norfloxacin. *Canadian J. Vet Res.* **54**: 195-197.

Pu-Shi Jin, Feng-Qi Hui, Pu S. J. and Feng, G. H. 1999. Ciprofloxacin pharmacokinetics and its concentration in the udder and milk of dairy goats. *Chinese J Vet Sci* **19**: 408-411.

Raina, R., Uppal, R. P., Vinod Kumar, Garg, B.D. (2000). Pharmacokinetics and dosage of ciprofloxacin in buffalo calves. *Indian J Ani Sci.* **70**: 475-77.

Received on: 15.10.2013
Accepted on 12.12.2013

TOXICODYNAMIC INTERACTION OF ACETAMIPRID WITH ANTIEPILEPTICS IN SWISS ALBINO MICE

V. KARTHIKEYAN AND S. K. JAIN*

Department of Pharmacology and Toxicology, LLR University of Veterinary and Animal Sciences
Hisar - 125 004, Haryana, India
*Corresponding author:E-mail: drskjainhau@gmail.com

ABSTRACT

The extensive use of acetamiprid in agricultural practices has increased the chances of toxicity in humans and animals. So, the better understanding of toxic mechanism and suitable remedial measures against toxicity is very important. Therefore, the present study was conducted to assess the neuropsychobehavioural toxicodynamic interactions of two doses i.e. 9.2 mg/kg and 18.4 mg/kg of acetamiprid with antiepileptics in Swiss albino mice. Acetamiprid prolonged the pentobarbitone induced hypnosis. Prior administration of sodium valproate decreased the sign and symptoms induced by acetamiprid. Carbamazepine decreased the effect of lower dose of acetamiprid. However, prior administration of diazepam and phenytoin had no effect on acetamiprid induced sign and symptoms. Fluoxetine did not alter the acetamiprid induced behaviour in mice. In conclusion, the results of present study suggested some inhibitory action of acetamiprid on hepatic metabolising enzymes, and possibility of involvement of GABAnergic system in modulating the effects/actions of acetamiprid. However, the involvement of serotonergic mechanisms in acetamiprid induced behaviour in mice may be ruled out.

Keywords: Acetamiprid, antiepileptics, mice, toxicodynamic interaction.

INTRODUCTION

Pesticide toxicity is a global problem and more than three million incidents of the acute pesticide poisonings per annum have been reported worldwide (Jayaratnam, 1990). India accounted for one third of the total pesticidal poisoning cases of the world (Dhaliwal and Atwal, 1997). The last decade has seen advent of a new class of highly effective insecticides, neonicotinoids. Neonicotinoids, potent broad spectrum insecticides, represent the fastest growing class of insecticides introduced to the market since commercialization of pyrethroids (Nauen *et al.,* 2002). The discovery of neonicotinoids can be considered a milestone in insecticide research. Like, the naturally occurring nicotine, all neonicotinoids acts on the insect central nervous system as agonist of the post-synaptic nicotinic acetylcholine receptors and induce abnormal excitement in the insect by interrupting the normal synaptic transmission, consequently, insects suffer from excitation and paralysis, followed by death (Bai *et al.,* 1991; Zhang *et al.,* 2000; Nauen *et al.,* 2001). Neonicotinoids have favourable mammalian safety characteristics (Tomizawa *et al.,* 2000), so they are also important for the control of subterranean pests and for veterinary use (Mencke and Jeschke, 2002).

Acetamiprid (a pyridyl methylamine nicotinoid insecticide) is a new member of the neonicotinoid insecticide family. Although, the compound is neonicotinoid, it possesses characterstic insecticidal properties different from others in the same category of chemical structure and exhibits a broad insecticidal spectrum (Hidemitsu *et al.*, 1998). This compound is widely used in agricultural practices and thus detailed toxicodynamic information on this compound is of upmost importance and need of the hour. Therefore, the present study was conducted to assess the neuropsychobehavioural toxicodynamic interactions of acetamiprid with antiepileptics in Swiss albino mice, so that the toxic mechanism can be understandable in better way, and suitable remedial measures against toxicity can also be developed.

MATERIAL AND METHODS

Experimental design

Healthy male mice (Swiss albino strain, weighing 20-25 g) were procured from Disease Free Small Animal House of LLR University of Veterinary and Animal Sciences, Hisar, Haryana, India. They were housed in the polyacrylic cages (five animals per cage) and maintained on a 12:12 h light dark cycle in a climatically controlled room (temperature of 22 ± 2ºC) with free access to standard feed and water. Animals were acclimatization for a period of a week before the commencement of trials. All the experiments were conducted in noise free laboratory conditions. The experimental protocol was approved by the Institutional Animal Ethical Committee. All animals received humane care in accordance with National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* (NIH Publication N. 85-23, revised 1985). The acetamiprid used in the present study was of technical grade and procured from Topical Agrosystem(India) Pvt. Limited, Chennai.

Mice were divided into three groups in all the

Table 1:
Effect acetamiprid on pentobarbitone induced hypnosis in mice.

Dose mg/kg, i.p.	Time of onset of sleep (min)	Duration of sleep (min)	%Mortality
Control	4.8±0.37^{AB}	24.8±0.37^{A}	0
9.2	4.2±0.37^{A}	25.8±0.66^{A}	0
18.4	5.8±0.37^{B}	51.0±4.00^{B}	0

Values are Mean±SEM of five observations and means bearing different superscripts differ significantly (P<0.05).

interactions study. Each group contained five mice. Group one served as control, and second and third group were treatment groups, as 9.2 mg/kg [1/5th of Maximum Tolerated Dose(MTD)] and 18.4 mg/kg (2/5th of MTD) of acetamiprid was administered, repectively. The MTD of acetamiprid in mice *i.e.* 46 mg/kg, i.p. was determined in our earlier study (Karthikeyan *et al.,* 2012).

Interaction of acetamiprid with antiepileptics

Pentobarbitone induced hypnosis

Acetamiprid was tested for its effects on duration of hypnosis induced by pentobarbitone (40 mg/kg, i.p.) in mice. The criterion for hypnosis was the loss of righting reflex i.e. when the animal could not correct its posture at least three times within fifteen seconds when placed on its back. Animals treated with pentobarbitone were placed on their back as soon as they lost the righting reflex. Time was noted when they spontaneously corrected their posture. Criterion for awakening i.e. regaining of righting reflex was the correction of posture at least three times within fifteen seconds, when they were placed on their back. The interval between the loss of righting reflex and the awakening of animal was taken as sleeping time (duration of sleep) which was recorded in min.

Three groups of five mice each were taken. The control group was administered 10 ml/kg of gum acacia solution (2%). Treatment groups received 9.2 or 18.4 mg/kg (1/5th or 2/5th of MTD) of acetamiprid i.p. thirty min before administration of pentobarbitone. Time taken for induction and duration of sleep was noted. Mortality, if any, was recorded.

Sodium valproate

Interaction of acetamiprid with sodium valproate administered at the rate of 300mg/kg, i.p. was studied in mice. Mice were randomly divided in four groups each containing five animals. First two groups were administered only 9.2 or 18.4 mg/kg (1/5th or 2/5th of MTD) of acetamiprid i.p. and used as a control in this experiment and also for next four interaction studies. Remaining two groups were administered 9.2 or 18.4 mg/kg (1/5th or 2/5th of MTD) of acetamiprid i.p. 45 min later after administration of sodium valproate.

Scoring of characteristic behavioural symptoms induced by acetamiprid treatment in these animals (tremors, respiratory rate, body posture and limb position, restlessness) was done as per Irwin schedule as described by Turner (1965) at 15, 30 and 45 min after acetamiprid administration.

Diazepam

Acetamiprid was studied for its interaction with diazepam administered at the rate of 2.5mg/kg, i.p. in mice. Mice were randomly divided in two groups of five animals each and were kept off feed for 12 h before study. The animals in two groups were administered 9.2 or 18.4 mg/kg (1/5th or 2/5th of MTD) of acetamiprid i.p. 45 min after the administration of diazepam and its value was compared with animals which were treated only with acetamiprid at the rate of 9.2 or 18.4 mg/kg (1/5th or 2/5th of MTD) i.p. which were kept as control in the previous interaction study.

Scoring of characteristic behavioural symptoms induced by acetamiprid treatment in these animals (tremors, respiratory rate, body posture and limb position, restlessness) was done as per Irwin schedule as described by Turner (1965) at 15, 30 and 45 min after acetamiprid administration.

Carbamazepine

Carbamazepine-acetamiprid interaction was studied in mice. Mice were randomly divided in two groups of five animals each and were kept off feed for 12 h before study. The animals in two groups were administered 9.2 or 18.4 mg/kg (1/5th or 2/5th of MTD) of acetamiprid i.p. 45 min after the administration of carbamazepine (8 mg/kg i.p.) and its value was compared with animals which were treated only with acetamiprid at the rate of 9.2 or 18.4 mg/kg (1/5th or 2/5th of MTD) i.p. which were kept as control in the previous interaction study. Scoring of some characteristic behavioural symptoms induced by acetamiprid treatment in these animals (tremors, respiratory rate, body posture and limb position, restlessness) was done as per Irwin schedule as described by Turner (1965) at 15, 30, and 45 min after acetamiprid administration.

Phenytoin

Interaction of acetamiprid with phenytoin was studied in mice. Mice were randomly divided in two groups of five animals each and were kept off feed for 12 h before study. The animals in two groups were administered 9.2 or 18.4 mg/kg (1/5th or 2/5th of MTD) of acetamiprid i.p. 1 h after the administration of phenytoin (10 mg/kg.i.p) and its value was compared with animals which were treated only with acetamiprid at the rate of 9.2 or 18.4 mg/kg (1/5th or 2/5th of MTD) i.p. which were kept as control in the

Table 2:
Effect of antiepileptic drugs on some characteristic behavioural symptoms induced by acetamiprid in mice

Dose (mg/kg,i.p.)	Score for severity of symptoms at different time intervals (min)											
	Restlessness (0)			Respiratory rate (4)			Body posture and limb position (4)			Tremors (0)		
	15	30	45	15	30	45	15	30	45	15	30	45
Sodium valproate (300 mg/kg, i.p.)												
Acetamiprid 9.2	0.0±0.00	0.0±0.00	0.0±0.00	4.2±0.20	4.6±0.24	4.8±0.37	4.0±0.00	4.0±0.00	4.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Sodium valproate + acetamiprid 9.2	0.00±0.00	0.0±0.00	0.0±0.00	4.0±0.00	4.0±0.00	4.0±0.00	4.0±0.00	4.0±0.00	4.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Acetamiprid18.4	0.0±0.00	0.0±0.00	0.0±0.00	5.0±0.00	6.0±0.00	6.0±0.00	4.0±0.00	4.0±0.00	4.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Sodium valproate +acetamiprid 18.4	0.0±0.00	0.0±0.00	0.0±0.00	4.0±0.00	4.0±0.00	4.0±0.00	4.0±0.00	4.0±0.00	4.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Diazepam (2.5 mg/kg, i.p.)												
Acetamiprid 9.2	0.0±0.00	0.0±0.00	0.0±0.00	4.2±0.20	4.6±0.24	4.8±0.37	4.0±0.00	4.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Diazepam + acetamiprid 9.2	0.0±0.00	0.0±0.00	1.0±0.00	4.0±0.00	5.0±0.00	6.0±0.00	4.0±0.00	4.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Acetamiprid 18.4	0.0±0.00	0.0±0.00	0.0±0.00	5.0±0.00	6.0±0.00	6.0±0.00	4.0±0.00	4.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Diazepam +acetamiprid 18.4	0.0±0.00	0.0±0.00	1.0±0.00	4.0±0.00	5.0±0.00	6.0±0.00	4.0±0.00	4.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Carbamazepine (8 mg/kg, i.p.)												
Acetamiprid 9.2	0.0±0.00	0.0±0.00	0.0±0.00	4.2±0.20	4.6±0.24	4.8±0.37	4.0±0.00	4.0±0.00	4.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Carbamazepine + Acetamiprid 9.2	0.0±0.00	0.0±0.00	0.0±0.00	4.0±0.00	4.0±0.00	4.0±0.00	4.0±0.00	4.0±0.00	4.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Acetamiprid18.4 0.00±0.00	0.0±0.00	0.0±0.00	0.0±0.00	5.0±0.00	6.0±0.00	6.0±0.00	4.0±0.00	4.0±0.00	4.0±0.00	0.0±0.00	0.0±0.00	
Carbamazepine +Acetamiprid 18.4	0.0±0.00	0.0±0.00	0.0±0.00	5.0±0.00	5.0±0.00	6.0±0.00	4.0±0.00	4.0±0.00	4.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Phenytoin (10 mg/kg, i.p.)												
Acetamiprid 9.2	0.0±0.00	0.0±0.00	0.0±0.00	4.2±0.20	4.6±0.24	4.8±0.37	4.0±0.00	4.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Phenytoin + Acetamiprid 9.2	0.0±0.00	0.0±0.00	0.0±0.00	4.2±0.20	4.6±0.24	4.8±0.37	4.0±0.00	4.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Acetamiprid18.4	0.0±0.00	0.0±0.00	0.0±0.00	5.0±0.00	6.0±0.00	6.0±0.00	4.0±0.00	4.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Phenytoin +Acetamiprid 18.4	0.0±0.00	0.0±0.00	0.0±0.00	5.0±0.00	6.0±0.00	6.0±0.00	4.0±0.00	4.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Flouxetine (5 mg/kg, i.p.)												
Acetamiprid 9.2	0.0±0.00	0.0±0.00	0.0±0.00	4.2±0.20	4.6±0.24	4.8±0.37	4.0±0.00	4.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Flouxetine + Acetamiprid 9.2	0.0±0.00	0.0±0.00	0.0±0.00	4.2±0.20	4.6±0.24	4.8±0.37	4.0±0.00	4.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Acetamiprid18.4	0.0±0.00	0.0±0.00	0.0±0.00	5.0±0.00	6.0±0.00	6.0±0.00	4.0±0.00	4.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Flouxetine +Acetamiprid 18.4	0.0±0.00	0.0±0.00	0.0±0.00	5.0±0.00	6.0±0.00	6.0±0.00	4.0±0.00	4.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00

Values are mean ± SEM of five observations.

previous interaction study. All the animals were observed for some characteristic behavioural symptoms induced by acetamiprid treatment. These symptoms included tremors, respiratory rate, body posture and limb position, restlessness and flexion of head and the scoring was done as per Irwin schedule as described by Turner (1965) at 15, 30, and 45 min after acetamiprid administration.

Interaction of acetamiprid with fluoxetine

Interaction of acetamiprid with fluoxetine was studied in mice. Mice were randomly divided in two groups of five animals each and were kept off feed for 12 h before study. The animals in two groups were administered flouxetine (5 mg/kg i.p.). one hour after the same animals were administered with 9.2 or 18.4 mg/kg (1/5th or 2/5th of MTD) of acetamiprid i.p. and its value was compared with animals which were treated only with acetamiprid at the rate of 9.2 or 18.4 mg/kg (1/5th or 2/5th of MTD) i.p. which were kept as control in the previous interaction study. All the animals were observed for some characteristic behavioural symptoms induced by acetamiprid treatment. These symptoms included tremor, respiratory rate, body posture and limb position and restlessness and the scoring was done as per Irwin schedule as described by Turner (1965) at 15, 30 and 45 min after acetamiprid administration.

RESULTS

Effect of acetamiprid on pentobarbitone induced hypnosis

The effect of 9.2 mg/kg and 18.4 mg/kg, i.p. of acetamiprid on pentobarbitone induced hypnosis is presented in Table 1. Higher dose of acetamiprid significantly enhanced the onset and duration of sleep as compared to lower dose and control group. No mortality was noticed in all groups.

Interaction with antiepileptic drugs

The effect of 45 min prior administration of sodium valproate on some characteristic behavioural symptoms induced by 9.2 mg/kg and 18.4 mg/kg, i.p. of acetamiprid is presented in Table 2. Sodium valproate and acetamiprid administered group animals were quite normal and no abnormalities like slight disturbances in spontaneous movement and disturbance in respiration were observed as seen in acetamiprid administered animals. Sodium valproate antagonized the effect of acetamiprid in both higher and lower doses treated animals.

The effect of 30 min prior administration of diazepam on some characteristic behavioural symptoms induced by 9.2 mg/kg and 18.4 mg/kg, i.p. of acetamiprid is given in Table 2. Diazepam did not antagonize the effect of acetamiprid. However, it increased the effect of acetamiprid at both the doses levels, for causing the respiratory disturbance and restlessness. Nonetheless, animals became normal after 75 min, indicating delaying in the diazepam activity.

The effect of 45 min prior administration of carbamazepine on some characteristic behavioural symptoms induced by 9.2 mg/kg and 18.4 mg/kg, i.p. of acetamiprid is presented in Table 2. Carbamazipine antagonized the effect of 1/5th of MTD (9.2 mg/kg) of acetamiprid but not the 2/5th of MTD (18.4 mg/kg).

The effect of 1 h prior administration of phenytoin on some characteristic behavioural symptoms induced by 9.2 mg/kg and 18.4 mg/kg, i.p. of acetamiprid is presented in Table2. Phenytoin did not show any effect on acetamiprid induced behavioural profiles.

The effect of 1 h prior administration of flouxetine on some characteristic behavioural symptoms induced by 9.2 mg/kg and 18.4 mg/kg, i.p. of acetamiprid is presented in Table 2. Flouxetine did not produce any effect on acetamiprid induced behavioural profiles in the treated animals.

DISCUSSION

In present study, acetamiprid increased the duration of effect of pentobarbitone in mice. Similar increase in pentobarbitone induced sleeping time by imidacloprid (Premlata, 2001) and isoproturon (Sarkar, 1990) has been reported. Prolonged barbiturate sleeping time can be due to effect on brain permeability (Paulet *et al.*, 1957) or inhibition of barbiturate metabolizing enzymes (Murphy and Duboi, 1956). It has been observed that hexobarbital impaires metabolism and not alters the sensitivity of brain to barbiturate and this is responsible for increased hexobarbital sleeping time (Stevens *et al.,* 1972). Since acetamiprid prolonged the sleeping time of pentobarbitone, it indicates the inhibition of hepatic metabolizing enzymes by acetamiprid.

In present study, sodium valproate antagonized the effect of acetamiprid in treated mice. Sodium valproate inhibits sustained repetitive firing induced by depolarization of mouse cortical or spinal cord neurons (Mclean and Macdonald, 1986). This appears to be mediated by a prolonged recovery of voltage-activated Na^+ channels from inactivation. Another potential mechanism that may contribute to valproate antiseizure actions involves metabolism of GABA. *In vitro,* valproate can stimulate the activity of GABA synthetic enzyme, glutamic acid decarboxylase (Philips and Fowler, 1982), and inhibits GABA degradative enzymes GABA transaminase and succinic semialdehyde dehydrogenase (Chapman *et al.*, 1982). However, in the present study, sodium valproate antagonized the characteristic behavioural symptoms viz. restlessness, respiratory rate, body posture and limb position and tremors.

Diazepam exerts its effects by enhancing the inhibitory effect of GABA. Diazepam did not antagonize the acetamiprid induced symptoms in present study.

Acetamiprid treated animals showed restlessness and increase in respiration. But, animals were observed to be quiet and normal like control animals after 90 min, indicating that acetamiprid delayed the effect of diazepam.

Carbamazepine limits the repetitive firing of action potential evoked by a sustained depolarization of mouse spinal cord or cortical neurons by slowing the rate of recovery of voltage-activated Na^+ channels from inactivation (Mclean and Macdonald, 1986). Carbamazepine antagonized the effect of lower dose of acetamiprid, but not the high dose in mice.

Phenytoin produces its action by stabilization of neuronal membrane, thus prolonging recovery of inactivated Na^+ channels. Thus it limits the repetitive firing of action potential evoked by sustained depolarization (Mclean and Macdonald, 1986). Phenytoin did not antagonize the effects of acetamiprid at both the dose levels in treated mice. However, at higher concentration, phenytoin enhances responses to GABA (Mclean and Macdonald, 1986).

Fluoxetine is highly selective and powerful inhibitor of 5-HT reuptake into presynaptic serotonergic nerve terminals, thereby potentiating the effect of 5-HT in CNS (Azmitia and Whitaker-Azmitia, 1995). Fluoxetine did not alter the acetamiprid induced behaviour in mice. This showed that there is no involvement of serotonergic mechanism in acetamiprid induced behaviour in mice.

In summary, acetamiprid increased the duration of pentobarbitone induced hypnosis, which might be due to the inhibition of hepatic metabolising enzymes. Sodium valproate, carbamazepine and Phenytoin acts in a similar manner. Our study showed that sodium valproate completely antagonized the effect of acetamiprid. Carbamazepine antagonized the lower dose of acetamiprid, but phenytoin and diazepam did not show any effect on acetamiprid induced symptoms in mice. It suggested the possibility of involvement of GABAnergic system in modulating the effects/actions of acetamiprid. Furthermore, fluoxetine did not alter the acetamiprid induced behaviour in mice, so the involvement of serotonergic mechanisms in acetamiprid induced behaviour in mice may be ruled out.

REFERENCES

Azmitia, E. C. and Whitaker-Azmitia, P.M. (1995). Anatomy, cell biology and plasticity of serotonergic system. In, *Psychopharmacology: the Fourth Generation of progress*. (Bloom, F.e., and Kupfer, D.L., eds) Raven Press, New York. pp. 443-449.

Bai, D., Lummis, S.C. R., Leicht, H. W. Breer, and Sattelle, D. B. (1991). Actions of imidacloprid and related mitromethylene on cholinergic receptors of an identified insect motor neuron. *J. Pesticide Sci.* **33**: 197-204.

Chapman, A., Kane, P.E., Meldrum, B.S., Simiand, J., and Vernieres, J.C. (1982). Mechanism of anticonvulsant action of valproate. *Progress in Neurobiol.* **19**: 315-359.

Dhaliwal, G.S. and Atwal, A.S. (1997). Agricultural pest of south asia and their management. Kalyani Publishers, Ludhiana, pp. 486.

Ecobichon, D. J. (1996). Toxic effects of pesticides. In: Klassen, C.D. (Ed.), Casarett and Doull's Toxicology: The basic science of poisons. 5th edition. Mcgraw-Hill, New York, pp. 643-690.Everett, H.C. 1963. Tremor produced by drugs. *Nature* (London). **177**: 1238.

Hidemitsu, T., Nubuo, T., Junjii, S. and takashi, K. (1998). Development of a new insecticide, Acetamiprid. *J. Pesticide Sci.* **23**: 193-200.

Jeyaratnam, J. (1990). Acute pesticide poisoning: a major global health problem. WHO Stastistics quarleft. **43**: 139-140.

Karthikeyan, V., Jain, S.K. and Punia, J.S. (2012). Acute toxicity and neurobehavioural effects of acetamiprid in mice. *J. Vet. Pharmacol. Toxicol.* **11**: 43-49.

Mclean, M.J. and Macdonald, R.L. (1986). Sodium valproate, but not ethosuximide, produces use and voltage dependent limitation of high frequency repetitive firing of action potentials of mouse central neurons in cell culture. *J. Pharmacol. Exp. Therapeut*. **237**:1001-1011.

Mencke, N. and Jeschke, P. (2002). Therapy and prevention of parasitic insects in veterinary medicine using imidacloprid. *Current Topics in Medicinal Chem.* **2**: 701-715.

Murphy, S.D. and Duboi, K.P. (1956). Metabolic conversion of ethyl p-nitrophenyl thinobenzene phosphonate (EPN) to an anticholinesterase agent. *Federation Proceedings*. **15**: 462.

Nauen, R., Stumpf, N. and Elbert, A. (2002). Toxicological and mechanistic studies on neonicotinoid cross resistance in Q-type Bemisia tabaci (Hemiptera: Aleyrodidae). *Pest Management Sci.* **58**: 53-57.

Nauen, R.,Ebbinghaus-kintscher, A., Elbert, A., Jeschke, P. and Tietjen, K. (2001). Acetylcholine receptorsas sites for developing neonicotinoid insecticides. In: Iishaaya, I.(Ed.), Biochemical Sites of insecticides Action and Resistence. Springer, New York, pp. 77-105.

Paulet, G., Marsol, H and Caq, H. (1957). Cholinesterase et Permeabilite de la barrier hematoencephalique. *J. Physiol.* **49**: 342.

Philips, N.L. and Fowler, L.J. (1982). The effects of sodium valproate on gamma-aminobutyrate metabolism and behaviour in naive and ethanolamine-O-sulphate pretreated rats and mice. *Biochem.*

Pharmacol. **31**: 2257-2261.

Premlata. (2001). Pharmacological and toxicological studies of Imidacloprid - a nitroguanidine insecticide. M.V.Sc. Thesis, CCS Haryana Agricultural University, Hisar.

Sarkar, S.N. (1990). Toxicological investigation of isoproturon with special reference to fetal toxicity. Ph.D. Dissertation, Indian Veterinary Research Institute, Izatnagar, U.P.

Stevens, J.T., Stitzel, R.E. and Mcphillips, J.J. (1972). Effect of anticholinesterase insecticides on hepatic microsomal metabolism. *J. Pharmacol. Exp. Therapeut.* **181**: 576-583.

Tomizawa, M., Lee, D.L. and Casida, J.E. (2000). Neonicotinoid insecticides: molecular feature conferring selectivity for insect versus mammalian nicotinic receptors. *J. Agricultural and Food Chem.* **48**: 6016-6024.

Turner, R.A. (1965). Screening methods in pharmacology. Academic press, New York and London. pp 22-41.

Zhang, A., Kayser, H., Maienfisch, P. and Casida, J.E. (2000). Insect nicotinic acetylcholine receptor: conserved neonicotinoid specifity of [3H] imidacloprid binding site. *J. Neurochem.* **75**: 1294-1303.

Received on: 16.11.2013
Accepted on: 22.12.2013