TOXICOLOGICAL EVALUATION OF ENROFLOXACIN AND CIPROFLOXACIN WITH SPECIAL REFERENCE TO GENOTOXICITY IN RATS

Thesis

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(Srinivasu M)
Author

CERTIFICATE

This is to certify that the thesis entitled **"TOXICOLOGICAL EVALUATION OF ENROFLOXACIN AND CIPROFLOXACIN WITH SPECIAL REFERENCE TO GENOTOXICITY IN RATS"** submitted in partial fulfillment of the requirements for the degree of **Master of Veterinary Science** with major in **Veterinary Pharmacology and Toxicology** and minor in **Veterinary Physiology** of the College of Post Graduate Studies, G. B. Pant University of Agriculture & Technology, Pantnagar, is a record of *bonafide* research carried out by **Mr. Srinivasu M, Id. No. 49574,** under my supervision and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged.

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We, the undersigned, members of the advisory committee of **Mr. Srinivasu M, Id. No. 49574,** a candidate for the degree of **Master of Veterinary Science** with major in **Veterinary Pharmacology and Toxicology** and minor in **Veterinary Physiology** agree that the thesis entitled "**TOXICOLOGICAL EVALUATION OF ENROFLOXACIN AND CIPROFLOXACIN WITH SPECIAL REFERENCE TO GENOTOXICITY IN RATS"** may be submitted in partial fulfillment of the requirements of the degree.

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(S.K. Rastogi)
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LIST OF ABBREVIATION

%	:	Percent
@	:	At the rate of
°C	:	Degree centigrade
µg	:	Microgram
µl	:	Microlitre
µm	:	Micrometer
A: G	:	Albumin:globulin ratio
ALT	:	Alanine aminotransferase
AST	:	Aspartate aminotransferase
b wt	:	Body weight
BUN	:	Blood urea nitrogen
CYP	:	Cyclophosphamide
dl	:	Deci liter
DMSO	:	DIMETHYL sulfoxide
DTNB	:	5,5-Dithiobis (2-Nitro-Benzoic acid)
E	:	Beta
EDTA	:	Ethylene diamine tetra acetic acid
FBS	:	Fetal bovine serum
g	:	Gram
GSH	:	Reduced glutathione
h	:	Hours
Hb	:	Hemoglobin
HCl	:	Hydrochloric acid
IU/L	:	International unit per lite
kg	:	Kilogram
l	:	Litre

LPO	:	Lipid peroxidation
M	:	Molar
MDA	:	Malondialdehyde
mg	:	Milli gram
ml	:	Milli litre
mM	:	Millimole
NCE	:	Normochromatic erythrocyte
nm	:	Nano metre
nM	:	Nanomole
NSS	:	Normal saline solution
OTM	:	Olive tail movement
PBS	:	Phosphate buffer saline
PCE	:	Polychromatic erythrocyte
PCV	:	Packed cell volume
RBC	:	Red blood cells
ROS	:	Reactive oxygen species
rpm	:	Rotation per minute
SOD	:	Superoxide dismutase
TBA	:	Thiobarbituric acid
TCA	:	Trichloroacetic acid
TEC	:	Total erythrocytes count
TLC	:	Total leucocytes counts
TSP	:	Total serum protein
WBC	:	White blood cell

Introduction

Chapter 1 INTRODUCTION

The fluoroquinolones are widely used in antimicrobial therapy. The therapeutic activity of fluoroquinolones is due to their ability to inhibit a specific prokaryotic enzyme DNA gyrase, which subsequently results in negative supercoiling of DNA leading to the cessation of proliferation of bacteria. A combination of DNA gyrase and topoisomerase-I is required to correct DNA topology during replication and transcription. The inhibition of DNA gyrase by quinolones triggers replication arrest leading to microbial death. Fluoroquinolones also have the inhibitory effect on functionally related mammalian topoisomerase-II. As a consequence of these effects, fluoroquinolones have shown genotoxic effects in pro and eukaryotic cells. However, higher concentrations of fluoroquinolones are normally required for topoisomerase-II inhibition.

Enrofloxacin and ciprofloxacin belong to fluoroquinolone family which is a subfamily of quinolone. The addition of a fluoro atom on the C-6 position led to the revolution in the quinolone family with the development of fluoroquinolones, which rendered broad antibacterial spectrum. Nalidixic acid was the first quinolone which was used in animals in the beginning of 1980s and enrofloxacin was the first fluoroquinolone patented in 1984 for antimicrobial therapy. Enrofloxacin is effective on most of the gram-negative and gram-positive bacteria but has negligible action against on anaerobic bacteria**.** Being sold at 3.6 million tons per year, fluoroquinolones presently have substantiated share in antibacterial therapy and thus are an important family of antimicrobials in human and veterinary medicine.

Enrofloxacin is well tolerated **(Aral *et al*., 2008)** with fewer side effects in comparison to their benefits. The most common side effects of enrofloxacin include digestive disorders such as nausea, abdominal discomfort, vomiting and diarrhea and inflammatory reaction at the site of injection for injectable forms, particularly in pigs. Enrofloxacin also targets central nervous system, ocular system and reproductive system and pose serious adverse effects on joints in juveniles. Arthropathy, articular cartilage degeneration, tendonitis and other forms of tendon injury are the unfavorable impacts of enrofloxacin in young animals.

Ciprofloxacin is a broad spectrum fluoroquinolone antibacterial agent. It was introduced in animal treatment in 1980s, since then it has been successfully used against most of the gram-negative bacteria and has moderate activity against gram-positive bacteria. Ciprofloxacin is widely distributed in most of the tissues and body fluids. It has shown good clinical efficacy during its clinical use with limited adverse effects. Ciprofloxacin is more potent antimicrobial in man and animals than nalidixic acid and achieves sufficient serum concentrations readily after oral administration to treat many systemic infections with unique spectrum of activity.

Ciprofloxacin is effective in the treatment of severe gastrointestinal infections, sexually transmitted diseases like gonorrhea and chancroid, urinary tract infections and skin and bone infections. It has also shown the bactericidal effect on bacteria causing febrile neutropenia, lower respiratory tract infections including those in patients with cystic fibrosis and malignant external otitis. It can be used in intra abdominal infections in combination with an anti-anaerobic agent in case of patients with mixed infections with Streptococcal pulmonary infections caused by gram negative organisms. Ciprofloxacin has shown usefulness with its broad spectrum antibacterial activity in treating the wide variety of gram positive and gram negative infections in man and animals.

The molecular mechanism responsible for the toxicity of enrofloxacin and ciprofloxacin has not been fully understood. However, experimental evidence suggests their inhibitory activity against bacterial topoisomerase I or DNA gyrase which is functionally and structurally related mammalian topoisomerase II. Consequently, these fluoroquinolones have been correlated with genotoxic effects in both prokaryotic and eukaryotic test systems **(Herbold *et al*., 2001)**. Genetic factors are important factors in the etiology of symptoms and determination of toxicity potential in the patients.

Substances that have a toxic effect on the genetic material of cells and thus can alter organism's DNA are said to be genotoxic. Such substances increase the error rate in the reduplication of the genome and induce mutation by damaging the DNA of the organism. Mutation in the germ cells is passed on to the organism's offspring and can cause congenital or hereditary defects. Mutation in somatic cells can result in an increased risk of diseases and specially the cancer or may lead to cell death.

Gene mutations, chromosomal aberrations and DNA effects are the major types of genotoxic effects. Because no single *in vitro* assay is capable of detecting genotoxic effects, a battery of tests are recommended such as gene mutation and chromosomal aberration tests, bone marrow micronucleus test (MNT) and comet assay. The conventional genotoxic techniques permit both qualitative and quantitative assessment of DNA damage in any eukaryotic cell of population. These methods have standard protocols to evaluate genotoxic effects of xenobiotics and are also accredited by drug regulatory authorities.

The present endeavor was undertaken in view of the fact that enrofloxacin and ciprofloxacin are widely used in small and large animal practice in our country and scarcity of literature on their toxic effects, particularly on their genotoxic effects, in animals, the present investigation was designed to study sub-acute toxicity and genotoxicity of enrofloxacin and ciprofloxacin in Wistar rats with the following objectives.

- To evaluate clinical and hematobiochemical effects of enrofloxacin and ciprofloxacin after 28 days of administration in rats.
- To evaluate oxidative stress induced by enrofloxacin and ciprofloxacin after 28 days of oral administration in rats.
- To assess the enrofloxacin and ciprofloxacin induced genotoxic effects in rats.
- To evaluate gross and microscopic alterations during after of exposure of enrofloxacin and ciprofloxacin in rats.

Review of Literature

Chapter 2 REVIEW OF LITERATURE

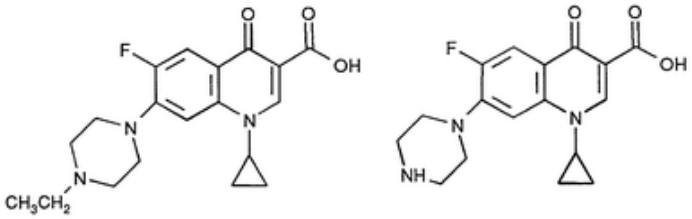
2.1 History

Quinolones were discovered in 1962 by altering the structure of the compound isolated from anti-malarial drug chloroquine **(Martinez *et al*., 2006)**. Nalidixic acid was the first drug in this class and was approved in 1965 for clinical use. Initially, it was limited to treat only urinary infections because of its narrow spectrum and poor absorption by oral route. To improve absorption and bioavailability, its structure was modified in 1980s by adding a fluorine molecule to the basic quinolones structure for synthesis of fluoroquinolones. Norfloxacin and ciprofloxacin were the first fluoroquinolones approved for the clinical use in 1980 and enrofloxacin was approved in 1989 for clinical use in cats and dogs.

2.2 Physicochemical properties

Enrofloxacin and ciprofloxacin belong to 7-piperazinyl fluoroquinolones. The molecular formula of enrofloxacin is $C_{19}H_{22}FN_3O_3$ and IUPAC name as 1-cyclopropyl-7-(4-ethylpiperazin-1-yl)-6-fluoro-4-oxoquinoline-3-carboxylic acid. IUPAC name of ciprofloxacin is 1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid with molecular formula as $C_{17}H_{18}FN_3O_3$. The molecular weights of enrofloxacin and ciprofloxacin are 359.4 and 331.35, respectively. Fluoroquinolones have poor solubility in lipids **(Anon, 1991).** The very poor aqueous solubility and wettability of the drug gives rise to difficulties in the design of pharmaceutical formulations and leads to variable bioavailability **(Seedher and Agarwal, 2009).**

The *p*Ka values of carboxyl and amine groups of enrofloxacin are 6.0 and 8.8, respectively and *p*Ka values for carboxyl and amine groups of ciprofloxacin are 6.1 and 7.8 respectively **(Papich and Riviere, 2009).** These compounds are stable in the environment in both parenteral and oral dosage forms but they are highly sensitive to light and thus must be protected from light for long term usage to preserve the antimicrobial activity of these antibiotics **(Belal *et al.*, 1999).** The structure of enrofloxacin and ciprofloxacin are depicted in Figure 2.1.



Enrofloxacin Ciprofloxacin

Fig 2.1. Structure of enrofloxacin and ciprofloxacin.

2.3 Mechanism of Action

Fluoroquinolones exhibit their bactericidal activity by interacting with two enzymes of topoisomerase family, DNA topoisomerase I (DNA gyrase) and topoisomerase IV resulting in inhibition of DNA synthesis and as well as RNA synthesis at higher concentration **(Hooper, 2001)**. Even though these enzymes are present in eukaryotes, quinolones have higher affinity for bacterial topoisomerase than the eukaryotic topoisomerase **(Levine et al., 1998)**. Initial genetic studies in nalidixic acid resistant *E.coli* mutants classified DNA gyrase as the first target of quinolones. DNA gyrase is a tetramer consisting of two GyrA and two GyrB genes **(Hane and Wood, 1969)**, and discovery of *E.coli* topoisomerase IV revealed that, it was also a tetramer which consisted two ParC and two ParE genes which are homologous to GyrA and GyrB respectively **(Kato *et al*., 1990).**

DNA gyrase plays a vital role in the life of bacteria through modification of the structure of spiral DNA. For transcription, separation of DNA strands is a prerequisite and separation of strands become more difficult once positive supercoiling starts as DNA is stabilized by positive supercoiling **(Anselmi *et al*., 1998).** Positive supercoiling leads to the decline in transcription **(Chong *et al*., 2014).** With the help of C-terminal domain gyrase binds and wraps around DNA strands **(Gubaev and Klostermeier, 2014)** and it cleaves each of GyrA genes by catalytic tyrosine Tyr 122 **(Horowitz and Wang, 1987).** By binding with each 5' phosphoryl terminus of both strands tyrosine forms the covalent phosphotyrosyls during the reaction **(Tse *et al*., 1980).** As a result, there is a gap formation in the sequence called G-DNA. GyrB gene holds the other DNA sequence called as transported DNA or T-DNA which passes through the opened G-DNA **(Wang, 1998).** Finally, the reaction results in formation of negative coiling after closure of G-DNA by an ATP dependent reaction **(Gellert *et al*., 1976).**

The topoisomerase IV also follows the same path as DNA gyrase, the major difference between the reaction of these two is unlike DNA gyrase, at the end of the replication by condensing the DNA, topoisomerase IV forms an intermolecular strand passage **(Peng and Marians, 1995).** DNA topoisomerase IV segregates the two daughter DNA molecules after the replication **(Peng and Marians, 1993)** more efficiently than DNA Gyrase **(Zechiedrich and Cozzarelli, 1995**).

In vivo, inhibition of DNA synthesis by quinolones occurs rapidly by interacting with DNA gyrase **(Drlica *et al*., 1980)** but inhibition shows some delay in case of topoisomerase IV **(Fass *et al*., 1999).** This is attributed by occupying different positions on the bacterial chromosome by DNA gyrase and topoisomerase IV. DNA gyrase localized in front of DNA replication forks so the formation of quinolone- gyrase-DNA complex occurs very quickly leading to inhibition of DNA synthesis. In contrast, topoisomerase IV will be localized behind the DNA replication forks thus it will not collide with the replication fork until subsequent DNA replication cycle **(Hooper, 2001**).

2.4 Pharmacokinetic properties

Kaartinen *et al*. (1995) reported that volume of distribution (V_d) was estimated as 1 L/kg and total clearance 0.81L/kg/h following administration of enrofloxacin @5mg/kg b wt in lactating cows. Mean absorption time (MAT) for both i.m. and s.c. administration was 7h. Bioavailability (F) after i.m. and s.c. administration were 70% and 100%, respectively. Long MAT and mean residence time (MRT) indicated the presence of strong flip-flop phenomenon after slow absorption from the site of injection which reduced the rate of elimination. About 23% of enrofloxacin was metabolized to ciprofloxacin based on AUC values of both agents after i.v. injection.

Rahal *et al.* (2006) reported that V_d for enrofloxacin was about 2.97 L/kg in sheep and goats after administering enrofloxacin @ 5mg/kg intramuscularly. Half life of enrofloxacin ranged from 89 to 169 minutes in mice and cows respectively. Volume of distribution was large in all species studied, with values ranging from 1.5 L/kg in sheep to10.5 L/kg in mice. Body clearance ranged from 68.1 ml/min/kg for mice to 4.6 ml/min/kg for sheep **(Bregante *et al*., 1999).**

Table 2.1: Pharmacokinetics properties of enrofloxacin and ciprofloxacin (Kaartinen *et al.,* 1995)

Enrofloxacin	**Intravenous route**	**Intramuscular route**	**Subcutaneous route**
AUC (mg/l ×h)	6.37±0.45	4.46±0.33	8.28±0.99
MRT (h)	0.77±0.11	7.83±1.77	7.82±1.57
C_{max} (mg/l)		0.70±0.07	0.91±0.09
T_{max} (mg/l)		1.31±0.30	1.25±0.33
$T_{1/2(\beta)}$ (h)	0.74±0.11	6.99±2.41	5.86±1.40
Ciprofloxacin			
AUC (mg/l ×h)	1.48±0.22	1.37±0.25	3.17±0.47
MRT (h)	1.27±0.09	7.90±2.24	12.05±1.95
C_{max} (mg/l)	1.24±0.09	0.14±0.03	0.21±0.01
T_{max} (mg/l)	0.12±0.01	2.00±0.55	3.20±0.49
$T_{1/2(\beta)}$ (h)	0.94±0.22	5.98±0.85	7.61±1.24

2.4.1 Absorption

Bioavailability of enrofloxacin varies from 10% to 80% in both polygastric and monogastric animals, respectively, after oral administration **(Nielsen and Gyrd-Hansen, 1997),** resulting in oral formulations of enrofloxacin and ciprofloxacin that are being used in case of pigs, poultry, carnivores and calves and only injectable formulations in cattle. Bioavailability mainly depends on animal feeding status whether animals are in full stomach or empty stomach **(Steinman *et al*., 2006).** It also depends on the presence or absence of ion, because formation of a complex between cation and fluoroquinolones makes them less permeable through the digestive barrier **(Ziółkowski *et al*., 2014).** Usage of hard water in case poultry to dilute the drug and feeding for birds also reduces the bioavailability **(Sumano *et al*., 2004).** Lipophilic compounds

tend to increase the per oral bioavailability of fluoroquinolones **(Coulet *et al*., 2005).** Active transport has an important role in intestinal absorption, even though enrofloxacin lipophilicity follows passive diffusion **(Dautrey *et al*., 1999).** Thus both active and passive transports play role in excretion of fluoroquinolones. After 3 hours of i.m. administration enrofloxacin reached 96% of bioavailability. Solid lipophilic nanoparticles enhance intramuscular bioavailability of enrofloxacin and this enhances the duration of retention of enrofloxacin in plasma **(Fauchier, 2013).**

2.4.2 Distribution

Enrofloxacin and ciprofloxacin preferably diffuses well into tissues and blood and distribution mainly depends on amount of unbound drug. Binding of drug to proteins and amount protein present in the blood determines the free drug concentration.

Table 2.2: The difference in protein binding between ciprofloxacin and enrofloxacin

Species	**Protein binding of enrofloxacin (%)**	**Protein binding of ciprofloxacin (%)**
Dogs	34	18
Dairy Cows	59.4	33.7
Steers	60	49.6
Chicken	23	Nd
Pigs	31.1-37.1	35

The above table reflects the role of enrofloxacin as a prodrug. As in some species, ciprofloxacin does not bind to proteins completely and is available freely to produce effective action. Enrofloxacin interaction with protein binding drugs like flumixin meglumine increases its clearance and shortens its antibacterial action **(Ogino *et al*., 2005).**

Tessa and Lefebvre (2016) reported that volumes of distribution of enrofloxacin and ciprofloxacin were more than 1 in all the species (Table 2.4), which reflects their prompt diffusion into tissues and cells.

2.4.3 Metabolism

After administration, the active metabolite enrofloxacin is converted to ciprofloxacin by de-ethylation of the ethyl on the piperazine ring **(Tyczkowska *et al*., 1989)**. Enrofloxacin is also converted into other metabolites but they don't have any therapeutic importance **(Papich and Riviere, 2009).** In most of the species, major part of enrofloxacin is metabolized into ciprofloxacin except poultry as only a small part is converted into ciprofloxacin metabolite and only 7% of enrofloxacin is converted to a metabolite in the first hepatic pass **(Cester and Toutain, 1997).**

Pasquali and Manfreda (2007) reported that ciprofloxacin was more potent than enrofloxacin in antibacterial action.

2.4.4 Elimination

Inter species difference can be seen in the elimination of enrofloxacin and ciprofloxacin. It is highly evident in pigs as half life for ciprofloxacin was 2.6 h and for enrofloxacin, it was 26 h **(Nouws *et al*., 1988**). Ciprofloxacin eliminated five times faster than the enrofloxacin in chickens. This differences might be attributed to a different of route of elimination in different species.

Hwang *et al*. (2009) reported that enrofloxacin is mainly excreted through kidneys and proved in rat by nephrectomy and in goat with probenecid **(Rao *et al*., 2002**). Probenecid reduced the renal excretion of fluoroquinolones **(Neuman, 1986).** On the other hand, ciprofloxacin was eliminated by both renal and hepatic routes **(Martinez *et al*., 2006**). For both molecules, there is an intestinal recirculation *via* the bile excretion, however, no significant difference was observed in the concentration of enrofloxacin in the intestinal content after two hours of administration by both oral and intramuscular routes **(Wiuff *et al.,* 2002).** In comparison with steers, lactating dairy cows showed two fold increase in clearance of enrofloxacin and ciprofloxacin which was attributed to the phenomenon of ion trapping **(Idowu *et al*., 2010).**

Table 2.3 Pharmacokinetic parameters of enrofloxacin and its metabolite ciprofloxacin in different species.

Species	t ½ (h)		Cl mL/min/kg		V_d L/kg	
	Enro	Cipro	Enro	Cipro	Enro	Cipro
Dogs	2.3	2.8	12.16	7.8	2.45	1.92
Dairy cows	3.69	2.96	24.16	ND	1.56	ND
Steers	5.5	7.60	11.6	ND	1.59	ND
Chickens	6.99	3.11	3.30	15.45	1.98	4.04
Pigs	26.6	2.60	3.0	17.30	6.40	3.80
Goats	1.39	1.82	22.18	19.59	1.27	3.33

t ½ =elimination half-live, Cl=clearance, Vd=volume of distribution at the steady state. Enro: enrofloxacin, Cipro: ciprofloxacin.

2.5 Clinical Uses

Fluoroquinolones are considered as broad spectrum antimicrobials which are used to treat infection of different body systems. Enrofloxacin and ciprofloxacin are used in wide range of local and systemic infections caused by both Gram-negative and Gram-positive bacteria **(CVMP, 2007).** Enrofloxacin is very effective against bacteria's causing urinary, digestive, genital, joint, mammary and dermal infections but it is most indicated in case of respiratory infections **(Fauchier, 2013).**

2.6 Adverse effects

Fluoroquinolones are very safe as compared to other antibacterials with only a few adverse effects **(Aral *et al*., 2008).** Gastro intestinal disturbances such as nausea, vomiting, diarrhea are the common adverse effects of fluoroquinolones **(Westropp *et al*., 2012).**

Hooper and Wolfson (1985) reported mild gastrointestinal symptoms like nausea, vomiting, anorexia and central nervous system symptoms like lightheadedness, headache, drowsiness, insomnia which disappeared after termination of the therapy.

Fauchier (2013) reported inflammatory signs in pigs after administration of enrofloxacin at the site of injection**.** The most common and serious adverse effects of quinolones targeted reproductive system, ocular system, central nervous system and juvenile joints and cartilage. **Hayem (1994)** reported tendonitis, articular cartilage degeneration, arthropathy and various tendon injuries in young animals after enrofloxacin therapy. Quinolone related arthropathy has been shown by all the fluoroquinolones **(Yabe *et al.,* 2004). Maślanka *et al*. (2009)** reported that quinolones induced arthropathy was less seen in birds as compared to mammals.

Al-Nazawi (2008) reported that enrofloxacin at therapeutical concentration (10, 20 and 40mg/kg b wt) does not affect parameters such as the weight of testes, wattles and combs, sperm motility, and the testicular concentration of testosterone, ascorbic acid, total protein and cholesterol. **Aral *et al*. (2008)** reported that @ 150mg/kg b wt dose enrofloxacin damaged the structural integrity of testicular tissue leading to the disruption in spermatogenesis with content, motility and morphology of sperm being affected.

Gelatt *et al.* (2001) reported retinal degeneration and blindness in cats following administration of enrofloxacin at more than 5mg.kg b wt p.o. Side effects such as hypersalivation, anorexia and lethargy were also observed in dogs following administration of enrofloxacin @ 18-20mg/kg b wt p.o. for 3 days **(Westropp *et al*., 2012).**

2.7 Effect on haemato-biochemical profile

After ciprofloxacin administration @ 25mg/kg orally for 14 days in dogs, PCV, hemoglobin, RBC, WBC counts were decreased initially for a week then subsequently increased above the baseline. Lymphocyte and neutrophil counts decreased significantly which gradually returned to normal after 5 days. On contrary monocytes and eosinophil count increased initially which subsequently declined after 7 days. Mean platelet count was also initially decreased between day 0 and day 5 but returned to baseline levels by 7^{th} day of the study **(Oridupa *et al*., 2013)**.

Pourabbas and Feizi (2015) reported that there was no change in TLC and DLC. However the low percentage of heterophil was observed in poultry.

Durgut *et al*. (2016) reported that there was a significant increase in MCHC and decline in MCV at 30 min which increased after 60 min post administration of 50mg/kg bwt of enrofloxacin in the rabbits. AST, LDH, and CK enzyme activities and cardiac troponins were not altered after treatment of enrofloxacin in the rabbits. This suggests that enrofloxacin in rabbits did not have adverse effect on enzyme activities at the dose rate of 50mg/kg b wt.

2.8 Effect on oxidative stress

Oxidative stress is defined as an imbalance between oxidant and antioxidant levels **(Lykkesfeldt and Svendsen, 2007).**

Rawi *et al*. (2011) reported increased Lipid peroxidation (LPO) levels throughout the experimental periods of 3, 7 and 14 days after the oral administration of ciprofloxacin @ 80mg/kg b wt in rats as compared to the control values. Superoxide dismutase (SOD) levels also increased significantly after treatment with ciprofloxacin in rats.

Veerareddy (2011) reported the significant increase in lipid peroxidase levels at different days after 500mg of ciprofloxacin tablets administration in humans, which almost doubled from day 1 to day 5. SOD decrease up to 73.3% and glutathione also decreased to 25.5% level after repeated administration of ciprofloxacin for 5 days. There was significant decrease in plasma antioxidant status (DPPH) as compared to levels before initiation of therapy.

Barski *et al*. (2011) observed no significant changes in the levels of SOD and catalase in rats following oral administration of enrofloxacin @5mg/kg b wt. **Yazar and Tras (2001)** administered enrofloxacin @ 10mg/kg b wt SC in mice and observed significant increase in SOD activity in liver and decline of GSH in liver. Decreased activity of catalase in liver and GSH in RBCs were observed in chickens following oral administration of enrofloxacin @ 10mg/kg b wt (**Benzer *et al*., 2009).**

2.9 Toxicity

Only vomiting and anorexia was seen in dogs which received ten times the standard dose 400mg of enrofloxacin twice daily for at least 14 days, but dogs fed with 25 times the standard dose for 11 days showed the lethal effect **(Wolfson and Hooper, 1985).**

Altreuther (1987) studied that higher doses of enrofloxacin had an impact on joints in rats, histologically vesicles developed in articular cartilage, which progressively ruptured leading to erosions in cartilage. **Bauditz (1987)** reported the same effect in dogs to cause cartilage erosion**.**

In addition to retinal degeneration blurred vision and permanent blindness had been reported by **Brown, (1996)** in fluoroquinolone eye formulations which contain more than 0.5% concentration.

Fluoroquinolones exert some toxic effects on the cardiovascular, gastrointestinal, and central nervous systems and they are also responsible for reproductive and developmental toxicity, chondrotoxicity, genotoxicity, carcinogenicity and phototoxicity **(Christ *et al*., 1988; Stahlmann and Lode, 1998).** Photocarinogenecity of ciprofloxacin and other fluoroquinolones are tested *in vivo* in mice and proven positively by exposing mice to UV radiation after ciprofloxacin therapy **(Klecak *et al*., 1997).**

Altreuther (1987) reported that there was no evidence of teratogenic effects in 6 to 15 pregnant rats after daily treatment of enrofloxacin @ 875mg/kg. However reduction in fetal weights and litter size was noticed after termination of pregnancy. Development of crystalluria was observed after high doses of fluoroquinolones (1200 to 1400mg) in humans **(Swanson *et al*., 1983)**.

2.9.1 Arthropathy

Gough *et al*. (1985) and Ingham *et al*. (1977) reported administration of fluoroquinolones in young rats, mice, rabbits and dogs produced lesions in the cartilage of diarthrodial joints. It was observed that younger animals were more affected than adults. In 3-4 month old dogs, 30mg/kg b wt dose of ciprofloxacin caused damage to weight bearing joints after 7-28 days of administration and cartilages affected by these lesions were incapable of regeneration leading to arthropathia deformans in dogs.

2.9.2 Nephrotoxicity

Fluoroquinolones are not nephrotoxic but at higher doses, they have some adverse effects on renal system. **Sisca *et al*. (1984)** reported variable nephrotoxic potential of fluoroquinolones, in different species of animals. Interstitial nephritis, crystalluria, occult blood in urine, increased renal weight and decreased renal function are some of the adverse effects seen after administration of fluoroquinolones.

Thorsteinsson *et al*. (1986) reported ciprofloxacin @ 500 mg and 1000mg induced crystalluria at urine pH of 6.8 in humans. More severe cases of acute tubular necrosis have also been linked to fluoroquinolone therapy (**Dichiara *et al*., 2008).**

2.9.3 CNS toxicity

Fraser and Harrower (1977) reported various CNS reactions such as seizures, depression, anxiety, euphoria, somnolence and insomnia. Following administration of fleroxacin @ 800mg/kg b wt in mice convulsions were observed. In long-term studies of over one year administered with flumequine @ 50 mg/kg/day was associated with convulsions in a dose-dependent manner in adult beagle dogs **(Christ *et al*., 1988).**

2.9.4 Toxicity in male reproduction system

Long term toxicity studies with fluoroquinolones have reported testicular damage, testicular degeneration and impaired spermatogenesis. In these investigations, the drugs were administered orally @ 100 mg/kg b wt for a longer period of more than 3 months (**Caesar and Stille, 1984).**

2.9.5 Ecotoxicity

The environmental fate of ciprofloxacin is of raising importance as it has been found in wastewater, sewage treatment plants and hospital effluents, where it can be removed partially by adsorption **(Golet *et al.,* 2002, 2003; Lindberg *et al*., 2006),** however, its concentration decreases once it reaches surface water **(Golet *et al*., 2001).** Ciprofloxacin was not biodegraded by sewage bacteria, and may have toxic effects on microorganisms and continuous exposure lead to development of resistance (**Halling-Sørensen *et al*., 2000 and Kümmerer *et al*., 2001).**

2.9.6 Genotoxicity of enrofloxacin and ciprofloxacin

Ciprofloxacin was shown to possess inhibitory activity against the mammalian topoisomerase II which may results in genotoxic effects (**Herbold *et al*., 2001).**

Gürbay *et al.* (2002) showed that although the antibacterial activity of ciprofloxacin was attributed to inhibition of bacterial DNA topoisomerases, yet ciprofloxacin did not produce any cytotoxicity in human fibroblasts incubated with ciprofloxacin for 24h at different concentrations. However **Jun *et al.* (2003)** at a very low concentration of ciprofloxacin @ 2.5 µg/ml reported that Jurkat T cells showed apoptotic changes.

DNA gyrase is structurally and functionally similar to the topoisomerase II of eukaryotic cells. Therefore, due to this homology genotoxic potential of fluoroquinolones should also be assessed in eukaryotic organisms **(Wyckoff *et al*., 1989).**

Mukherjee *et al.* (1993) reported the findings of *in vivo* bone marrow chromosomal aberration assay after injecting ciprofloxacin at 0.6, 6 and 20 mg/kg concentrations intraperitonealy in mice. There was an increase in number of aberrant cells up to 10 at 6 and 20 mg/kg after 3, 6, 12 and 24 h after treatment. The level of chromosomal damage was comparable at all four sampling times.

Metronidazole and fluoroquinolones are the main antimicrobial agents which are responsible for genotoxic effects **(Simon and Stille, 1993 and Mersch-Sundermann *et al*., 1994). Al-Ahmad *et al.* (1999)** subjected ciprofloxacin to closed bottle test (CBT) in which ciprofloxacin failed to get biodegraded. In hospital effluents, ciprofloxacin residue level was detected at concentration between 0.7 and 124.5 µg/l. Hospital effluents enriched with ciprofloxacin were found positive for genotoxic effects measured with the umuC test **(Hartmann *et al*., 1999).**

Gibson *et al.* (1998) reported after treatment with nalidixic acid and ciprofloxacin, Chinese hamster ovary cells or Syrian hamster embryo cells showed cytotoxic effects by formation of micro nucleus. *In vitro* study with human lymphocytes treated with enrofloxacin and ciprofloxacin produced increase in the chromosomal aberrations such as fragmentation, chromosome breaks and gaps **(Gorla *et al*., 1999)**. Neonatal alterations in articulation cartilages, bone growth and tendons are some other effects caused by fluoroquinolones in both humans and animals **(Stahlmann, 2003; Lemus *et al*., 2009).**

The antibacterial activity of quinolones is due to their ability to inhibit bacterial DNA gyrase**.** However, their cross-reactivity with mammalian topoisomerase II leads to undesired toxicity in mammalian cells **(Von Rosentiel and Adam, 1994)**. The early quinolone drugs were narrow spectrum and easily developed resistance in bacteria with low serum concentrations, low potency and short half lives **(Von Rosentiel and Adam, 1994).** However, they had very minimal cytotoxic effects on mammalian DNA and cytotoxic effects were observed only at a concentration which was 1000 fold higher

than the bacteria **(Albertini *et al.,* 1995).**Newer class of quinolones developed by altering their chemical structure. This increased the chances of cross reactivity with topoisomerase II to occur in mammalian cell. The inhibition of mammalian topoisomerase II leads to DNA strand breaks during replication, condensation of chromosome and disjunction during mitosis, these resulting in genotoxicity **(Ralph *et al*., 1994 and Ferguson and Baguley, 1994)**. Fluoroquinolones are capable of inducing unscheduled DNA synthesis, structural chromosome aberrations, and micronucleus formation **(Ciaravino *et al.,* 1993 and Curry *et al.,* 1996).**

The antimicrobial activity and toxicity of quinolones were greatly improved after structural alterations of quinolones by substituting different moieties **(Domagala, 1994)**. This led to a development of new quinolones drugs with improved antibacterial spectrum, reduced development of resistance and reduced toxicity to humans. In addition to changing the moieties to improve activity and lessen toxicity, they were coupled with β-lactam, forming quinolonyl-lactams to increase the activity **(Demuth *et al*., 1991; Hamilton-Miller, 1994)**. Quinolonyl-lactams are multifunctional in activity, as they both inhibit bacterial DNA gyrase and bind to bacterial penicillin-binding proteins.

In vitro micronucleus assay was considered an appropriate method for comparing the genotoxicity of quinolones as they have been shown to induce both structural chromosome aberrations **(Curry *et al*., 1996)** and aneuploidy **(Ferguson and Whiteside, 1995).** Due to its ease of use and its ability to detect both structural chromosome breaks as well as chromosome loss *in vitro* micronucleus assay is gaining increased significance as a potential alternative to *in vitro* metaphase analysis assay (**Marzin, 1997, Kirsch-Volders, 1997 and Miller *et al*., 1997).**

Takayama *et al.* (1995) reported *in vitro* genotoxicity of ciprofloxacin with sister chromatid exchange and unscheduled DNA synthesis. **Pino (1995)** demonstrated *in vivo* genotoxicity of ciprofloxacin with micronucleus test and **Gorla *et al*. (1999)** reported the genotoxic effects of enrofloxacin and ciprofloxacin in chromosomal aberration test in human lymphocytes. Genotoxic effects of ciprofloxacin were also reported by **Mukarjee *et al*. (1993)** and **Basaran *et al*. (1993)** in rats. However, **Herbold *et al*. (2001)** did not observe chromosomal aberrations in patients treated with ciprofloxacin @ 400mg for 10 weeks.

Mamber *et al*. (1993) observed cytotoxicity, SOS response and arrest of DNA synthesis in the *Salmonella typhimurium* TA 102 after treatment with ciprofloxacin. These results are indicated the capacity of quinolones to inhibit bacterial enzyme gyrase, which is involved in DNA-replication.

Curry *et al*. (1996) reported that ciprofloxacin exhibited structural chromosome aberrations, which was evident by chromatid and chromosome breaks and exchanges. There was significant increase in percent of cells with structural chromosome aberrations following exposure of ciprofloxacin @ 200-600pg/ml concentration.

2.10 Assessment of genotoxicity

Heddle *et al.* (1981) reported the chromosomal aberrations are one of the important causes of bone marrow toxicity. Chromosomal effects like non disjunction, numerical aberrations like aneuploidy, polyploidy are rarely observed by chemical exposure, but the structural alterations in chromosome may be lethal to cell directly and may produce genotoxicity. Micronucleus test is one of the test which is very useful in detecting structural changes caused by chemical agent by utilizing polychromatic erythrocytes. Recent advances and changes made in the protocol of micronucleus test are very useful in detecting clastogens with a high success rate.

The *in vitro* micronucleus test is a genotoxicity test which detects the micronuclei in the cytoplasm of interphase cells. Micronucleus originates from acentric chromosome fragments or whole chromosomes that are unable to migrate to the poles during the anaphase stage of cell division **(OECD 2010)**. Thus, genetic damage becomes evident in the newly formed daughter cell **(Reifferscheid *et al*., 2008).**

Rishi and Grewa (1995) performed chromosome aberration test for dichlorovas toxicity using a fish model of *Channapunctatus* an ophlocephalid (2n = 32). The results were positive with many aberrations like chromatid gaps, centromeric gaps, separation of chromatids, sub-chromatid gaps, precocious and polyploidy.

Johnson *et al.* (1998) reported that there are mainly three major types of genotoxic effects: gene mutations, chromosomal aberrations and DNA effects. A battery of tests is required to assess genotoxicity, as no single *in vitro* assay is capable of detecting all three types. Chromosomal aberration tests and gene mutation tests

detect the actual lesions in the DNA, however cytotoxic effects on cell can be detected by measuring DNA damage.

Banu *et al.* (2001) reported that one of the best and effective methods to detect DNA damage caused by metals is alkaline comet assay. It is also called as single cell gel electrophoresis because the individual cell DNA migration patterns produced by this assay resembled with the image of comet having head and followed by a blaze of tail indicating the damaged DNA migration. In comet assay, the cells embedded in low melting agarose were laid in between two normal agarose layers on a microscopic slide (one served as bedding layer while the other one as covering layer). Cells were lysed by detergents and high concentration of salts in alkaline environment and DNA will be liberated and this was electrophoresed under alkaline conditions (pH>13). Cells with more DNA damage display the increased migration towards anode. DNA damage was quantitated by staining with syber green dye and calculating the tail moment per cell. The concept of tail moment (tail moment = a measure of tail length x a measure of DNA in the tail) as a metric was introduced by **Olive *et al.* (1990)** and expressed in arbitrary units.

Jena *et al.* (2002) reported that most human carcinogens are genotoxic in nature. Chemicals which induce mutations in various experimental models are of genotoxic importance and they conceivably affect the incidence of heritable mutations in man. Genotoxicity tests can be defined as i*n vitro* or *in vivo* tests designed to detect drugs, which can induce genetic damage directly or indirectly by various mechanisms of action. Bone marrow metaphase analysis detects the numerical and structural chromosomal aberrations such as breaks, gaps, deletion, exchanges and pulverization. Positive and negative controls are concurrently used with the test compound. Low doses of positive compounds are generally tested to detect the sensitivity of test system. Previous positive and negative control data provided these are homogenous increase the statistical significance of the test results.

Comet assay (single cell gel electrophoresis or SCGE) is used for the detection of both single and double DNA strand breaks **(Jena *et al.* 2002).** Comet assay has manifold applications in fundamental research for DNA damage and repair, in genotoxicity testing of novel chemicals and pharmaceuticals, environmental biomonitoring and human population monitoring **(Speit and Hartman, 2005).**

Bone marrow micronucleus test (MNT) has been most suitable genotoxic test along with simultaneous use of chromosomal aberration, peripheral blood micronucleus and sperm morphology test would give 100% accurate genotoxicity testing **(Rao and Narayana, 2005).**

2.11 Cyclophosphamide

Cyclophosphamide is a antimetabolite drug which is used in cancer chemotherapy. It is an alkylating agent of oxazaphosphorine group and damages the genetic material by attaching an alkyl group at N-7 to the guanine base of imidazole ring, thus producing DNA crosslikns. Cyclophosphamide is metabolized in liver by cytochrome P450 (CYP) enzymes to produce an active metabolite called as 4-hydroxy cyclophosphamide which has anti cancerous activity and can be used to treat autoimmune disorders.

2.11.1 Mechanism of action

Cells having the lowest concentration of aldehyde dehydrogenase (ALDH) produce metabolite phosphoramide mustard, which forms irreversible crosslinks between and within DNA at guanine N-7 positions leading to apoptosis **(Hall and Tilby, 1992).** Cyclophosphamide has little effects in cells which have higher concentration of ALDH such as bone marrow and liver. ALDHs converts aldophosphamide to carboxy-cyclophosphamide, a non toxic metabolite to cells and prevent the formation of phosphoramide mustard and carboxy-cyclophosphamide cannot be eliminated. This prevents the nitrogen mustard activation and subsequent alkylation to produce its anticancerous activity **(Emadi *et al.,* 2009).**

2.11.2 Pharmacokinetics

Cyclophosphamide after oral absorption rapidly metabolized to active metabolites in the liver by cytochrome P450 system **(Cohen and Jao, 1970).** 4-hydroxycyclophosphamide which is active metabolite exits in equilibrium with aldophosphamide. ALDH converts aldophosphamide to carboxy-cyclophosphamide, and trace amounts of aldophosphamide decompose to phosphoramide mustard and acrolein **(Boddy and Yule, 2000).** Active metabolites of cyclophosphamide are widely distributed all over the body, and may cross placenta and pass onto breast milk **(Wiernik and Duncan, 1971)**.

Haubitz *et al.* (2002) reported cyclophosphamide metabolites are excreted in the urine in unchanged form and this should be taken into consideration in patients with renal dysfunction while administering the drug. Drugs which induce the hepatic microsomal enzyme activity will accelerate the metabolism of cyclophosphamide, subsequently causing pharmacologic and toxic effects of the drug. Drugs like corticosteroids, tricyclic antidepressants or allopurinol inhibit hepatic microsomal enzymes leading to slower conversion of cyclophosphamide, resulting in its reduced therapeutic and toxic effects **(Donelli *et al.*, 1976).**

2.11.3 Genotoxicity due to cyclophosphamide

Cyclophosphamide targets DNA primarily to exert teratogenecity, mutagenicity and antineoplastic action and its toxic effects are seen in mammalian, somatic and germ cells **(Anderson *et al*., 1995).** It exerts its cytotoxic action by crosslinking of DNA, DNA-Protein crosslinks and intrastrand and interstrand cross links (**Enckson *et al*., 1980).** Cylcophosphaomide also causes G_o/G_1 and S phase arrest in cell cycle **(Isabella *et al*., 1977).**

Murata *et al*. (2004) reported cyclophosphamide and its metabolites induced damage to DNA which was evident by chromosome breaks, micronucleus formation and cell death. Cyclophosphomide causes chromosomal damage, sister chromatid exchanges and other genotoxic effects and is now considered as a well documented standard genotoxic compound **(Frank *et al*., 2005; Hosseinimehr *et al*., 2008).**

2.11.4 Clinical uses

Cyclophosphamide is an anticancerous drug which is mainly used in lymphoproliferative disorders and solid tumors and as an immunosuppressant in the treatment of autoimmune diseases such as nephritic syndrome, systemic lupus erythematosus and rheumatoid arthritis **(Morais *et al*., 1999).**

It is also used in bone marrow transplants and as well as organ transplants to suppress immune system **(Demirer *et al.*, 1996).** Clinically it can be used to treat wide range of cancers such as malignant lymphomas, myeloma, leukaemia, mycosis and breast carcinoma **(Kovarsky, 1983)**.

Materials and Methods

Chapter 3 MATERIALS AND METHODS

The study was undertaken for toxicological evaluation including genotoxicity of following oral administration of enrofloxacin and ciprofloxacin at 75mg/kg b wt and 150mg/kg b wt and 50mg/kg b wt and 100mg/kg b wt respectively for 28 days period.

3.1 Drugs and chemicals

Enrofloxacin and ciprofloxacin were procured from the local market with trade name Enrox® which contains 10% enrofloxacin solution, Ciprox® which contains 10% ciprofloxacin powder. Cyclophosphamide, colchicines and other chemicals of analytical grade were procured from Himedia Laboratories Pvt. Ltd.

3.2 Experiment and animals

The rats weighing around 250 gms of 6-8 weeks were procured from IVRI as per approval of IAEC. As per CPCSEA guidelines, for a period of seven days rats were kept in laboratory conditions for acclimatization and quarantine Rats were provided with standard pellet diet and deionized water *ad libitum* throughout the experiment. All the experimental animals were kept under constant observation during the entire period of study.

3.3 Experimental design

Thirty six Wistar rats were divided equally and randomly into six groups as given in the table. Enrofloxacin @ 75 and 150 mg/kg b wt and ciprofloxacin @ 50 and 100 mg/kg b wt were administered orally by gavage for the period of 28 days. Cyclophosphamide was used as a standard genotoxic drug and was given @ 20mg/kg b wt intraperitonially 24h before sacrifice.

Table 3.1: Experimental design for toxicological evaluation of enrofloxacin and ciprofloxacin with special reference to genotoxicity in rats.

Groups	Treatment	Dose/kg b wt	Days of administration
1	Control	1ml of distilled water p.o.	Once daily for 28 days
2	Positive Control	cyclophosphamide @20mg/kg b wt i.p	24 hrs before sacrifice
3	Enrofloxacin -1	75mg/kg b wt p.o	Once daily for 28 days
4	Enrofloxacin-2	150mg/kg b wt p.o	Once daily for 28 days
5	Ciprofloxacin-1	50mg/kg b wt p.o	Once daily for 28 days
6	Ciprofloxacin-2	100mg/kg b wt p.o	Once daily for 28 days

Animals were observed daily for clinical signs, behavioral changes and mortality. Samples were collected at the end of treatment and the study after 28 days.

3.4 Collection of blood samples

After completion of 28 days study rats were sacrificed humanely after anesthetizing with diethyl ether blood was collected from the tail vein by puncturing with the needle in sodium-EDTA vials for hematological examination and without sodium-EDTA vials for serum separation. Samples were stored at $4^{o}C$ for hematological and biochemical examination.

3.5 Collection of tissue samples

Rats were sacrificed after 28 days of trial by cervical dislocation. Different organs like spleen, kidney, brain, liver and heart were collected and blotted with tissue paper and weighed. Gross changes in the visceral organs were observed. Oxidative stress related parameters were determined by processing liver, spleen, kidney and heart. The tissues were stored at $-80^{0}C$ untill further processing. For histopathological examination, the above samples were fixed in 10% neutral buffered formalin.

3.6 Clinical signs

Rats of all groups were closely observed throughout the period of experiment twice a day for clinical signs and mortality if any for 28 days.

3.7 Body weights and organ weights

Body weights of each rat were recorded every week for 28 days. At the end of the experiment percent body weight gain was calculated. In addition to body weights, weights of the vital organs like liver, spleen and kidney were noted after collection and blotting the tissue with tissue paper. Relative organ weights (gm per 100 gm body weight) were calculated as mentioned below.

$$Relative\ organ\ weight(g/100gm) = \frac{Absolute\ organ\ weight}{Total\ body\ weight} \times 100$$

3.8 Hematological examination

The blood samples were analyzed immediately after collection for following hematological parameters, using standard laboratory procedures as described by **Jain (1986)**.

3.8.1 Hemoglobin (Hb)

Hemoglobin concentration was estimated using spectrophotometer by cyanomet-hemoglobin (Drabkin's) method and results were expressed in g/dl of blood.

3.8.2 Total erythrocytes count (TEC)

The total erythrocyte count was done using Hayem's fluid and results were expressed in million per micro liter ($x10^6$/µl).

3.8.3 Packed cell volume (PCV)

PCV was determined as per micro-hematocrit method and the results were expressed in percentage (%).

3.8.4 Erythrocytic indices

Following erythrocytic indices were calculated as per formula given below:

Mean corpuscular volume (MCV) = PCV X 10/TEC (expressed in femto liter)

Mean corpuscular hemoglobin (MCH) = Hb X 10/TEC (expressed in pictogram, pg)

Mean corpuscular hemoglobin concentration (MCHC) = Hb X 100/PCV (expressed in %)

3.8.5 Total leukocyte count (TLC)

TLC was estimated with the help of hemocytometer using Thomas diluting fluid and results were expressed as thousands per micro liter ($x10^3/\mu l$).

3.8.6 Differential leukocyte count (DLC)

Thin blood smear was made over a clean grease free glass slide using a drop of blood. The smear was air-dried and stained with Giemsa stain. The different leukocytes were counted by Battlefield method and values were expressed in percentage using blood cell counter.

3.9 Biochemical parameters

Biochemical parameters were analyzed after collection of serum after 28 days of study. Serum enzymes such as AST (Aspartate aminotransferase), ALT (Alanine aminotransferase) and ALP (Alkaline phosphatase) were determined by IFCC method using diagnostic kits (Erba Mannheim). Serum analytes such as total proteins (Biuret method), albumin (BCG Dye method), triglycerides (GPO-Trinder method), glucose (God-Pod method), creatinine (Jaffe's method), bilirubin (Diazo method), cholesterol (Chod-Pap method) and blood urea nitrogen (GLDH-Urease method) were estimated employing standard methods by UV-VIS spectrophotometer (Bio Rad, USA) using diagnostic kits (Erba Mannheim).

3.10 Antioxidative parameters

Oxidative stress in erythrocytes, liver, kidney, spleen and heart were estimated using UV- VIS Spectrophotometer (Bio Rad, USA).

3.10.1 Separation of RBCs

Preparation of RBC pellet was carried out by using heparinized blood which was subjected to centrifugation at 2000rpm for 15min. After discarding plasma and buffy coat erythrocyte pellet was obtained which was washed with 0.15 M NaCl. To prepare 33 % RBC solution, RBC pellet was dissolved in PBS (pH 7.4) which was used for lipid peroxidation and reduced glutathione. The 33 % RBC solution in PBS (pH 7.4) was stored at 4^0C till further analysis. Solution of 10 % RBCs in PBS was prepared using RBC pellet and used for estimation of catalase activity in RBC.

3.10.2 Lipid peroxidation (LPO)

LPO in RBCs expressed as nM.MDA/gram by measuring malondialdehyde (MDA) formation by the method of **Rehman** (**1984**).

Reagents

1. 10 % Trichloroacetic acid (TCA) solution: dissolved 10 g of trichloroacetic acid in 100 ml of distilled water.
2. 0.67% Thiobarbituric acid (TBA) was freshly prepared by dissolving 0.67 g of thiobarbituric acid in 100 ml warmed distilled water for dissolving TBA.

Procedure

1 ml of 10 % TCA was added to 1 ml of 33 % packed RBC and was mixed thoroughly. This mixture was centrifuged at 2000 rpm for 10 min. Supernatant was separated. 1 ml of supernatant was mixed with 1 ml of 0.67 % TBA and boiled for 10 min in water bath. It was cooled and diluted with 1ml of water. Blank was prepared by adding all the reagents except the packed RBCs. The OD was taken at 535 nm by the UV-VIS spectrophotometer (Bio Rad, USA).

Calculation

The concentration of LPO (nM.MDA) by applying molar extinction coefficient (EC) of MDA-TBA complex, 1.56 x 10^8 $M^{-1}cm^{-1}$ was determined by following formula.

$$LPO(nM.{}^{MDA}/_{ml}) = \frac{OD\ of\ test \times total\ volume\ of\ reaction\ mixture \times 10^9 \times DF \times IT}{EC \times volume\ of\ sample\ taken}$$

Where,

DF = dilution factor (10). IT =incubation time (2 hours), OD = optical density and EC = Extinction coefficient (1.56 x 10^8/ M/cm).

3.10.3 Reduced glutathione (GSH)

GSH was estimated by the method given by **Prins and Loos** (**1969**) using 5, 5' dithiobis (2-nitrobenzoic acid) (DTNB) and expressed as GSH (mM/mL).

Reagents

1. H_2SO_4-0.08 N in distilled water
2. Tungstate solution: containing 0.3 M Na_2WO_4 and 0.1 M EDTA
3. Tris buffer (pH 8.0): solution containing 1M tris-hydroxy methyl amino methane and adjusted with HCl to pH 8.0
4. DTNB reagent: mixture of 0.009 M Na_2HPO_4, 0.14 M NaCl, NaH_2PO_4, 0.000013 M and 40 mg/ 100 ml DTNB (freshly prepared).

Procedure

4 ml of 0.08 NH_2SO_4 was mixed with 0.2ml of packed erythrocytes (33 % dilution in PBS) carefully. After 10 min of incubation at room temperature, to clear the brown hemolysate and added 0.5 ml of tungstate solution to it and mixture was shaken for 5 min. The suspension was then centrifuged at 2000rpm for 15 min . Supernatant was separated and 2.5 ml of tris buffer was added to 2ml of supernatant. 0.2 ml of DTNB reagent was added to it and mixed well. The OD was recorded within a minute after the addition of DTNB reagent at 412 nm against blank in which 2 ml of distilled water was substituted for the supernatant.

Calculation

The concentration (mM GSH per ml of sample) was calculated was done by using the extinction co-efficient (EC = 13100/ M/cm) and results were expressed in.

$$GSH\left({}^{mM}/_{mL}\right) = \frac{OD \times Total\ volume\ of\ reaction\ mixture \times 1000 \times DF}{EC \times Volume\ of\ sample\ taken}$$

3.10.4 Superoxide dismutase (SOD)

SOD determined by using the method given by **Madesh and Balasubramanian (1998).** The principle behind the test is production of superoxide by auto oxidation of pyrogallol and subsequent inhibition of superoxide dependent reduction of MTT to its formazan, which was quantified at 570 nm. The reaction was stopped by the adding DMSO which helped to solubilize the formazan. The colour appeared was stable for several hours and was measured as SOD Units (amount (mg) of protein required to inhibit the MTT reduction by 50% corresponds to one unit of SOD).

Reagents

1) Pyrogallol (100 µM): For 100 ml solution, 0.12 mg pyrogallol was dissolved 10 ml of distilled water.
2) 2.58 mg MTT was dissolved in 5 ml to prepare 1.25 mM of distilled water.
3) Phosphate buffer saline (pH 7.4).

Procedure

Reagents added to be in sample, control and blank are given in the table.

The OD was taken at 570 nm by using distilled water as blank.

	Sample	**Control**	**Blank (Duplicate)**
PBS	0.65µl	0.65µl	0.65µl
MTT	30µl	30µl	30µl
Homogenate	10µl	-	-
Pyrogallol	75µl	75µl	75µl

The sample, control and blank were incubated for 5 min at room temperature.

DMSO	0.75ml	0.75ml	0.75ml
Homogenate	-	10µl	-

Calculation

Superoxide dismutase was expressed as SOD units/mg of Hemoglobin

$$Y\% = \frac{OD\ of\ Test}{OD\ of\ Control} \times 100$$

$$SOD(U/mg\ of\ Hb) = \frac{mg\ of\ Hb\ in\ 0.01ml\ packed\ RBC \times 50 \times DF}{Y}$$

Where,

Y = percent inhibition of MTT reduction by SOD protein

DF = dilution factor.

3.10.5 Catalase

Catalase was estimated in RBCs by the method suggested by **Aebi (1988)** using spectrophotometer and expressed as mM H_2O_2 utilized Min/mg/Hb.

Reagents

1. Phosphate buffer (50 mM, pH 7.0): 50 mM KH_2PO_4 and 50 mM Na_2HPO_4 were mixed in 1:1.5 (V/V) and the pH was adjusted to 7.0.

   (a) 50 mM KH_2PO_4 – 1.37 g in 200 ml

   (b) 50 mM Na_2HPO_4 – 1.42 g in 200 ml

2. H_2O_2 (10 mM): 0.1 ml of 30 % H_2O_2 was diluted to 100 ml distilled water.

Procedure

10 µl of RBC pack (1:10 dilution in PBS) was added to 2 ml of phosphate buffer and the contents were transferred to the cuvette. The OD was measured at every 30 seconds for 3 min at 240 nm wave length (UV-VIS Spectrophotometer) against water blank after adding 1 ml of H_2O_2 directly into the cuvette.

Calculation

The activity of catalase was expressed as mM H_2O_2 utilized Min/mg/Hb and calculated using the formula.

$$Catalse = \frac{\frac{\Delta OD}{Time} \times Total\ volume\ of\ reaction\ mixture}{0.067 \times Sample\ volume \times mg\ of\ Hb\ in\ 0.01ml\ of\ packed\ RBC}$$

3.11 Assessment of oxidative stress in tissues

Oxidative stress parameters were determined in different tissues like liver, kidney, spleen and heart. A double beam UV-VIS Spectrophotometer (Bio Rad, USA) was used for recording the absorbance of the test samples.

3.11.1 Preparation of liver, spleen, kidney, heart and brain homogenates

About 500 mg each of liver, kidney, spleen, heart and brain tissues of each animal were weighed and taken into 5 ml of ice-cold PBS (pH 7.4) and for estimation of reduced glutathione 200 mg of sample was weighed separately and transferred into two ml of 0.02 M ethylenediamine tetra acetic acid (EDTA) solution. The 10% tissue

homogenates were prepared with IKA homogenizer (Germany) under ice-cold water and centrifuged at 3000 rpm for 10 min. The supernatant was stored at -20^{0}C until further analysis of different oxidative stress related parameters.

3.11.2 Lipid peroxidation (LPO)

The tissue homogenate of various organs was centrifuged for 10 min at 3000 rpm. Supernatant was used for estimation of LPO. The method was followed similarly as for RBCs which was described previously. Reagent and procedure were same taking tissue homogenate in place of RBC pellet.

3.11.3 Reduced glutathione (GSH)

The level of GSH was determined by employing DTNB method suggested by **Sedlak and Lindsay** (**1968**) through estimating free-SH radicals and expressed as (nM/gram of wet tissue). For GSH, 10 % homogenates were prepared in 0.02 M EDTA.

Reagents

1. DTNB (0.01 M): dissolve 99 mg of DTNB in 25 ml of methanol
2. 1 M tris buffer (pH 8.9): 121 g of tris base in 950 ml of distilled water and make up the volume to 1000 ml.
3. TCA (50 %): 50 g of TCA in 100 ml distilled water.

Procedure

To 1 ml of tissue homogenate, 0.2 ml of TCA and 0.8 ml of distilled water were added and kept for incubation for 15min at room temperature. Then, the mixture was centrifuged for 15 min at 3000 rpm. Supernatant was separated and 0.8 ml of tris buffer (1 M) was added followed by 0.2 ml DTNB (0.01 M). The absorbance was recorded at 412 nm within 5 min. For blank, there was no sample added whereas DTNB was not added to sample blank

Calculation

The concentration of GSH (nM of GSH/gram of wet tissue.) was calculated by using the formula:

$$GSH(mM/g) = \frac{OD \times Total\ vloume\ of\ reaction\ mixture(1.4ml) \times 1000 \times DF(3)}{EC \times Volume\ of\ sample\ taken(0.2ml)}$$

3.11.4 Superoxide dismutase (SOD)

SOD was estimated by employing the method **Madesh and Balasubramanian (1998)** and it corresponds to mg of protein required to inhibit the MTT reduction by 50%. Reagent and procedure were same as described for the estimation of SOD in the previous experiment and tissue homogenate was used in place of RBC pellet

Calculation

Superoxide dismutase was expressed as SOD units/mg of protein

$$Y\ \% = \frac{OD\ of\ test}{OD\ of\ control} \times 100$$

$$SOD(U/mg\ of\ protein) = \frac{mg\ of\ protein\ Hb\ in\ 0.01ml\ tissue\ homogenate\ \times 50 \times DF}{Y}$$

Where,

Y is % inhibition of MTT reduction by SOD protein and DF = dilution factor

3.11.5 Catalase (CAT)

The enzyme catalase (CAT) converts H_2O_2 into H_2O. The catalase activity in tissue supernatant was measured by taking OD at 240 nm and calculating the rate of degradation of H_2O_2 which serve as the substrate of CAT **(Aebi, 1988)** and expressed as mmole H_2O_2 utilized/min/mg protein it was followed similarly as in for RBCs.

Calculation

The activity of catalase was expressed as mmole H_2O_2 utilized/min/mg protein and calculated by the following formula:

$$Catalase = \frac{\frac{\Delta OD}{Time} \times Total\ volume\ of\ reaction\ mixture}{0.067 \times Sample\ volume \times mg\ of\ protein\ in\ 0.01ml\ of\ homogentae}$$

3.12 Genotoxicity test procedures

Following tests are recommended by OECD for determination of genotoxic potential of the xenobiotics.

1. Chromosomal aberration and gene mutation tests to detect actual DNA damage
2. Bone marrow micronucleus test (MNT) is the most reliable test for genotoxicity testing.

3. The single cell gel electrophoresis (SCGE) assay, also known as the Comet assay is used to measure and analyze extent of DNA damage in cells. It require less resource as compared to conventional genotoxicity tests and evaluates DNA damage both qualitatively and quantitatively.

These methods are accepted as the standard methods to evaluate the xenobiotics induced genotoxic effect.

3.12. 1 Chromosomal aberration test

Chromosomal aberration assay was carried out in bone marrow cells as per the methods suggested by **Malhi and Grover (1987)** and **Chauhan *et al.* (2000).**

Reagents -

1) Hank's balanced salt solution (HBSS): Dissolved 9.8 g of HBSS in 1 liter of distilled water and pH was adjusted to 7.2.

2) Potassium chloride KCl (KCl; 0.56% w/v) solution: Dissolved 0.56 g of KCl in 100 ml of distilled water.

3) Carnoy's fixative: Methanol and glacial acetic acid were mixed in the ratio 3:1.

4) Phosphate buffer (pH 6.8): 56.86 g of Na_2HPO_4 was dissolved in 500 ml distilled water (solution A). 43.31g of KH_2 PO_4 was dissolved in 500-ml distilled water (solution B). Five ml of each of solution A and B were mixed and made up the volume to a 1 liter using distilled water. The pH was adjusted to 6.8.

5) Giemsa stock solution: Giemsa stain powder (1 g) was added in 66 ml glycerol and kept for 3 h at 55° to 60°C. After cooling up to room temperature, 66ml methanol was added and stain was stored as stock solution.

6) Giemsa working solution: It was prepared by mixing 2 ml of stock Giemsa solution with 3 ml phosphate buffer in 95 ml distilled water (pH 6.8).

To arrest mitosis process, 2 hours prior to sacrifice of animals, colchicine was injected at the dose rate of 4 mg/kg, intraperitoneally. After sacrificing the rats as per standard protocol, skin and muscle were removed to collect both femurs. Using bone snips femur bones were cut at the both the ends. Bone marrow washings were collected with HBSS (pH 7.2). Collected washings were centrifuged for 10 min at 1000 rpm. Supernatant discarded and bone marrow pellet was resuspended in hypotonic solution

of 0.56 % (w/v) KCl for 30 min to permit osmotic swelling of cells at 37°C. Supernatant was discarded and cells were centrifuged for 10 min at 1000 rpm and the pellets were resuspended in chilled Carnoy's fixative.

Cells were subjected to centrifugation for 10 min at 1000 rpm after 2 h of fixation, and resuspended in the same fixative for 24 h. From a height of about 2-2.5 feet fixed cells were dropped on chilled slides. The slides were air dried for 24 h, stained with 2% Giemsa stain for 15-20 min, dehydrated for five min in acetone: xylene (1:1) and for pure xylene again for five min. For scoring, fifty well spread metaphase plates per rat were observed and classified for chromosomal aberration.

3.12.2 Bone marrow micronucleus test

Micronuclei assay was carried out in bone marrow cells by the method suggested by **Hayashi *et al.* (1983)** and **Chauhan *et al.* (2000).**

Reagents-

1) Hank's balanced salt solution (HBSS): dissolved 980 mg of HBSS, 1g of BSA and 150 mg of EDTA in 100 ml of distilled water and pH was adjusted to 7.2.
2) Phosphate buffer (pH 6.8): 0.92 g of NaH_2PO_4 was dissolved in 100 ml distilled water (solution A). 0.95 g of Na_2HPO_4 was dissolved in 100-ml distilled water (solution B). 51 ml of solution A and 49 ml of solution B were mixed and pH was adjusted to 6.8.
3) Giemsa working solution (5% v/v): It was prepared by mixing 3.5 ml of stock Giemsa solution with 66.5 ml phosphate buffer.
4) May Grunewald stain: 500mg of May Grunewald stain powder was mixed thoroughly in 200 ml of methanol and filtered before use.

Bone marrow washings as collected for micronucleus assay were centrifuged at 1000 rpm for 10 min to obtain single cell suspension by discarding supernatant and the cells in the sediment were mixed carefully. A drop of cell suspension was taken and smeared on a clean, grease free glass slide. Smears were fixed using methanol for 5 min and stained with a combination of undiluted and diluted May Grunewald Stain for 5 min each.

Slides were stained with 5% Giemsa after washing in distilled water for 10 min. Slides were air dried and 2000 polychromatic erythrocytes (PCEs) per animal were scored to determine micronucleus frequencies and 1000 erythrocytes were examined to calculate the ratio of PCEs to normochromatic erythrocytes (NCEs).

3.12.3 Comet assay

The single cell gel electrophoresis (SCGE) / comet assay was carried out in blood samples of rats by method suggested by **Singh *et al,* (1988)** and T**ice *et al.* (2000).**

Reagents-

1. Phosphate buffer saline:

80 g of NaCl, 2 g of KCl, 2 g of KH_2PO_4, 29 g of NA_2HPO_4. 7 H_2O were dissolved in 900 ml distilled and pH adjusted to 7.4. Solution was autoclaved after adjusting volume to 1000 and stored.

2. Lysing solution

2.5 M NaCl 146.1 g, 100 mM EDTA 37.2 g, 10 mM trizma base 1.2 g and 8 g NaOH were dissolved in 700ml distilled water. After adjusting pH to 10, using concentrated HCl or NaOH total volume was made to 1000 ml with dH_2O and stored at room temperature.

Final lysing solution was prepared by adding fresh 1% Triton X-100 and 10% DMSO, and then refrigerated at 4°C before use.

3. Electrophoresis buffer (300 nM NaOH / 1 mM EDTA)

Stock solutions for electrophoresis buffer were prepared as follows.

I. 10N NaOH : 200 g NaOH dissolved in 500 ml distilled water.

II. 200 mM EDTA (14.89 g / 200 ml distilled water, pH=10)

Final electrophoresis buffer was made fresh by adding 30 ml of 10N NaOH to 5 ml of 200mM EDTA and final volume made to 1000 ml with distilled water. Prior to use pH of the buffer was ensured to be more than 13.

4. Neutralization buffer (0.4 M Tris)

48.5 g Tris was dissolved in 800 ml distilled water pH was adjusted to 7.5 with concentrated HC1 and final volume was made to 1000 ml with distilled water and stored at room temperature.

5. Staining solution

Sybergreen of 10X was prepared by dissolving 10 mg in 1000 ml distilled water and stored at room temperature. For working 1 x solution, 1 ml of 10X stock was mixed with 9 ml distilled water.

6. 1 % Low melting point agarose (LMPA)

1g of LMPA was taken in 100 ml PBS, heated in a microwave till boiling and agarose was dissolved.

7. 0.5 % Low melting point agarose (LMPA)

0.5g of LMPA was taken in 100 ml PBS, heated in a microwave till boiling and agarose was dissolved.

8. 1.0 % Normal melting agarose (NMA)

1g of NMA was taken in 100 ml mili water, heated in a microwave till boiling and agarose dissolved.

1) Preparation of base slides

Conventional one end frosted slides were dipped in methanol and burnt on blue flame to clean. 1% NMA was melted and slide was dipped in hot NMA up to one third the frosted area and removed gently. Underside was wiped off with lint free tissue paper to remove agarose. Slides were laid on flat surface to dry and were stored at room temperature in dust free environment avoiding high humid condition. They were prepared one day before use.

2) Viability Assay

10 µl of blood was taken in micro centrifuge tube and 10µl of 0.1% Trypan blue dye was gently mixed with it. Mixture was allowed to stand for at least two minutes and then placed on the slide and cover slip was applied. 100 cells were scored per blood sample and number of viable cells (shiny) and dead cells (blue) were recorded.

3) Preparation of slides:

1% LMPA was weighed and prepared in phosphate buffer saline. LMPA was put in to microwave till it boiled and then placed in water bath to maintain a steady temperature of 37°C. 100 µl of LMPA was mixed gently with same amount of whole blood and 80 ul of suspension was layered on the base slide in two replicates and cover slips were placed on it. These slides were put on ice pack until agarose solidified (5-10 min). 0.5% LMPA was prepared in PBS and maintained at 37°C. Once agarose solidified, cover slip was gently taken off and 80 µl of 0.5% LMPA was added to the slide. Cover slip was replaced and slides were again put on the ice pack for solidification of agarose.

4) Lysing and electrophoresis:

Cover slips were removed and slides were slowly lowered in to opaque coupling jars containing cold, freshly made lysing solution in order to avoid exposure to light. Slides were kept in lysing solution for minimum for 2 h.

But for optimum lysis, they were kept in lysing solution overnight and refrigerated at 4°C. After lysis slides were gently removed from lysing solution and placed side by side in gel box with agarose ends towards anode and frosted ends towards cathode. Electrophoresis gel apparatus was filled with freshly prepared cold electrophoresis buffer (pH>13) until the liquid level completely covered the slides. Slides were allowed to be in alkaline buffer for 20 minutes for unwinding of DNA. After unwinding, electrophoresis was done by supplying power of 24 volts and current of 300 mAmps adjusted by raising or lowering the buffer level, for 30 minutes for optimum migration.

5) Neutralization

Slides were gently lifted and placed on dry tray, flooded with neutralization buffer (pH-7.5) and allowed to sit for 5 minutes. They were drained and procedure was repeated twice.

6) Staining

Slides were stained with 75µl of 1X SYBER green, and left for 5 minutes and excess stain was removed by dipping slides in chilled distilled water. The cover slip

was placed over it and slides were scored immediately by using fluorescent microscope.

Qualitative and quantitative extent of DNA damage in the cells was evaluated by visual scoring method by observing extent of DNA migration (tail length) and percentage of migrated DNA. 100 randomly selected cells were analyzed per sample.

3.13 Pathological examination

After the completion of experimental period of 28 days, all the rats from all the groups were euthanized humanely. All the rats were subjected to detailed post mortem examination and the gross lesions if any, in liver, spleen, kidney and brain were recorded. Representative samples were collected from these organs in 10 % buffered formalin. The samples were processed for routine histopathological examination by dehydrating in ascending grades of alcohol followed by clearing in xylene and then blocks are embedded in paraffin. Tissues were cut in 4-5 microns thick sections and stained in of tissues were cut and stained with hematoxyline followed by staining in eosin for histopathological examination **(Lillie, 1965)**.

3.14 Statistical analysis

The data was analyzed for statistical significance difference by graph pad prism version 7 employing ANOVA. Comparisons among the treated groups were made. Statistical difference between respective means for various parameters was evaluated using appropriate statistical tests at 5% level of significance **t**he data were analyzed .

Results and Discussion

Chapter 4 RESULTS AND DISCUSSION

The study was undertaken for evaluating toxicological potential including genotoxicity of enrofloxacin and ciprofloxacin @ 75mg/kg b wt and 150mg/kg b wt and 50mg/kg b wt and 100mg/kg b wt, respectively, following oral administration for 28 days in rats. Cyclophosphamide was used as a standard genotoxic compound and administered @ 20mg/kg b wt i.p 24h before sacrifice after 28 days of study.

4.1 Clinical signs

In the treatment groups clinical symptoms like weakness, lethargy and depression of mild intensity were observed in higher dose treatment groups as compared to control group I. The body weights (g) of rats were recorded at weekly intervals (Table 4.1 and Figure 4.1.) and there was no significant ($P<0.05$) difference in body weights among groups were observed at 7th, 14th, 21th and 28th days during the 28 day trial. There was non significant reduction in the body weight gain after administration of enrofloxacin @ 75mg/kg b wt and 150mg/kg b wt. Even groups administered with ciprofloxacin @ 75mg/kg b wt and 100mg/kg b wt showed non significant reduction in the body weight gain. However, **Mulligan (1997)** reported reduced body weight gain in males (20%) and females (23%) following enrofloxacin administration @ 550 mg/kg b wt in males and 690mg/kg b wt in females.

The absolute organ weight and relative organ weight were measured at the end of the 28 days of experiment (Table 4.3 & 4.4 and Fig 4.3 & 4.4.) There was significant ($P<0.05$) decrease in absolute and relative liver weights of cyclophosphamide treated group II as compared to group I (control). Group VI treated with ciprofloxacin @ 100mg/kg b wt and group IV treated with enrofloxacin @150mg/kg b wt showed significant ($P<0.05$) decrease in the organ weight of liver as compared to groups I and II. There was no change in organ weights of kidney, spleen, heart and brain. Except group IV all the treatment groups showed significant ($P<0.05$) decrease in relative organ weights of liver as compared to control group I. Significant ($P<0.05$) reduction in liver weight in this study might be due to toxic effect of enrofloxacin and ciprofloxacin on hepatic system at higher doses.

However, **Kashida *et al*. (2002)** reported an increase in both absolute and relative weight of liver following the administration fluroquinolones @ 40mg/kg b wt for 2 weeks.

4.2 Hematological parameters

The effect of enrofloxacin and ciprofloxacin on Hb (mg/dl) is presented in the Table 4.4 and Figure 4.4. Significant ($P<0.05$) reduction in Hb value was observed in higher dose group. There was nonsignificant ($P<0.05$) decrease in Hb in cyclophosphamide treated group II as compared to control group I. A significant ($P<0.05$) decrease was noted in all the treatment groups except group V as compared to control group I. The reduction in Hb level in treatment groups might have been due to their toxic effect on hematopoietic system including bone marrow which resulted in decreased biosynthesis of Hb **(Palma-Carlos *et al.*, 1971).** This drop in Hb level generally results from either direct toxicity or immune-mediated destruction of erythrocytes **(Blum *et al*., 1994).**

The effect of enrofloxacin and ciprofloxacin on PCV (%) value is presented in the Table 4.5 and Figure 4.5. Significant($P<0.05$) decline in PCV values in all the treatment groups as compared to group I (control) was observed after 28 days. The higher dose groups showed decrease in PCV values as compared to lower dose groups which showed a little decrease in PCV values. The reduction in PCV is indication of lower level of Hb observed in this study.

The effect of enrofloxacin and ciprofloxacin on total erythrocyte counts (TEC, $\times 10^6/\mu l$) are presented in the Table 4.6 and Figure 4.6. A significant ($P<0.05$) fall in TEC values was observed in all groups except enrofloxacin low dose group III as compared to control group I. Whereas **Oridupa *et al*. (2013)** reported the reduced red blood cell counts in dogs treated with 25mg/kg b wt ciprofloxacin for 14 days. **Packman (2001)** reported three main mechanisms responsible for drug mediated anemia. Firstly, hapten-drug adsorption mechanism, where drug binds to RBC membrane covalently leading to binding of anti-drug antibody to the drug resulting in destruction of cells by spleenic macrophages. Secondly, the formation of trimolecular complex which consists of the drug, RBC membrane antigen and antibody capable of recognizing the complex formed by drug and RBC membrane. Thirdly, the drug

Table 4.1: Effect on body weight (g) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Body weight (g)				
			0^{th} day	7^{th} day	14^{th} day	21^{th} day	28^{th} day
I.	Control	1 ml of distilled water, po	245.8±4.55	251.0±5.13	264.3±4.62	282.3±2.5	299.3±4.9
II.	CYP	20 mg/kg b wt, ip	224.3±6.66	231.7±6.66	240.7±6.54	257.8±8.5	288.0±5.1
III.	Enrofloxacin	75 mg/kg b wt, po	252.0±7.65	245.7±5.72	247.0±4.15	235.5±4.2	229.0±3.7
IV	Enrofloxacin	150 mg/kg b wt, po	246.3±4.59	240.7±4.63	242.0±2.91	220.0±5.3	204.3±2.49
V.	Ciprofloxacin	50 mg/kg b wt, po	262.3±7.60	240.0±8.16	234.3±7.82	233.3±3.2	215.8±2.6
VI.	Ciprofloxacin	100 mg/kg b wt, po	250.0±8.56	245.0±9.57	232.7±8.8	220.7±4.4	198.3±4.3

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide.

Table 4.2: Effect on organ weight (g) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Absolute organ weight (g)				
			Liver	**Kidney**	**Spleen**	**Brain**	**Heart**
I.	Control	1 ml of distilled water, po	12.5±0.55	2.54±0.14	0.96±0.04	1.75±0.03	1.95±0.09
II.	CYP	20 mg/kg b wt, ip	9.28±0.34[a]	1.84±0.52	0.85±0.02	1.62±0.08	1.05±0.01
III.	Enrofloxacin	75 mg/kg b wt, po	8.99±0.16[a]	1.84±0.85	0.98±0.08	1.45±0.02	1.05±0.04
IV	Enrofloxacin	150 mg/kg b wt, po	7.89±0.89[ab]	1.75±0.25	0.73±0.05	1.33±0.09	0.98±0.02
V.	Ciprofloxacin	50 mg/kg b wt, po	8.74±0.45[a]	1.52±0.05	0.64±0.01	1.23±0.04	0.95±0.06
VI.	Ciprofloxacin	100 mg/kg b wt, po	7.75±0.98[ab]	1.22±0.05	0.51±0.06	1.05±0.08	0.86±0.03

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide.

a =significant($P<0.05$)difference as compared to group I; b =significant($P<0.05$)difference as compared to group II

ANOVA Table	SS	df	MS
Treatment (between columns)	74.50	5	14.90
Residual (within columns)	18.30	30	0.6099
Total	92.80	35	

Table 4.3: Effect on relative organ weights (%) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Relative organ weight (%)				
			Liver	Kidney	Spleen	Brain	Heart
I.	Control	1 ml of distilled water, po	4.41±0.15	0.84±0.04	0.33±0.03	0.54±0.04	0.35±0.01
II.	CYP	20 mg/kg b wt, ip	4.10±0.44[a]	0.87±0.05	0.35±0.04	0.48±0.03	0.45±0.01
III.	Enrofloxacin	75 mg/kg b wt, po	3.74±0.06[a]	0.74±0.05	0.38±0.04	0.59±0.01	0.38±0.03
IV	Enrofloxacin	150 mg/kg b wt, po	4.36±0.83	0.71±0.08	0.23±0.02	0.48±0.05	0.38±0.02
V.	Ciprofloxacin	50 mg/kg b wt, po	3.85±0.15[a]	0.75±0.09	0.25±0.01	0.53±0.05	0.40±0.05
VI.	Ciprofloxacin	100 mg/kg b wt, po	4.05±0.81[a]	0.72±0.01	0.24±0.06	0.45±0.03	0.36±0.02

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide.

ANOVA Table	SS	df	MS
Treatment (between columns)	95.49	4	23.87
Residual (within columns)	4.498	25	0.1799
Total	99.98	29	

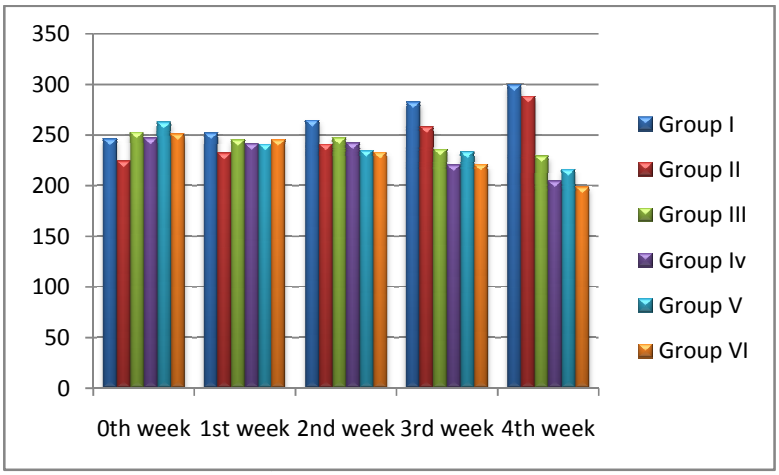


Figure 4.1 Effect on body weight (grams) following administration of enrofloxacin and ciprofloxacin for 28 day in rats.

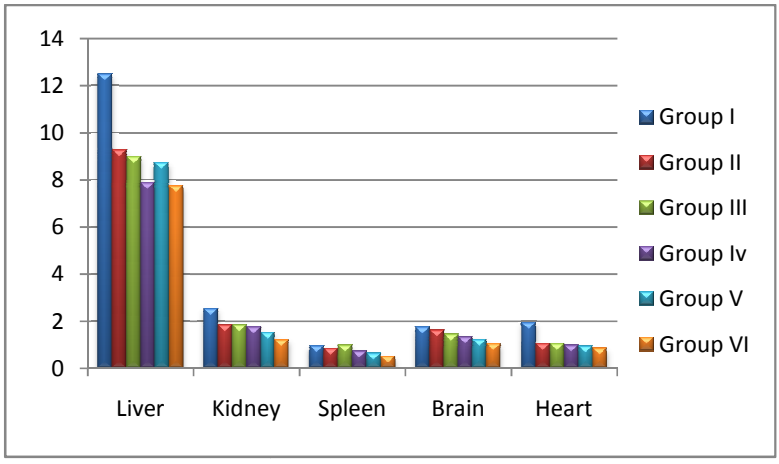


Figure 4.2 Effect on absolute organ weights (grams) following administration enrofloxacin and ciprofloxacin for 28 days in rats.

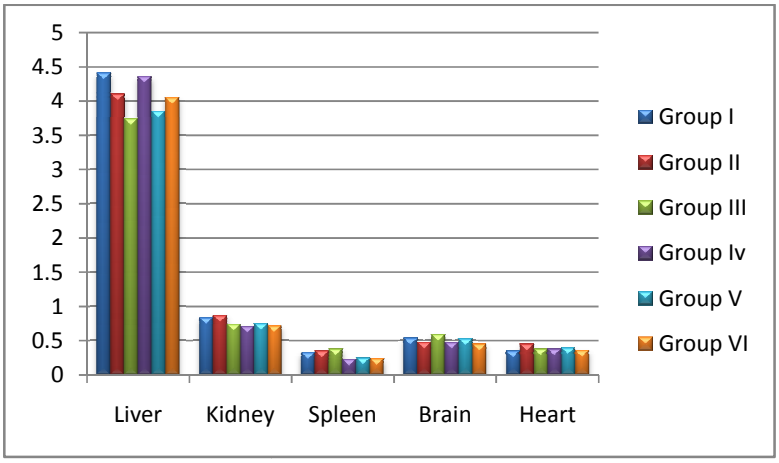


Figure 4.3 Effect on relative organ weights (grams/100 gm of tissue) following administration of enrofloxacin and ciprofloxacin for 28 day in rats.

induces the formation of true autoantibodies which are similar to those as seen in autoimmune disorders like hemolytic anemia **(Pierce and Nester, 2011)** with increased formation of lymphocytes. The third mechanism doesn't require the presence of a drug where as foresaid two mechanisms strictly require presence of drug in the site. Thus, the reduction in TEC might have occurred either due to direct hemolytic action of the drug or the drug induced auto immune disorder leading to anemia **(Packman, 2001).**

The effect of enrofloxacin and ciprofloxacin on mean corpuscular volume (MCV, femto litre) is presented in the Table 4.7 and Figure 4.7. Significant ($P<0.05$) increase was found in all the groups compared to control group I. An increase in group II can be noted as it was treated with cyclophosphamide with toxic dose. Significant ($P<0.05$) increase in MCV in rest of the treatment group can be attributed to the chemotherapy induced macrocytic anemia.

The effect of enrofloxacin and ciprofloxacin on mean corpuscular hemoglobin (MCH, picogram) is presented in the Table 4.8 and Figure 4.8. Significant ($P<0.05$) decrease in the MCH was evidenced in all the treatment groups as compared to control group I after 28 days. Group V showed significant ($P<0.05$) increase as compared to group III and IV.

The effect of enrofloxacin and ciprofloxacin on mean corpuscular hemoglobin concentration (MCHC %) is presented in the Table 4.9 and Figure 4.9. A significant ($P<0.05$) decrease in the percent of MCHC was observed in all the treatment groups as compared to both positive (group II) and negative control groups (group I) after 28 days. MCH and MCHC values are in agreement with the findings of Hb concentration in this study.

The effect of enrofloxacin and ciprofloxacin on total leukocyte count (TLC $\times 10^3/\mu l$) is presented in the Table 4.10 and Figure 4.10. A significant ($P<0.05$) decrease was seen in all the groups as compared to control group. Group II, treated with cyclophosphamide, showed a significant($P<0.05$) decrease in TLC as compared to control group after 28 days, which could be due to toxic effect of cyclophosphamide on proliferation and maturation of leukocytes.

The effect of enrofloxacin and ciprofloxacin on differential leukocyte count (DLC, %) is presented in the Table 4.10a. A significant (P<0.05) increase was found in lymphocyte count in all the treated groups from II to VI as compared to control group I, whereas percent neutophils and monocyte count showed significantly (P<0.05) lower count as compared to control groups I and II. In case of eosinophils, ciprofloxacin treated groups (V and VI) showed significantly (P<0.05) low count. Basophil count of higher dose groups of enrofloxacin and ciprofloxacin also showed significantly (P<0.05) low counts after 28 days. **Priyadarshini (2013)** reported reduction in various hematobiological values in rats after 400mg/kg bwt ciprofloxacin administration for 30 days. However, effects were reversed by concurrent administration of Vitamin A, E, and C and ciprofloxacin @ 150mg/kg also inhibited lymphocyte proliferation.

Thus, significant (P<0.05) reduction in different cell counts on DLC was observed in all treated groups (II to VI) after 28 days in the study indicating the direct hemotoxic effect at higher doses of antibiotics in rats. A significant (P<0.05) decrease in TLC, Hb, PCV, MCH and MCHC in cyclophosphamide treated group II indicated adverse effect of cyclophosphamide on hemopoietic organs. Due to impairment in heme synthesis, reduced Hb levels caused decrease in hematological parameters. This might be the result of increased destruction of RBC. TLC could be reduced due to suppression of bone marrows or by increased destruction of leukocytes.

4.3 Biochemical parameters

The effect of enrofloxacin and ciprofloxacin on total serum proteins (TSP, g/dl) concentration is presented in the Table 4.11 and Figure 4.11. There was a significant (P<0.05) reduction in all the treatment groups except III as compared to control group I after 28 days.

The effect of enrofloxacin and ciprofloxacin on albumin (g/dl) concentration is given in the Table 4.12 and Figure 4.12. There was significant (P<0.05) reduction in all the treatment groups except group III as compared to control group I after 28 days. The effect of enrofloxacin and ciprofloxacin on globulin (g/dl) concentration is presented in the Table 4.13 and Figure 4.13. A significant (P<0.05) reduction in globulin values was observed in all treated groups as compared to control group I after 28 days. There was a significant (P<0.05) increase in albumin: globulin ratio in all treated groups as compared to control group I after 28 days (Table 4.14 and Figure 4.14).

Table 4.4: Effect on Hemoglobin (g/dl) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Heamoglobin (g/dl)
			28th day
I.	Control	1 ml of distilled water, po	16.5±0.50
II.	CYP	20 mg/kg b wt, ip	14.92±1.24
III.	Enrofloxacin	75 mg/kg b wt, po	13.08±0.57[a]
IV	Enrofloxacin	150 mg/kg b wt, po	13.17±0.60[a]
V.	Ciprofloxacin	50 mg/kg b wt, po	13.28±0.44
VI.	Ciprofloxacin	100 mg/kg b wt, po	11.50±0.72[ab]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant(P<0.05)difference as compared to group I; b =significant(P<0.05)difference as compared to group II;

ANOVA Table	SS	df	MS
Treatment (between columns)	81.97	5	16.39
Residual (within columns)	88.83	30	2.961
Total	170.8	35	

Table 4.5:Effect on Packed cell volume (%) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	PCV (%)
			28th day
I.	Control	1 ml of distilled water, po	50.67±1.55
II.	CYP	20 mg/kg b wt, ip	45.33±1.47
III.	Enrofloxacin	75 mg/kg b wt, po	39.00±1.57[ab]
IV	Enrofloxacin	150 mg/kg b wt, po	37.67±0.88[ab]
V.	Ciprofloxacin	50 mg/kg b wt, po	38.30±1.37[ab]
VI.	Ciprofloxacin	100 mg/kg b wt, po	33.15±1.07[abc]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant (P<0.05) difference as compared to group I; b =significant (P<0.05) difference as compared to group II;
c =significant (P<0.05) difference as compared to group III.

ANOVA Table	SS	df	MS
Treatment (between columns)	1150	5	229.9
Residual (within columns)	232.3	29	8.010
Total	1382	34	

Table 4.6:Effect on total erythrocyte count ($\times 10^6/\mu l$) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	TEC($\times 10^6/\mu l$)
			28^{th} day
I.	Control	1 ml of distilled water, po	7.16±0.20
II.	CYP	20 mg/kg b wt, ip	6.11±0.38
III.	Enrofloxacin	75 mg/kg b wt, po	6.30±0.26
IV	Enrofloxacin	150 mg/kg b wt, po	5.95±0.17[a]
V.	Ciprofloxacin	50 mg/kg b wt, po	6.04±0.27[a]
VI.	Ciprofloxacin	100 mg/kg b wt, po	5.22±0.17[ac]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28^{th} day only.
a =significant (P<0.05) difference as compared to group I; c = significant (P<0.05) difference as compared to group VI.

ANOVA Table	SS	df	MS
Treatment (between columns)	10.34	5	2.068
Residual (within columns)	9.745	29	0.3360
Total	20.08	34	

Table 4.7: Effect on mean corpuscular volume (MCV, femto litre) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	MCV(femto litre)
			28th day
I.	Control	1 ml of distilled water, po	67.62±0.30
II.	CYP	20 mg/kg b wt, ip	82.36±0.51[a]
III.	Enrofloxacin	75 mg/kg b wt, po	66.46±0.26[ab]
IV	Enrofloxacin	150 mg/kg b wt, po	65.18±0.28[ab]
V.	Ciprofloxacin	50 mg/kg b wt, po	71.71±0.35[abcd]
VI.	Ciprofloxacin	100 mg/kg b wt, po	69.27±0.31[abcde]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant(P<0.05)difference as compared to group I; b =significant(P<0.05)difference as compared to group II;
c =significant(P<0.05)difference as compared to group III; d =significant(P<0.05)difference as compared to group IV;
e =significant(P<0.05)difference as compared to group V;

ANOVA Table	SS	df	MS
Treatment (between columns)	1197	5	239.4
Residual (within columns)	21.92	30	0.7307
Total	1219	35	

Table 4.8: Effect on mean corpuscular hemoglobin (MCH, picogram) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	MCH (picogram)
			28th day
I.	Control	1 ml of distilled water, po	24.70±0.16
II.	CYP	20 mg/kg b wt, ip	21.49±0.69[a]
III.	Enrofloxacin	75 mg/kg b wt, po	20.69±0.29[a]
IV	Enrofloxacin	150 mg/kg b wt, po	20.32±0.05[a]
V.	Ciprofloxacin	50 mg/kg b wt, po	22.70±0.41[acd]
VI.	Ciprofloxacin	100 mg/kg b wt, po	21.91±0.37[a]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant(P<0.05)difference as compared to group I; c =significant(P<0.05)difference as compared to group III;
d =significant(P<0.05)difference as compared to group IV.

ANOVA Table	SS	df	MS
Treatment (between columns)	76.06	5	15.21
Residual (within columns)	20.79	30	0.6931
Total	96.85	35	

Table 4.9: Effect on mean corpuscular hemoglobin concentration (MCHC, %) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	MCHC(%)
			28th day
I.	Control	1 ml of distilled water, po	36.12±0.24
II.	CYP	20 mg/kg b wt, ip	34.44±0.26[a]
III.	Enrofloxacin	75 mg/kg b wt, po	31.15±0.32[ab]
IV	Enrofloxacin	150 mg/kg b wt, po	30.60±0.35[ab]
V.	Ciprofloxacin	50 mg/kg b wt, po	30.82±0.24[ab]
VI.	Ciprofloxacin	100 mg/kg b wt, po	30.19±0.13[abc]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant(P<0.05)difference as compared to group I; b =significant(P<0.05)difference as compared to group II;
c =significant(P<0.05)difference as compared to group III.

ANOVA Table	SS	df	MS
Treatment (between columns)	175.9	5	35.19
Residual (within columns)	8.538	30	0.2846
Total	184.5	35	

Table 4.10: Effect on total leukocyte count (TLC, × 10^3/µl) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	TLC(× 10^3/µl)
			28th day
I.	Control	1 ml of distilled water, po	8.37±0.29
II.	CYP	20 mg/kg b wt, ip	7.51±0.28[a]
III.	Enrofloxacin	75 mg/kg b wt, po	7.56±0.25[a]
IV	Enrofloxacin	150 mg/kg b wt, po	7.33±0.14[a]
V.	Ciprofloxacin	50 mg/kg b wt, po	7.36±0.13[a]
VI.	Ciprofloxacin	100 mg/kg b wt, po	7.21±0.15[a]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant (P<0.05) difference as compared to group I.

ANOVA Table	SS	df	MS
Treatment (between columns)	4.185	5	0.8371
Residual (within columns)	3.563	30	0.1188
Total	7.748	35	

Table 4.10A: Effect on differential leukocyte count (DLC, %) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Differential Leukocyte count (%) 28th day				
			L	N	M	E	B
I.	Control	1 ml of distilled water, po	74.26±0.63	20.51±0.58	2.45±0.21	1.84±0.19	0.94±0.02
II.	CYP	20 mg/kg b wt, ip	74.68±0.54	19.65±0.18	2.97±0.25	2.25±0.27	0.45±0.05[a]
III.	Enrofloxacin	75 mg/kg b wt, po	79.95±0.15[ab]	15.28±0.52[ab]	2.04±0.40[ab]	1.85±0.05	0.88±0.07
IV	Enrofloxacin	150 mg/kg b wt, po	81.25±0.23[ab]	14.85±0.45[ab]	1.68±0.09[ab]	2.014±0.15	0.21±0.04[ac]
V.	Ciprofloxacin	50 mg/kg b wt, po	80.59±0.45[ab]	15.21±0.29[ab]	2.09±0.65[ab]	1.38±0.09[ab]	0.73±0.10
VI.	Ciprofloxacin	100 mg/kg b wt, po	83.56±1.08[ab]	13.25±0.89[ab]	1.69±0.26[ab]	1.16±0.21[ab]	0.44±0.03[b]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant (P<0.05)difference as compared to group I; b =significant(P<0.05)difference as compared to group II;

c =significant (P<0.05)difference as compared to group III.

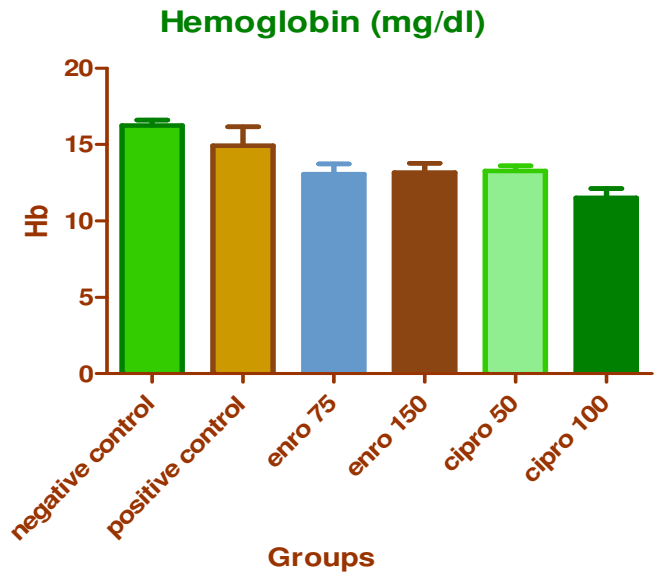


Figure 4.4 Effect on Hemoglobin (g/dl) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

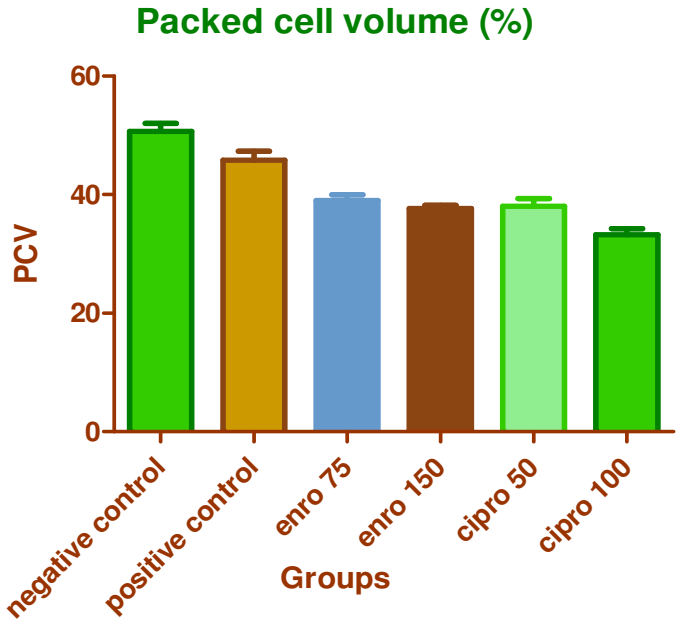


Figure 4.5 Effect on packed cell volume (%) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

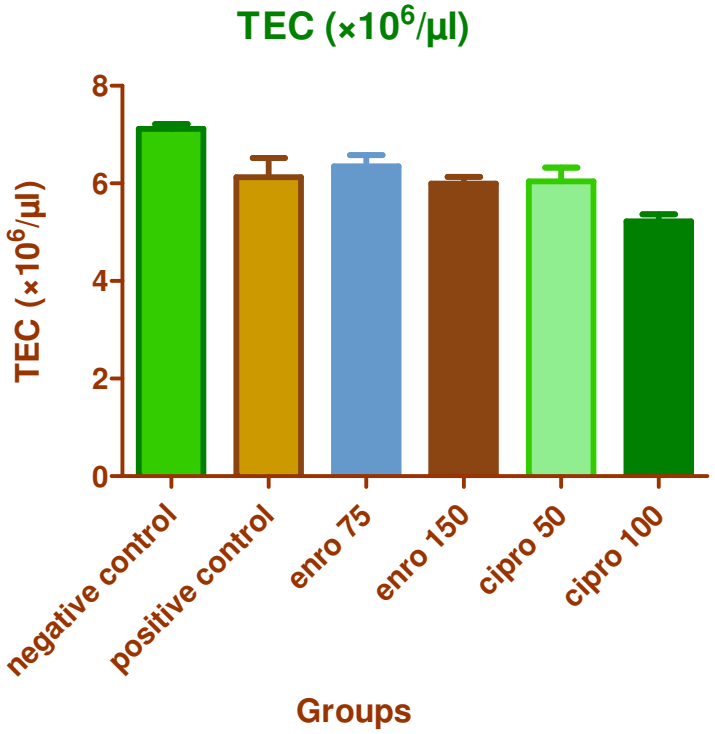


Figure 4.6 Effect on total erythrocyte count (× 10^6/µl) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

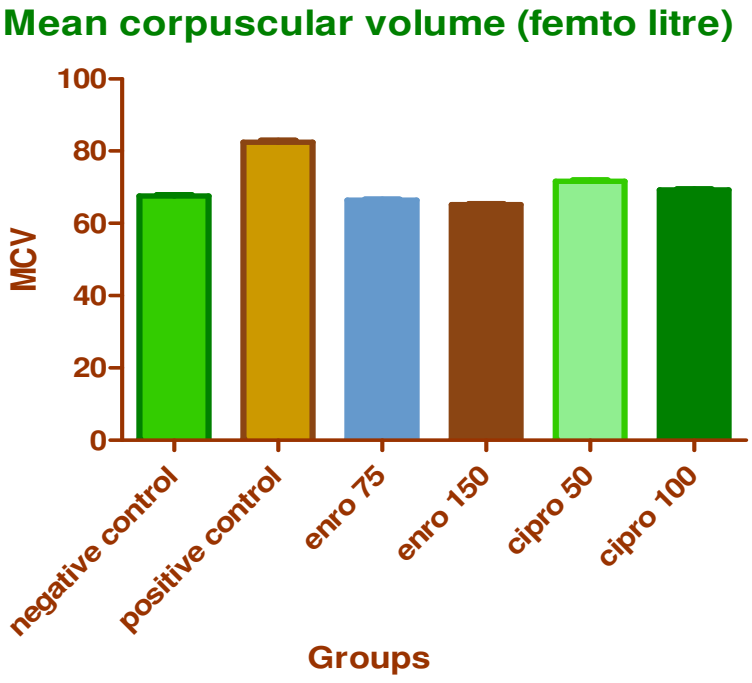


Figure 4.7 Effect on mean corpuscular volume (MCV, femto litre) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

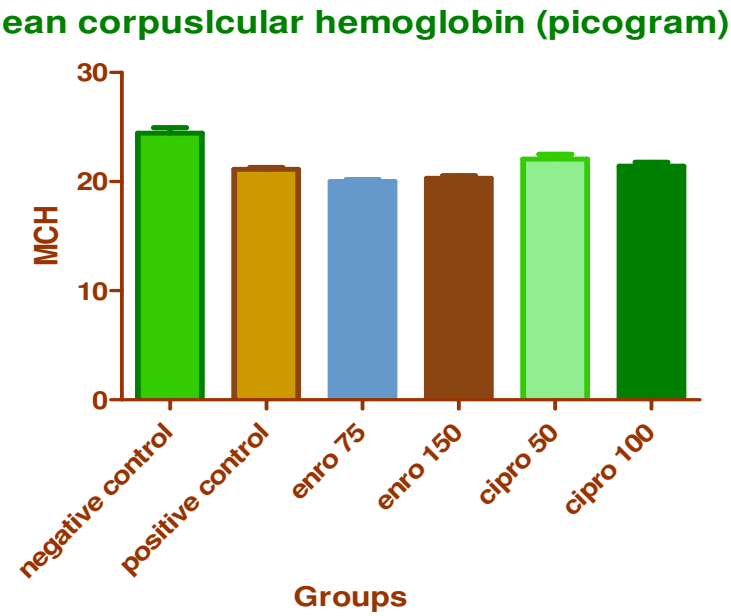


Figure 4.8 Effect on mean corpuscular hemoglobin (MCH, picogram) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

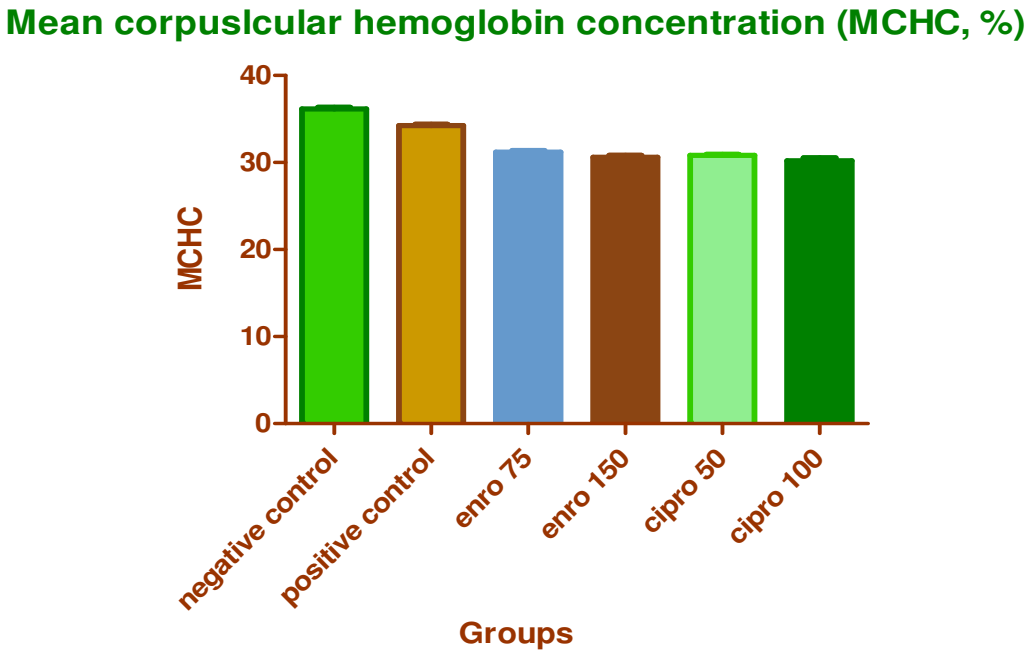


Figure 4.9 Effect on mean corpuscular hemoglobin concentration (MCHC, %) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

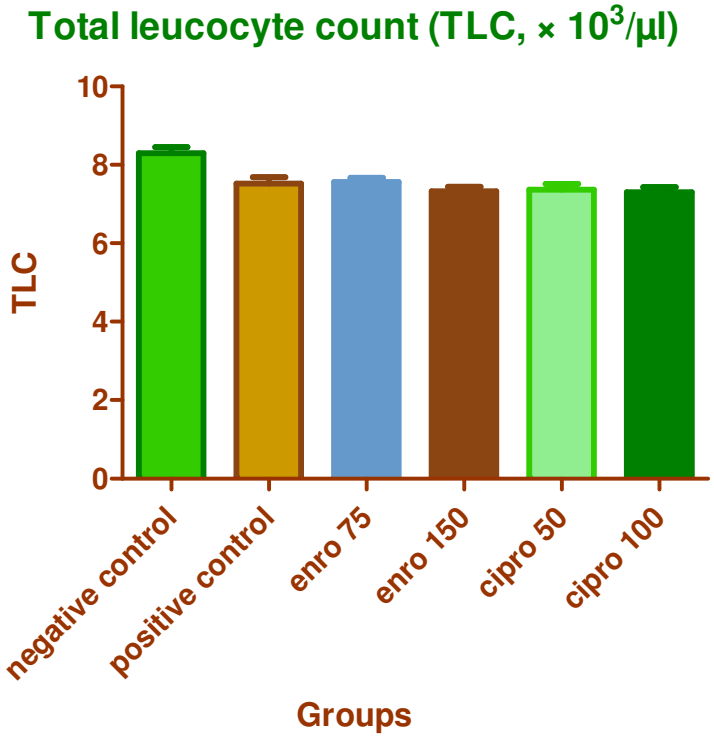


Figure 4.10 Effect on total leukocyte count (TLC, $\times 10^3/\mu l$) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

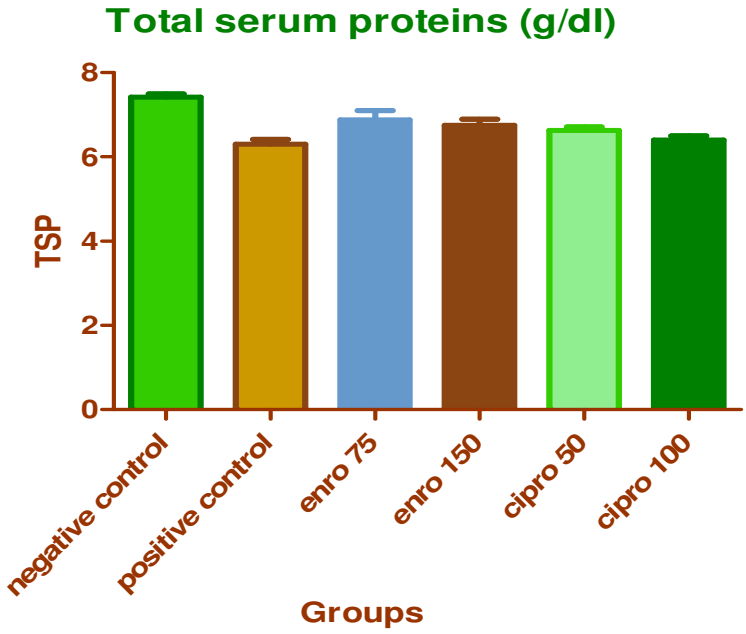


Figure 4.11 Effect on total protein level (g/dl) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Arivuchelvan *et al.* (2013) reported the significant (P<0.05) decrease in globulin levels in poultry after 4 weeks of administration of enrofloxacin in higher doses**. Tras *et al.* (2001)** reported no change in the serum protein levels following administration of enrofloxacin @ 5mg/kg b wt in dogs for 14 days. **Shayne *et al.* (2008)** reported total serum proteins, albumin, globulin and A: G ratio are the important clinical chemistry parameters which measure the hepatic and immune functions. Albumin and globulin are globular proteins, which are mainly synthesized by liver and other cells particularly by immune cells **(Dhinaa and Palanisamy, 2010)**.

Snover *et al.* (1989) reported hepatotoxic potential of cyclophosphamide occurring as a result of enhanced oxidative stress. Cyclophosphamide is metabolized into phosphoramide mustard and acrolein by hepatic microsomal cytochrome P450 mixed functional oxidase system, which induces the oxidative stress **(Storms *et al.*, 1999)** and subsequently responsible for induction of hepatotoxicity **(Selvakumar *et al.*, 2005)**. **Senthilkumar *et al.* (2006)** also reported significant reduction in TSP, albumin and globulin in male Wistar rats as treated with cyclophosphamide @ 150 mg/kg b wt for two days. **Ghaly *et al.* (2015)** reported that significant decrease in total serum proteins, albumin and globulin levels after administration of fluoroquinolones may be due to partial hydrolysis of proteins in blood. In this study cyclophosphamide treated rats showed significant decrease in total serum proteins, albumin and globulin which might have occurred either due to hepatotoxic effects of fluoroquinolones at high doses or direct hydrolytic action on their plasma proteins. In this study significant decrease in the serum proteins may be attributed to the hepatotoxic effect of enrofloxacin and ciprofloxacin.

The effect of enrofloxacin and ciprofloxacin on glucose (mg/dl) concentration is presented in the Table 4.15 and Figure 4.15. There was significant (P<0.05) decrease observed in treatment groups except positive control group II as compared to control group I.

Liver is one of the important organs in the body for regulation of glucose homeostasis. Thus alterations in liver function may affect the normal blood glucose homeostasis. **Mann and Bollman (1933)** reported that a total hepactotomy in a dog resulted in death within a few hours from hypoglycemic shock, signifying the role of liver in glucose metabolism. The findings in the present study are not in agreement

with the findings of **Aziz (2005)** who did not observe any change in glucose levels after ciprofloxacin administration. **Shailer *et al.* (1997)** reported reduced blood glucose levels in human following administration of clinofloxacin @ 400mg/kg through intravenous route.

4.4 Lipid profile

The effect of enrofloxacin and ciprofloxacin on triglyceride concentration is presented in the Table 4.16 and Figure 4.16. No significant ($P<0.05$) reduction was observed in all the treatment groups as compared to control group after 28 days. But non significant increase was noted in all the groups as compared to control group I, this can be due to partial liver damage. No significant($P<0.05$) change in serum cholesterol levels in the treatment groups was observed as compared to control group after 28 days study in rats(Table 4.17 and Figure 4.17). **Al Nazawi (2008)** also observed no changes in triglycerides and cholesterol levels after administering enrofloxacin @ 40mg/kg b wt for 10 days in broilers.

Serum cholesterol and triglyceride levels are indicators of hepatic function and lipid metabolism in the body. Liver is the major site of cholesterol synthesis and metabolism. Lipid metabolizing enzymes maintain the lipid homeostasis in the body **(Alberti *et al.*, 2001)**. Elevated triglycerides level in this study may be due to mild hepatic damage induced by the antibiotics. Thus non-significant ($P<0.05$) alterations in lipid profile is indication of mild alterations in liver function.

The effect of enrofloxacin and ciprofloxacin on BUN (mg/dl) concentration is presented in the Table 4.18 and Figure 4.18. A significant ($P<0.05$) increase in serum BUN values was observed in ciprofloxacin treated groups VI as compared to control I group after 28 days. However, other treatment groups showed non significant ($P<0.05$) increase in BUN values after 28 days.

The effect of enrofloxacin and ciprofloxacin on creatinine (mg/dl) concentration is presented in the Table 4.19 and Figure 4.19. No significant ($P<0.05$) difference was found in all the treatment groups as compared to control group after 28 days. A significant increase in creatinine levels in the treatment groups IV and VI was observed as compared to control group I after 28 days. The end product of protein metabolism is urea, nitrogenous compound produced by liver and distributed though the entire

Table 4.11: Effect on total protein level (g/dl) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Total Protein(g/dl)
			28^{th} day
I.	Control	1 ml of distilled water, po	7.41±0.76
II.	CYP	20 mg/kg b wt, ip	6.30±0.11[a]
III.	Enrofloxacin	75 mg/kg b wt, po	6.80±0.46
IV	Enrofloxacin	150 mg/kg b wt, po	6.75±0.25[a]
V.	Ciprofloxacin	50 mg/kg b wt, po	6.66±0.12[a]
VI.	Ciprofloxacin	100 mg/kg b wt, po	6.42±0.20[a]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28^{th} day only.
a =significant($P<0.05$)difference as compared to group I.

ANOVA Table	SS	df	MS
Treatment (between columns)	4.791	5	0.9583
Residual (within columns)	3.145	30	0.1048
Total	7.936	35	

Table 4.12: Effect on Albumin (g/dl) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Albumin(g/dl)
			28th day
I.	Control	1 ml of distilled water, po	5.06±0.58
II.	CYP	20 mg/kg b wt, ip	4.11±0.31[a]
III.	Enrofloxacin	75 mg/kg b wt, po	4.40±0.17
IV	Enrofloxacin	150 mg/kg b wt, po	4.06±0.21[a]
V.	Ciprofloxacin	50 mg/kg b wt, po	4.25 ±0.88[a]
VI.	Ciprofloxacin	100 mg/kg b wt, po	4.16±0.35[a]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant(P<0.05)difference as compared to group I.

ANOVA Table	SS	df	MS
Treatment (between columns)	3.352	5	0.6704
Residual (within columns)	4.484	29	0.1546
Total	7.835	34	

Table 4.13:Effect on globulin (g/dl) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Globulin(g/dl)
			28th day
I.	Control	1 ml of distilled water, po	4.13±0.08
II.	CYP	20 mg/kg b wt, ip	2.43±0.23[a]
III.	Enrofloxacin	75 mg/kg b wt, po	2.65±0.28[a]
IV	Enrofloxacin	150 mg/kg b wt, po	2.53±0.06[a]
V.	Ciprofloxacin	50 mg/kg b wt, po	2.35 ±0.03[a]
VI.	Ciprofloxacin	100 mg/kg b wt, po	2.30±0.02[a]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant(P<0.05)difference as compared to group I.

ANOVA Table	SS	df	MS
Treatment (between columns)	14.86	5	2.971
Residual (within columns)	1.877	30	0.06256
Total	16.73	35	

Table 4.14: Effect on albumin:globulin following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Albumin:Globulin
			28th day
I.	Control	1 ml of distilled water, po	1.19±0.12
II.	CYP	20 mg/kg b wt, ip	1.73±0.25[a]
III.	Enrofloxacin	75 mg/kg b wt, po	1.69±0.09[a]
IV	Enrofloxacin	150 mg/kg b wt, po	1.62±0.19[a]
V.	Ciprofloxacin	50 mg/kg b wt, po	1.82 ±0.26[a]
VI.	Ciprofloxacin	100 mg/kg b wt, po	1.64±0.32[a]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant(P<0.05)difference as compared to group I.

ANOVA Table	SS	df	MS
Treatment (between columns)	1.432	5	0.2864
Residual (within columns)	1.454	30	0.04846
Total	2.886	35	

Table 4.15:Effect on glucose level (mg/dl) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Glucose (mg/dl)
			28^{th} day
I.	Control	1 ml of distilled water, po	93.37±2.71
II.	CYP	20 mg/kg b wt, ip	86.67±2.85
III.	Enrofloxacin	75 mg/kg b wt, po	$73.63±2.57^{ab}$
IV	Enrofloxacin	150 mg/kg b wt, po	$73.33±3.45^{ab}$
V.	Ciprofloxacin	50 mg/kg b wt, po	$76.67 ±3.14^{a}$
VI.	Ciprofloxacin	100 mg/kg b wt, po	$72.00±2.97^{ab}$

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28^{th} day only.
a =significant(P<0.05)difference as compared to group I; b =significant(P<0.05)difference as compared to group II.

ANOVA Table	SS	df	MS
Treatment (between columns)	1733	5	346.7
Residual (within columns)	1099	29	91.56
Total	2832	34	

Table 4.16:Effect on triglycerides level (mg/dl) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Triglycerides(mg/dl)
			28^{th} day
I.	Control	1 ml of distilled water, po	53.67±4.87
II.	CYP	20 mg/kg b wt, ip	53.21±3.97
III.	Enrofloxacin	75 mg/kg b wt, po	59.17±6.67
IV	Enrofloxacin	150 mg/kg b wt, po	58.83±4.57
V.	Ciprofloxacin	50 mg/kg b wt, po	69.50 ±5.34
VI.	Ciprofloxacin	100 mg/kg b wt, po	64.07±6.98

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28^{th} day only.

ANOVA Table	SS	df	MS
Treatment (between columns)	1168	5	233.6
Residual (within columns)	5257	30	175.2
Total	6425	35	

Table 4.17:Effect on cholesterol level (mg/dl) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Cholesterol (mg/dl)
			28th day
I.	Control	1 ml of distilled water, po	51.67±1.59
II.	CYP	20 mg/kg b wt, ip	54.67±3.27
III.	Enrofloxacin	75 mg/kg b wt, po	53.67±6.58
IV	Enrofloxacin	150 mg/kg b wt, po	53.17±3.58
V.	Ciprofloxacin	50 mg/kg b wt, po	56.33 ±4.33
VI.	Ciprofloxacin	100 mg/kg b wt, po	55.80±7.73

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.

ANOVA Table	SS	df	MS
Treatment (between columns)	88.00	5	17.60
Residual (within columns)	3659	29	126.2
Total	3747	34	

Table 4.18: Effect on blood urea nitrogen level (mg/dl) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Blood Urea Nitrogen(mg/dl)
			28^{th} day
I.	Control	1 ml of distilled water, po	8.04±0.40
II.	CYP	20 mg/kg b wt, ip	8.62±0.21
III.	Enrofloxacin	75 mg/kg b wt, po	8.33±0.32
IV	Enrofloxacin	150 mg/kg b wt, po	8.65±0.45
V.	Ciprofloxacin	50 mg/kg b wt, po	9.14±0.26[a]
VI.	Ciprofloxacin	100 mg/kg b wt, po	9.66±0.08[ab]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28^{th} day only.
a =significant(P<0.05)difference as compared to group I; b =significant(P<0.05)difference as compared to group II.

ANOVA Table	SS	df	MS
Treatment (between columns)	10.17	5	2.033
Residual (within columns)	7.132	29	0.2459
Total	17.30	34	

Table 4.19: Effect on Creatinine (mg/dl) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Creatinine (mg/dl)
			28th day
I.	Control	1 ml of distilled water, po	0.51±0.05
II.	CYP	20 mg/kg b wt, ip	0.60±0.03
III.	Enrofloxacin	75 mg/kg b wt, po	0.61±0.08
IV	Enrofloxacin	150 mg/kg b wt, po	0.76±0.03[a]
V.	Ciprofloxacin	50 mg/kg b wt, po	0.73±0.03
VI.	Ciprofloxacin	100 mg/kg b wt, po	0.79±0.10[a]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant(P<0.05)difference as compared to group I.

ANOVA Table	SS	df	MS
Treatment (between columns)	0.3689	5	0.07378
Residual (within columns)	0.5033	30	0.01678
Total	0.8722	35	

Table 4.20: Effect on Bilirubin (mg/dl) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Bilirubin(mg/dl)
			28th day
I.	Control	1 ml of distilled water, po	0.46±0.03
II.	CYP	20 mg/kg b wt, ip	0.48±0.02
III.	Enrofloxacin	75 mg/kg b wt, po	0.52±0.08
IV	Enrofloxacin	150 mg/kg b wt, po	0.51±0.05
V.	Ciprofloxacin	50 mg/kg b wt, po	0.53±0.04
VI.	Ciprofloxacin	100 mg/kg b wt, po	0.58±0.13

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.

ANOVA Table	SS	df	MS
Treatment (between columns)	0.05867	5	0.01173
Residual (within columns)	0.2598	29	0.008957
Total	0.3184	34	

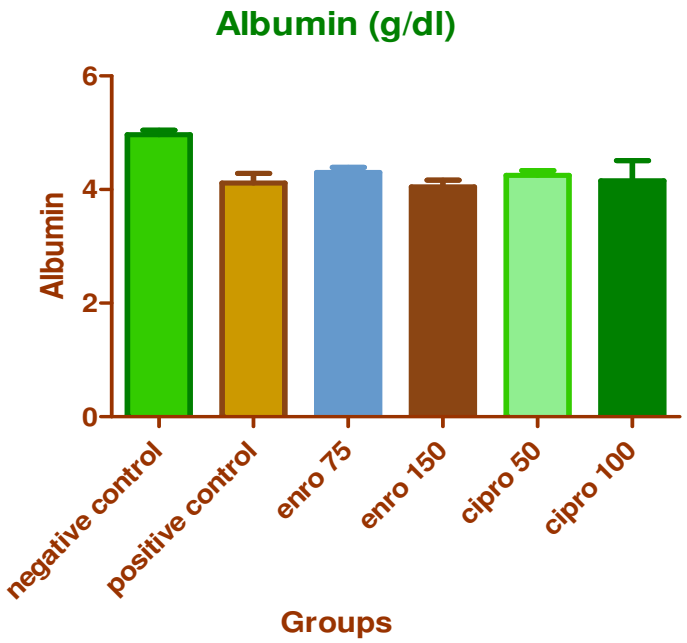


Figure 4.12 Effect on albumin (g/dl) following administration of enrofloxacin and ciprofloxacin, for 28 days in rats.

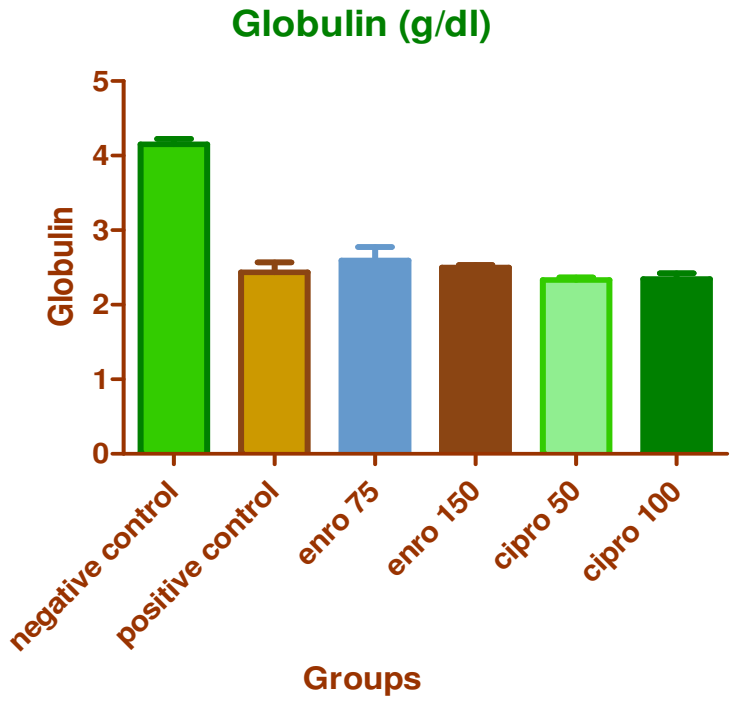


Figure 4.13 Effect on globulin level (g/dl) following administration of enrofloxacin and ciprofloxacin, for 28 days in rats.

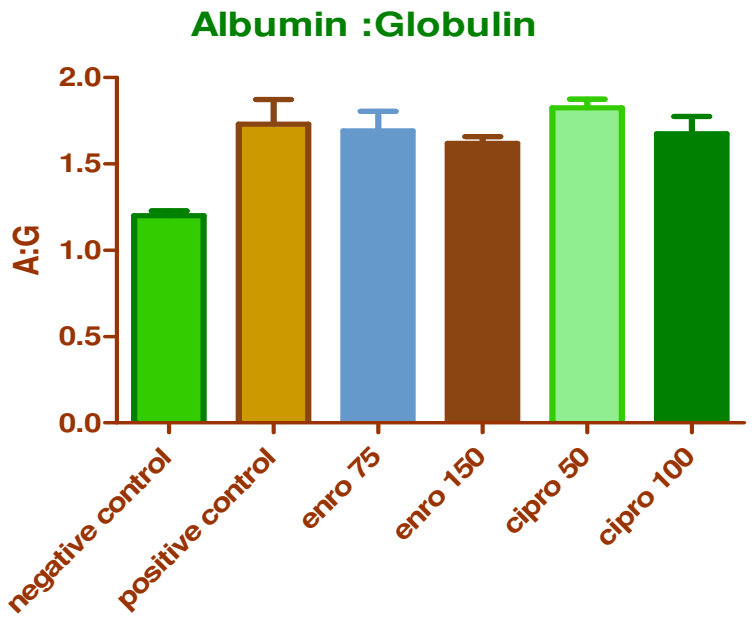


Figure 4.14 Effect on albumin: globulin ratio following administration of enrofloxacin and ciprofloxacin, for 28 days in rats.

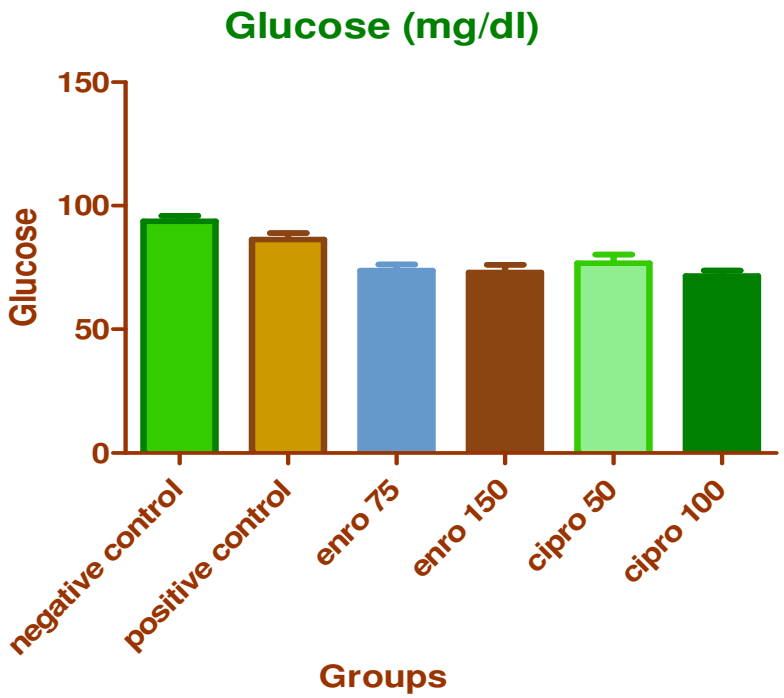


Figure 4.15 Effect on glucose level (mg/dl) following administration of enrofloxacin and ciprofloxacin, for 28 days in rats.

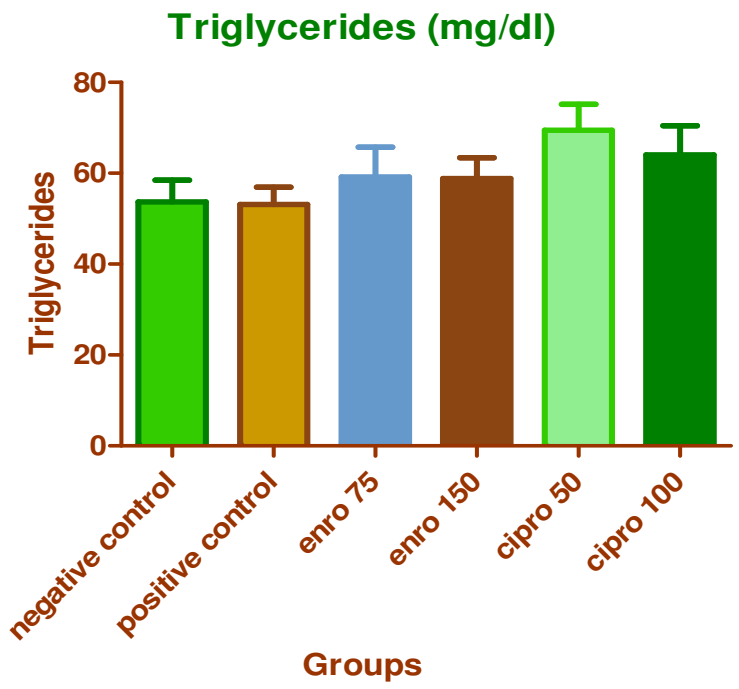


Figure 4.16 Effect on triglycerides level (mg/dl) following administration of enrofloxacin and ciprofloxacin, for 28 days in rats.

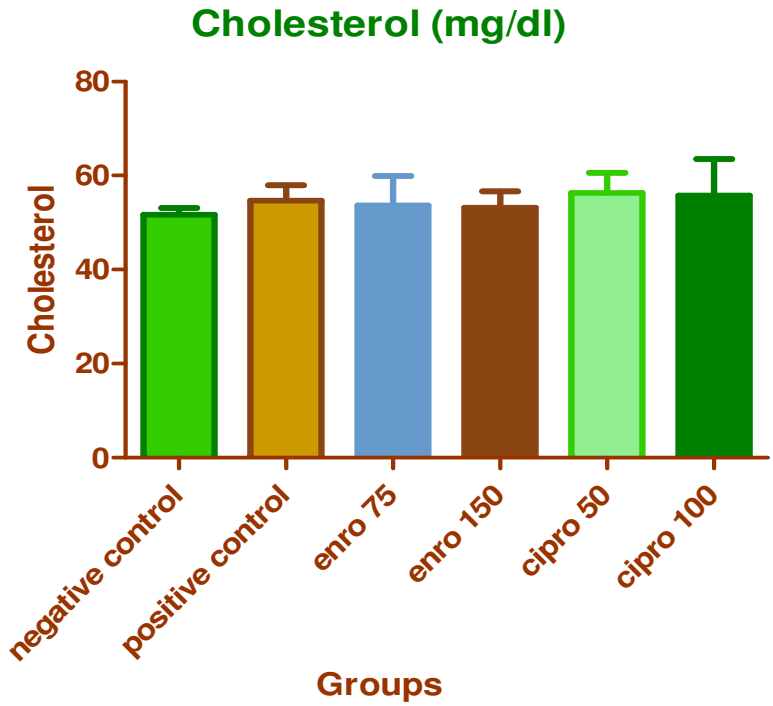


Figure 4.17 Effect on cholesterol level (mg/dl) following administration of enrofloxacin and ciprofloxacin, for 28 days in rats.

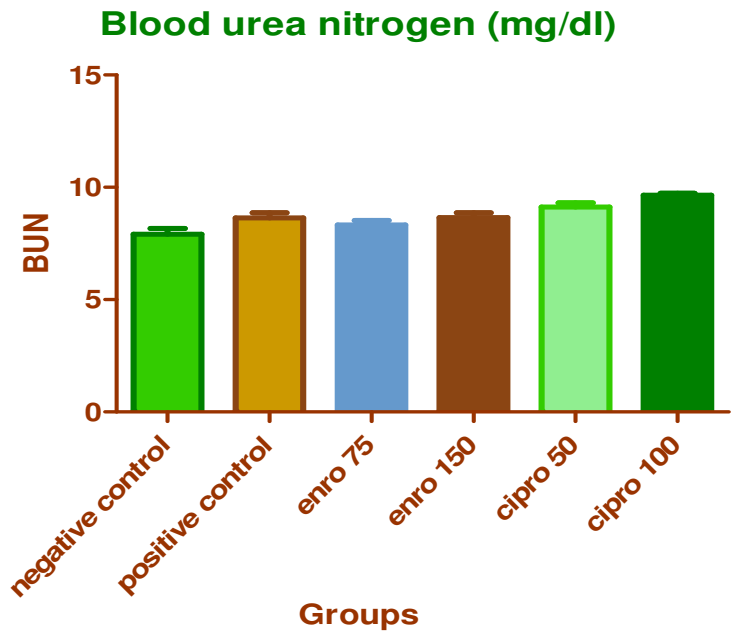


Figure 4.18 Effect on blood urea nitrogen level (mg/dl) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

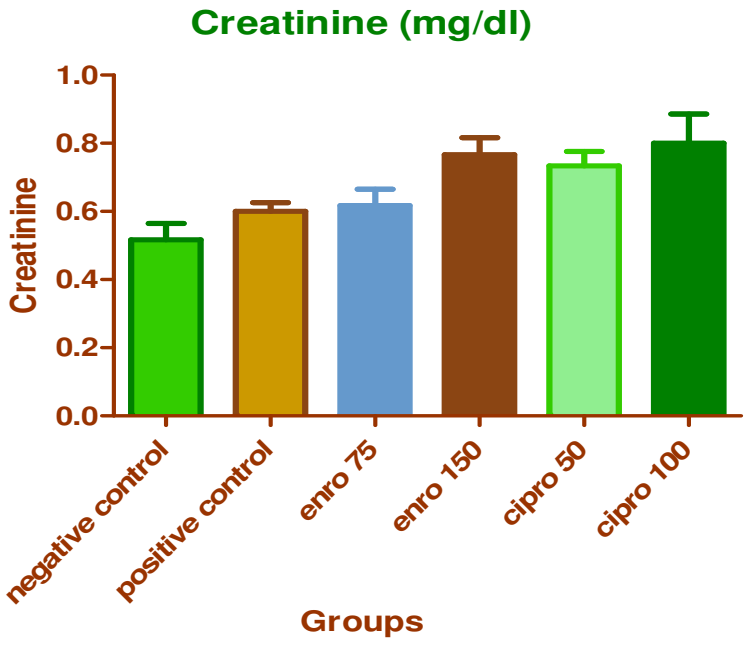


Figure 4.19 Effect on creatinine level (mg/dl) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

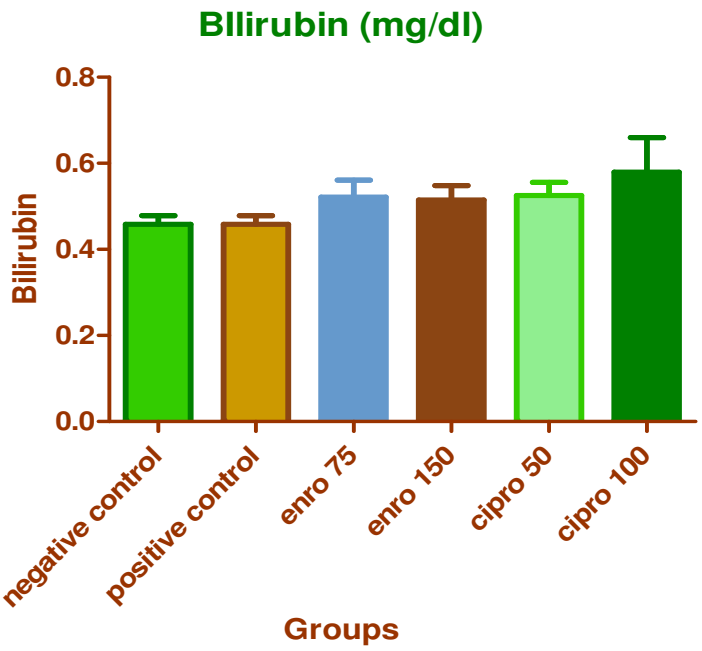


Figure 4.20 Effect on bilrubin level (mg/dl) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

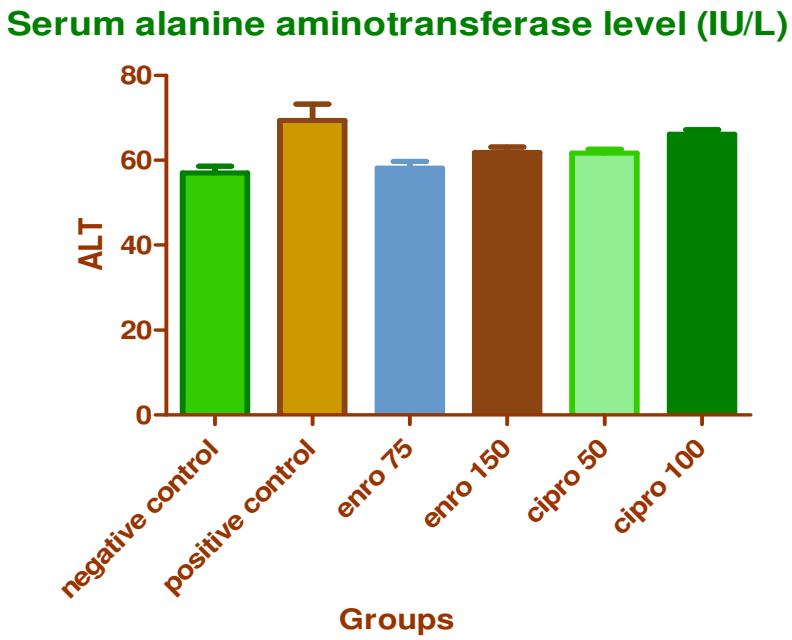


Figure 4.21 Effect on enzymatic activity of ALT (IU/L) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

extracellular and intracellular matrix. Filtration of urea takes place in glomerulus and in case of renal dysfunction it get reabsorbed in blood circulation with water **(Corbett, 2008)**. Thus, concentration of urea in blood is measured as an indicator for renal function **(Rosner and Bolton, 2006)**.

Creatinine is breakdown product of creatinine phosphate in muscle and its concentration depends on muscle mass and is considered as a biomarker for kidney damage **(Gowda *et al.*, 2010)**.

Basaran *et al.* (1993) who also reported no change in serum levels of BUN and creatinine after treatment with ciprofloxacin @20mg/kg b wt after 14 days in rats but there was an increase in BUN and creatinine values @ 200mg/kg b wt in rats. **Shaki *et al.* (2016)** reported significant (P<0.01) increase in the BUN and creatinine values in rats following administration of ciprofloxacin @ 100mg/kg b wt for 8 days. However, **Shoorijeh *et al.* (2012)** observed no change in the BUN and creatinine values after administration of enrofloxacin @25mg/kg b wt in cats for 21 days. The findings of the present study are in agreement with findings others (**Shaki *et al.*, 2016: Shoorijeh *et al.*, 2012**).

The effect of enrofloxacin and ciprofloxacin on bilirubin (mg/dl) concentration is given in the Table 4.20 and Figure 4.20. No significant (P<0.05) difference was observed between control and treatment groups after 28 days of study.

Bilirubin is a yellow compound produced from degradation of hemoglobin from RBC and mainly excreted through after metabolism in liver and bile. This process is important to excrete the bilirubin as waste metabolite which is produced from the destruction of aged RBCs. When hepatocytes are degenerated, excretion of bilirubin is inhibited leading to redistribution of bilirubin in blood and extracellular fluid. An increase in bilirubin level is due to decreased hepatic clearance which leads to jaundice and other hepatotoxic symptoms **(Saukkonen *et al.*, 2006)**. No change in the level of bilirubin is indicative of normal hepatic function during the period of trail in this study.

The effect of enrofloxacin and ciprofloxacin on ALT (IU/L) concentration is presented in the Table 4.21 and Figure 4.21. A significant (P<0.05) increase was observed in the activity of serum ALT in the group II and VI as compared to control group I after 28 days. Significant (P<0.05) increase in AST (IU/L) level was observed

in the groups II and VI as compared to control group after 28 days (Table 4.22 and Figure 4.22). Group VI showed significant ($P<0.05$) difference as compared to all treatment groups (III to V). The effect of enrofloxacin and ciprofloxacin on activity of ALP (IU/L) is presented in the Table 4.23 and Figure 4.23. The level of serum ALP activity was not altered in liver much in groups II to VI as compared with control group I. However, a non significant decline in ALP levels was observed in the treatment groups as compared to control groups.

Alanine aminotransferase (ALT; formerly serum glutamic pyruvic transaminase, SGPT) and aspartate amino transferase (AST; formerly serum glutamic oxaloacetic transaminase) are the important biomarkers for the hepatic function in the body **(Amacher, 2002)**. Estimation of these enzymes give the picture of liver status in the body. An increase in the levels of these enzymes attributes for the liver damage and estimation of ALT enzyme has significance as liver function tests because it is primarily found in liver **(Amacher, 2002)**.

The AST, a cytoplasmic enzyme, catalyses the reductive transfer of an amino group from aspartate to α-ketoglutarate to yield oxaloacetate and glutamate. Apart from liver it is also found in kidney, heart, muscle and brain and thus damage to any of these organs results in the release and elevated levels of enzyme in the blood **(Nathwani *et al.*, 2005)**. Because of its release from the organs other than liver, it is less specific for hepatic injury **(Dufour *et al.*, 2001)**.

Alkaline phosphatases are the enzymes which are widely distributed in various tissues. Serum ALP concentration is an indicator for hepatobilliary disorders and skeletal disorders. Liver or the billiary tract is the main source of ALP in the serum. An increase in the levels of ALP can be seen in bone disorders accompanied by increased osteoblastic activity.

Neer (1988) reported an increase in ALT, AST, and ALP levels following administration of enrofloxacin in a clinical study. But in contrast, **Altreuther (1987**) observed that even 10 times high dose of enrofloxacin did not reveal any significant increase in activity of ALT, AST, and ALP enzymes in the serum.

Channa and Janjua (2003) reported that the administration of ciprofloxacin caused degenerative changes in liver in rats. Many other workers also reported hepatotoxic potential of ciprofloxacin in humans and other species **(Alcalde *et al.*, 1995;**

Table 4.21: Effect on Serum alanine aminotransferase level (IU/L) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	ALT (IU/L)
			28th day
I.	Control	1 ml of distilled water, po	57.02±4.03
II.	CYP	20 mg/kg b wt, ip	69.77±2.21[a]
III.	Enrofloxacin	75 mg/kg b wt, po	58.01±3.25
IV	Enrofloxacin	150 mg/kg b wt, po	61±5.03
V.	Ciprofloxacin	50 mg/kg b wt, po	61.25±1.15
VI.	Ciprofloxacin	100 mg/kg b wt, po	66.33±2.02[a]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant(P<0.05)difference as compared to group I.

ANOVA Table	SS	df	MS
Treatment (between columns)	661.1	5	132.2
Residual (within columns)	707.2	30	23.57
Total	1368	35	

Table 4.22: Effect on Serum Aspartate amino transferase level (IU/L) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	AST (IU/L)
			28th day
I.	Control	1 ml of distilled water, po	59.17±2.16
II.	CYP	20 mg/kg b wt, ip	69.13±2.62[a]
III.	Enrofloxacin	75 mg/kg b wt, po	58.67±2.02[b]
IV	Enrofloxacin	150 mg/kg b wt, po	61.87±1.08[b]
V.	Ciprofloxacin	50 mg/kg b wt, po	62.33±5.54[b]
VI.	Ciprofloxacin	100 mg/kg b wt, po	68.69±4.47[acde]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant(P<0.05)difference as compared to group I; b =significant(P<0.05)difference as compared to group II;
c =significant(P<0.05)difference as compared to group III; d =significant(P<0.05)difference as compared to group IV;
e =significant(P<0.05)difference as compared to group V.

ANOVA Table	SS	df	MS
Treatment (between columns)	646.9	5	129.4
Residual (within columns)	224.0	29	7.725
Total	871.0	34	

Table 4.23: Effect on Serum Alkaline phosphatase level (IU/L) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	ALP (IU/L)
			28th day
I.	Control	1 ml of distilled water, po	114.7±9.81
II.	CYP	20 mg/kg b wt, ip	119.00±6.12
III.	Enrofloxacin	75 mg/kg b wt, po	117.70±9.41
IV	Enrofloxacin	150 mg/kg b wt, po	119.56±4.38
V.	Ciprofloxacin	50 mg/kg b wt, po	108.30±11.54
VI.	Ciprofloxacin	100 mg/kg b wt, po	121.30±5.78

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.

ANOVA Table	SS	df	MS
Treatment (between columns)	1203	5	240.6
Residual (within columns)	14149	30	471.6
Total	15352	35	

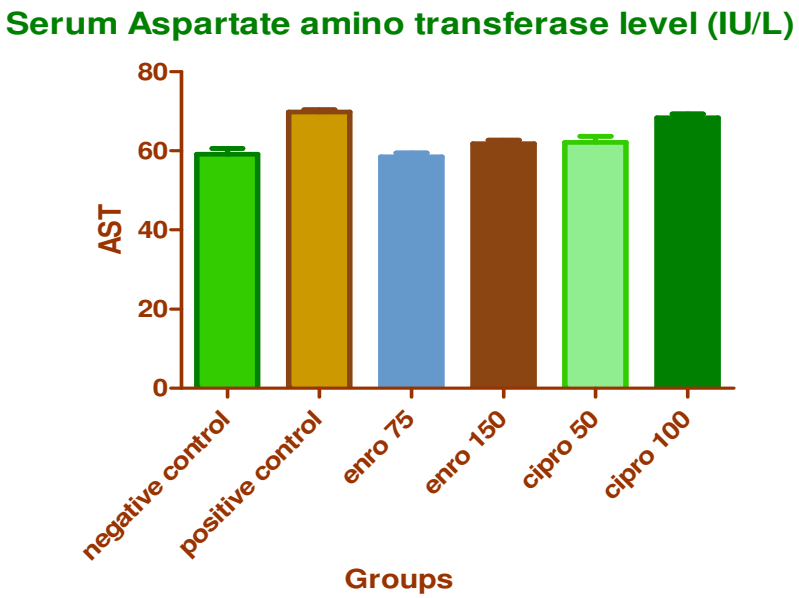


Figure 4.22 Effect on enzymatic activity of AST (IU/L) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

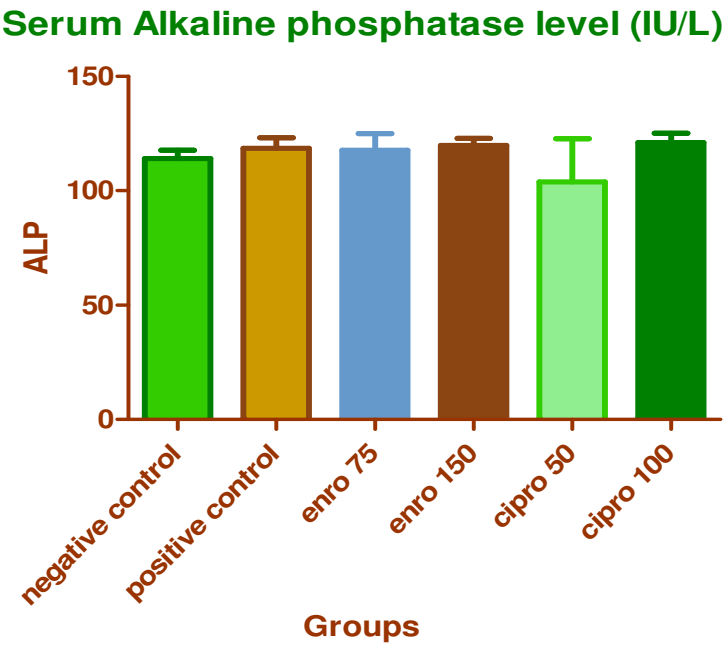


Figure 4.23 Effect on enzymatic activity of ALP (IU/L) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Cholongitas *et al*., 2001). In this study a significant increase in the levels of AST and ALT were attributed to the toxic effect of enrofloxacin and ciprofloxacin on liver function at higher doses. These findings are in agreement with the findings of **Greene *et al*. (2002)** who reported an increase in levels of these enzymes in dogs following treatment with enrofloxacin.

4.5 Antioxidant parameters

The effect of enrofloxacin and ciprofloxacin on activity of LPO (MDA. nM/ml) in RBCs is presented in the Table 4.24 and Figure 4.24. LPO was measured by determining the malondialdehyde in RBCs. A significant ($P<0.05$) increase in LPO status of RBCs was observed in group II, III, IV and V as compared to group I. All the groups (III to VI) showed significant ($P<0.05$) decrease in LPO values as compared to group II, which would have been due to oxidation of the drug by liver microsomal enzymes of the cytochrome P450 family.

The effect of enrofloxacin and ciprofloxacin on activity of GSH (mM/ml) in RBCs is presented in the Table 4.25 and Figure 4.24. A significant ($P<0.05$) increase in all the groups except group IV was noticed as compared to control group I. As the oxidative stress induced in these groups was high, an increase in the activity of GSH could be noticed. In group II treated with Cyclophosphamide induced maximal oxidative stress so GSH activity increased to scavenge the free radicals.

The effect of enrofloxacin and ciprofloxacin on activity of SOD (U/ml) in RBCs is given in the Table 4.26 and Figure 4.24. A significant ($P<0.05$) increase in SOD levels in RBCs were observed in all the groups as compared to control group I after 28 days of period. As compared to positive control group II, all the remaining groups showed significant ($P<0.05$) reduction in SOD activity.

A significant ($P<0.05$) rise in catalase activity of RBCs was observed in group II as compared to group I (control). Groups II, III and V also showed significant ($P<0.05$) increase in catalase activity as the drugs induced high oxidative stress in the rats (Table 4.27 and Figure 4.24).

4.5.1 Antioxidant status in liver, kidney, spleen, brain and heart

4.5.1.1 Lipid peroxidation

The effect of enrofloxacin and ciprofloxacin on activity of LPO (nM MDA/g of tissue) in different organs is presented in the Table 4.28 and Figure 4.25. In liver there

was a significant (P<0.05) increase in LPO values in group II, IV and group VI as compared to control group I after 28 days. There was significant (P<0.05) decrease in LPO in group III and group V as compared to group II. A significant (P<0.05) difference was observed between the higher dose group IV and lower dose group III of enrofloxacin.

In kidney, there was a significant (P<0.05) increase in LPO values in groups II, IV and VI as compared to control group I after 28 days. Significant (P<0.05) decrease in LPO values was observed in group III and group V as compared to group II and LPO suppression was significant (P<0.05) in the higher dose group VI than lower dose group V of ciprofloxacin.

A significant (P<0.05) higher level of LPO of spleen was observed in cyclophosphamide group II as compared to control group I. Groups IV and VI showed significantly (P<0.05) higher LPO of spleen than group I but less than group II. LPO was significantly higher in high dose groups than the low dose groups of enrofloxacin and ciprofloxacin (Table 4.28)

There was significantly (P<0.05) higher LPO in group II to VI as compared to control group I and II in myocardial tissue. Significantly (P<0.05) low level of LPO values were found in low dose groups III and group V as compared to high dose groups of enrofloxacin and ciprofloxacin.

It was concluded from this study that cyclophosphamide significantly (P<0.05) elevated in LPO, however, the level of LPO was less on higher dose groups than low dose groups after 28 days of study in rats.

4.5.1.2 Reduced glutathione (GSH)

The effect of enrofloxacin and ciprofloxacin on activity of GSH (mM/ml) in different organs is depicted in the Table 4.29 and Figure 4.26. In liver a significant (P<0.05) increase in GSH levels were found in all the groups except group IV as compared to control group I. Group II showed maximum increase in GSH level as cyclophosphamide is known to induce hepatic injury leading to increase in free radicals, there had been high amount of GSH levels were to scavange these radicals. All the treatment groups showed significant (P<0.05) reduction GSH values as compared to positive control group II. Findings of present study showed that high dose

groups revealed a distinction in GSH activity indicating high oxidative stress in the tissue as compared to low dose groups.

In kidney, significant ($P<0.05$) increase in GSH levels were observed in groups II to VI in comparison to control group I after 28 days. However, all the treatment groups showed significantly ($P<0.05$) lower GSH levels than positive control group II. The levels of GSH were higher in low dose groups than high dose groups of enrofloxacin and ciprofloxacin.

A significant ($P<0.05$) increase in GSH status of spleen was observed in group II to VI, with high concentration in group II and as compared to group I after 28 days. Low levels of GSH found in high dose groups than the low dose groups. Similar pattern of GSH was observed in myocardial tissue after 28 days of study in rats.

4.5.1.3 Super oxide dismutase (SOD)

The effect of enrofloxacin and ciprofloxacin on activity of SOD in different organs is presented in the Table 4.30 and Figure 4.27. The value of SOD was measured as unit/gram of tissues. All the tissues revealed similar pattern of SOD activity after 28 days of study. There was significant ($P<0.05$) increase in SOD values in group II to VI as compared to control group I after 28 days in liver, kidneys, spleen and heart. A significant ($P<0.05$) decline in SOD activity was observed in high dose groups IV and VI in comparison with low dose groups III and V. However, activity of SOD was highest in cyclophosphamide treated group II after the completion of the study.

4.5.1.4 Catalase

All the organs revealed similar pattern of catalase activity as it was maximum in cyclophosphamide treated group II in comparison to control group I and treatment groups from III to VI. The low dose groups III and V showed higher catalase activity than the higher dose groups IV and VI in liver, kidneys, spleen and heart for the 28 days study in rats (Table 4.31 and Figure 4.28).

Oxidative stress occurs in the body as a result of alteration in the homeostasis of production of reactive oxygen species (ROS) and these includes super oxide radical ion, hydrogen peroxide, and hydroxyl radicals etc. Lipid peroxidation, protein modification, and DNA strand breaks are the different types of damages caused due to

ROS. To avoid these ROS induced damages there are certain mechanisms that prevent formation of ROS or they remove existing ROS **(Sies, 1991; Halliwell, 1992).**

Cell injury caused by free radicals is responsible for aging, cancer, cardiovascular disease, immune system decline, brain and liver dysfunction. All free radicals are highly reactive and highly damaging to biological system **(Moein *et al*., 2007).**

Reduction in glutathione (GSH) indicates the oxidative stress. H_2O_2 in the body will be detoxified by GSH, with the enzymes GSH peroxidase and GSSG reductase to water and molecular oxygen and maintains the cysteinyl-thiols (R-CH_2–SH) groups of proteins in the reduced state which is often necessary for their functional integrity.

Dismutation of superoxide anion radical by SOD results in production of hydrogen peroxide. Catalase or isoenzymes of glutathione play a vital role in removal of hydrogen peroxide. At higher concentration of hydrogen peroxide catalase activity will be high which removes most of the compound. **Scandalios *et al.* (1993)** reported the role of superoxide dismutase in defending against oxidative stress in all aerobic organisms. Decrease in SOD activity results in enhancement of oxidative radicals which causes oxidative stress.

Becerra and Albesa (2002) and Albesa *et al*. (2004) reported that ciprofloxacin has ability of producing reactive oxygen species in bacterial cell. Phototoxic effect of fluoroquinolones is attributed by the production of singlet oxygen and superoxide anion by fluoroquinolones only (**Umezawa *et al*., 1997).** Higher value of antioxidants parameters also indicate the higher scavenging of oxidative radicals following enrofloxacin and ciprofloxacin treatment.

The significant elevation in LPO levels in treatment groups in the study may be due to oxidation of the drug by liver microsomal enzymes of the cytochrome P450 family resulting in formation of free radicals causing lipid peroxidation **(Carreras *et al*., 2004).** In addition, **Goswami *et al.* (2006)** also reported the involvement of H_2O_2 in antibacterial action of ciprofloxacin.

The significant increase in catalase activity of antibiotic treated group remained till the end of the experiment. This was with accordance with **Carreras *et al*. (2004)** who found significant differences in catalase activity in chicken after receiving the

Table 4.24: Effect on Lipid peroxidation (MDA.nM/ml) in RBCs following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	LPO(MDA.nM/ml)
I.	Control	1 ml of distilled water, po	47.18±3.20
II.	CYP	20 mg/kg b wt, ip	142.4±10.36[a]
III.	Enrofloxacin	75 mg/kg b wt, po	76.13±7.02[ab]
IV	Enrofloxacin	150 mg/kg b wt, po	104.30±7.03[ab]
V.	Ciprofloxacin	50 mg/kg b wt, po	64.06±4.462[bc]
VI.	Ciprofloxacin	100 mg/kg b wt, po	85.95±5.53[ab]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant(P<0.05)difference as compared to group I; b =significant(P<0.05)difference as compared to group II;
c =significant(P<0.05)difference as compared to group III.

ANOVA Table	SS	df	MS
Treatment (between columns)	34417	5	6883
Residual (within columns)	3907	29	134.7
Total	38325	34	

Table 4.25: Effect on Reduced glutathione in RBCs following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	GSH(nM/ml)
I.	Control	1 ml of distilled water, po	0.43±0.29
II.	CYP	20 mg/kg b wt, ip	2.16±0.28[a]
III.	Enrofloxacin	75 mg/kg b wt, po	1.08±0.76[ab]
IV	Enrofloxacin	150 mg/kg b wt, po	0.82±0.08[bc]
V.	Ciprofloxacin	50 mg/kg b wt, po	1.20±0.09[ab]
VI.	Ciprofloxacin	100 mg/kg b wt, po	0.72±0.08[abce]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant(P<0.05)difference as compared to group I; b =significant(P<0.05)difference as compared to group II;
c =significant(P<0.05)difference as compared to group III; e =significant(P<0.05)difference as compared to group V.

ANOVA Table	SS	df	MS
Treatment (between columns)	11.54	5	2.309
Residual (within columns)	4.507	30	0.1502
Total	16.05	35	

Table 4.26: Effect on Superoxide desmutase in RBCs following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	SOD(U/ml)
I.	Control	1 ml of distilled water, po	4.24±0.18
II.	CYP	20 mg/kg b wt, ip	9.38±0.38[a]
III.	Enrofloxacin	75 mg/kg b wt, po	6.89±0.70[ab]
IV	Enrofloxacin	150 mg/kg b wt, po	5.41±0.39[ab]
V.	Ciprofloxacin	50 mg/kg b wt, po	7.29±0.23[abd]
VI.	Ciprofloxacin	100 mg/kg b wt, po	5.39±0.42[abce]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant(P<0.05)difference as compared to group I; b =significant(P<0.05)difference as compared to group II;
c =significant(P<0.05)difference as compared to group III; d =significant(P<0.05)difference as compared to group IV;
e =significant(P<0.05)difference as compared to group V.

ANOVA Table	SS	df	MS
Treatment (between columns)	95.84	5	19.17
Residual (within columns)	11.08	29	0.3819
Total	106.9	34	

Table 4.27: Effect on Catalase in RBCs following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Catalase(U/ml)
I.	Control	1 ml of distilled water, po	2.11±0.11
II.	CYP	20 mg/kg b wt, ip	8.13±0.43[a]
III.	Enrofloxacin	75 mg/kg b wt, po	5.96±0.31[ab]
IV	Enrofloxacin	150 mg/kg b wt, po	2.62±0.29[bc]
V.	Ciprofloxacin	50 mg/kg b wt, po	4.72±0.62[abd]
VI.	Ciprofloxacin	100 mg/kg b wt, po	2.82±0.12[bce]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant(P<0.05)difference as compared to group I; b =significant(P<0.05)difference as compared to group II;
c =significant(P<0.05)difference as compared to group III; d =significant(P<0.05)difference as compared to group IV;
e =significant(P<0.05)difference as compared to group V.

ANOVA Table	SS	df	MS
Treatment (between columns)	163.2	5	32.64
Residual (within columns)	15.49	29	0.5343
Total	178.7	34	

Table 4.28: Effect on Lipid peroxidation (LPO) (MDA.nM/ml) in liver, kidney, spleen, and heart following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Liver	Kidney	Spleen	Heart
I.	Control	1 ml of distilled water, po	39.37±4.41	40.90±4.20	36.01±1.30	22.27±2.03
II.	CYP	20 mg/kg b wt, ip	128.50±5.99[a]	125.00±3.22[a]	109.35±1.74[a]	59.64±1.75[a]
III.	Enrofloxacin	75 mg/kg b wt, po	58.14±1.54[bc]	61.69±3.31[abc]	57.65±2.79[abc]	31.17±1.762[abc]
IV	Enrofloxacin	150 mg/kg b wt, po	79.33±1.78[bce]	87.69±3.27[bc]	85.15±2.48[bde]	42.59±1.49[bce]
V.	Ciprofloxacin	50 mg/kg b wt, po	54.41±3.11[bce]	50.38±0.87[bc]	44.51±1.98[bde]	25.85±0.49[bce]
VI.	Ciprofloxacin	100 mg/kg b wt, po	89.25±5.29[abd]	83.64±2.02[abd]	77.82±3.58[abd]	41.25±0.57[abd]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant(P<0.05)difference as compared to group I; b =significant(P<0.05)difference as compared to group II;
c =significant(P<0.05)difference as compared to group III; d =significant(P<0.05)difference as compared to group IV;
e =significant(P<0.05)difference as compared to group V.

Table 4.29: Effect on reduced glutathione (nM/g of tissue) in liver, kidney, spleen, and heart following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Liver	Kidney	Spleen	Heart
I.	Control	1 ml of distilled water, po	0.39±0.03	0.39±0.03	0.41±0.04	0.31±0.03
II.	CYP	20 mg/kg b wt, ip	8.72±0.36[a]	10.47±0.24[a]	8.94±0.41[a]	9.77±0.17[a]
III.	Enrofloxacin	75 mg/kg b wt, po	2.47±0.24[ab]	3.04±0.51[ab]	2.36±0.38[ab]	2.11±0.12[ab]
IV	Enrofloxacin	150 mg/kg b wt, po	1.46±0.23[b]	1.37±0.04[bc]	1.24±0.17[b]	1.20±0.15[abc]
V.	Ciprofloxacin	50 mg/kg b wt, po	3.74±0.09[abcd]	3.84±0.10[abd]	3.67±0.32[abc]	3.45±0.17[abc]
VI.	Ciprofloxacin	100 mg/kg b wt, po	2.64±0.23[abde]	2.84±0.10[abd]	2.55±0.24[ab]	2.07±0.06[abde]

Values in the Table are mean ±S.E. (n=6); CYP= cyclophosphamide, and it was administered on 28th day only.
a =significant(P<0.05)difference as compared to group I; b =significant(P<0.05)difference as compared to group II;
c =significant(P<0.05)difference as compared to group III; d =significant(P<0.05)difference as compared to group IV;
e =significant(P<0.05)difference as compared to group V.

Table 4.30: Effect on superoxide dismutase (U/g of tissue) in liver, kidney, spleen, and heart following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Liver	Kidney	Spleen	Heart
I.	Control	1 ml of distilled water, po	4.39±0.30	3.23±0.15	3.89±0.17	3.74±0.24
II.	CYP	20 mg/kg b wt, ip	9.19±0.59[a]	8.13±0.10[a]	8.41±0.44[a]	7.42±0.27[a]
III.	Enrofloxacin	75 mg/kg b wt, po	7.62±0.52[a]	6.16±0.12[ab]	6.10±0.08[ab]	5.98±0.72[ab]
IV	Enrofloxacin	150 mg/kg b wt, po	5.05±0.35[bc]	4.52±0.30[abc]	4.58±0.36[bc]	4.28±0.38[bc]
V.	Ciprofloxacin	50 mg/kg b wt, po	7.59±0.76[ad]	7.45±0.25[ac]	7.18±0.38[ad]	6.61±1.02[ad]
VI.	Ciprofloxacin	100 mg/kg b wt, po	5.95±0.39[b]	5.96±0.04[abde]	5.57±0.18[abe]	5.30±0.30[ab]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant(P<0.05)difference as compared to group I; b =significant(P<0.05)difference as compared to group II;
c =significant(P<0.05)difference as compared to group III; d =significant(P<0.05)difference as compared to group IV;
e =significant(P<0.05)difference as compared to group V.

Table 4.31: Effect on Catalase (mM H_2O_2/min/mg protein) in liver, kidney, spleen, and heart following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Liver	Kidney	Spleen	Heart
I.	Control	1 ml of distilled water, po	1.60±0.51	1.53±0.42	0.94±0.19	1.11±0.27
II.	CYP	20 mg/kg b wt, ip	7.03±0.37[a]	7.12±0.44[a]	6.92±0.19[a]	6.52±0.58[a]
III.	Enrofloxacin	75 mg/kg b wt, po	4.07±0.31[ab]	4.14±0.35[ab]	3.88±0.11[ab]	3.90±0.16[ab]
IV	Enrofloxacin	150 mg/kg b wt, po	2.75±0.07[b]	2.69±0.19[b]	2.73±0.09[abc]	2.78±0.17[ab]
V.	Ciprofloxacin	50 mg/kg b wt, po	5.19±0.26[abd]	5.19±0.49[abc]	5.07±0.33[abc]	4.46±0.37[abd]
VI.	Ciprofloxacin	100 mg/kg b wt, po	5.314±0.24[be]	3.68±0.02[ab]	3.02±0.34[abe]	3.45±0.29[ab]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant(P<0.05)difference as compared to group I; b =significant(P<0.05)difference as compared to group II;
c =significant(P<0.05)difference as compared to group III; d =significant(P<0.05)difference as compared to group IV;
e =significant(P<0.05)difference as compared to group V.

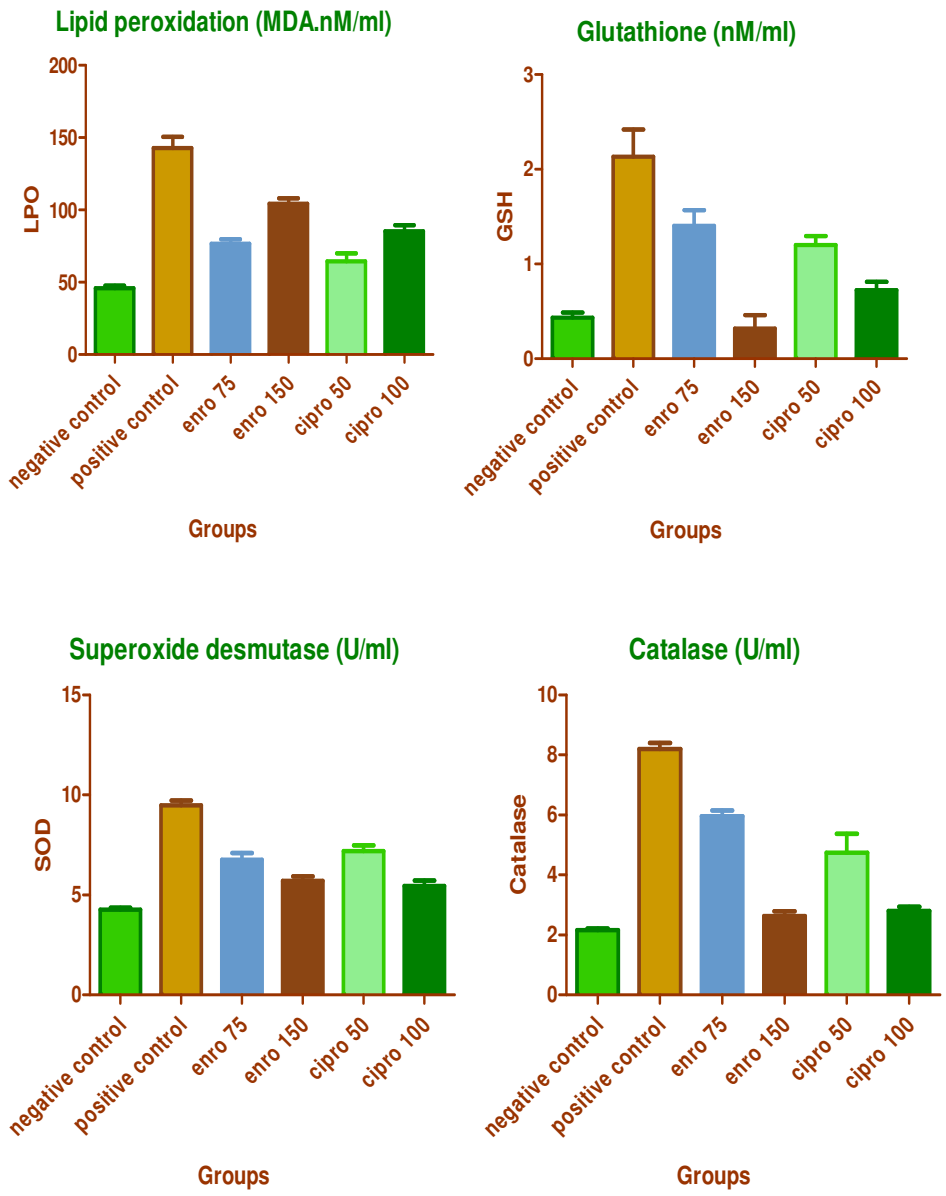


Figure 4.24 Effect of enrofloxacin and ciprofloxacin on oxidative stress related parameters in RBCs of rats following oral administration for 28 day.

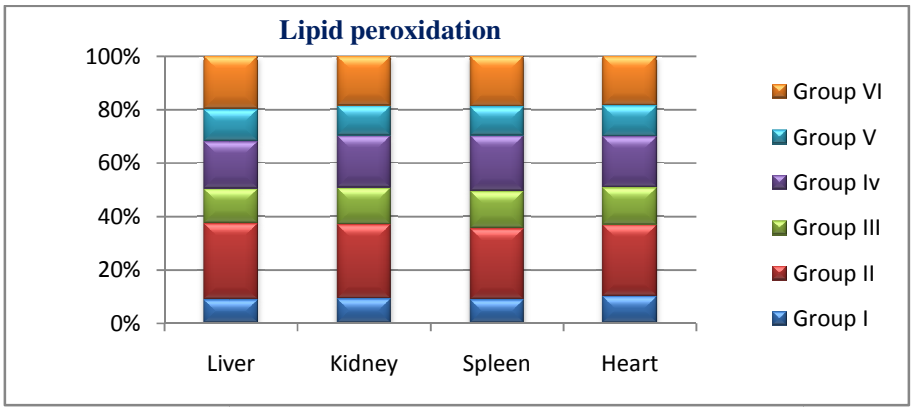


Figure 4.25 Effect on lipid peroxidation (MDAmalondialdehyde nmol/gm wet tissue) in liver, kidney, spleen, brain and heart following oral administration of enrofloxacin and ciprofloxacin for 28 days in rats.

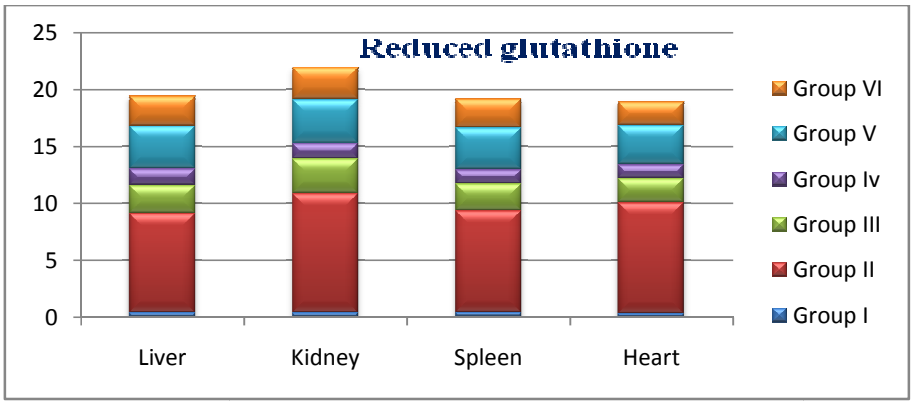


Figure 4.26 Effect on reduced glutathione (nM/g of tissue) in liver, kidney, spleen, brain and heart following oral administration of enrofloxacin and ciprofloxacin for 28 days in rats.

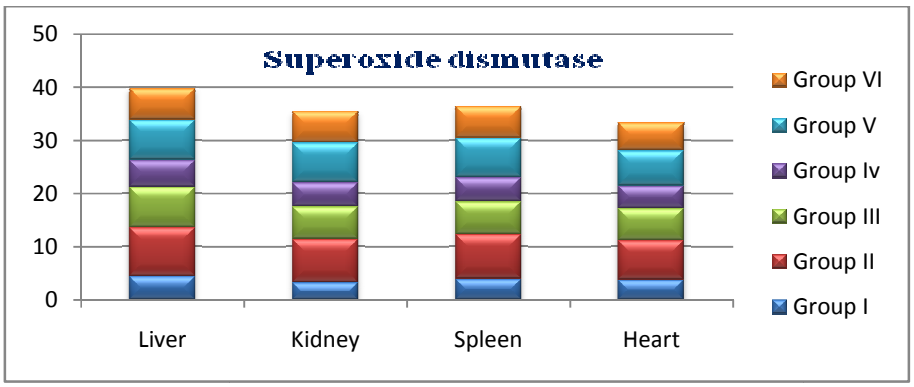


Figure 4.27 Effect on superoxide dismutase (U/g of tissue) in liver, kidney, spleen, brain and heart following oral administration of enrofloxacin and ciprofloxacin for 28 days in rats.

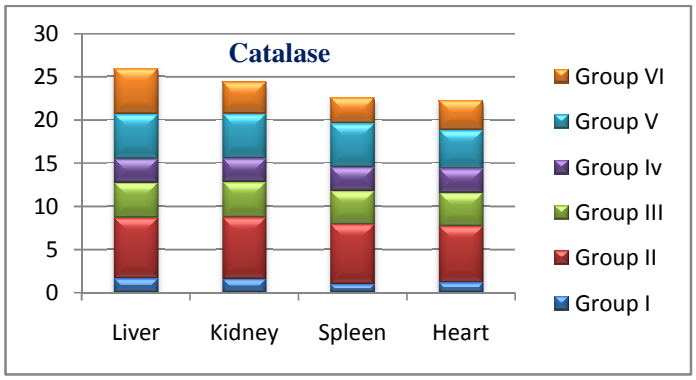


Figure 4.28 Effect on catalase (mM H2O2/min/mg protein) in liver, kidney, spleen, brain and heart following oral administration of enrofloxacin and ciprofloxacin for 28 days in rats.

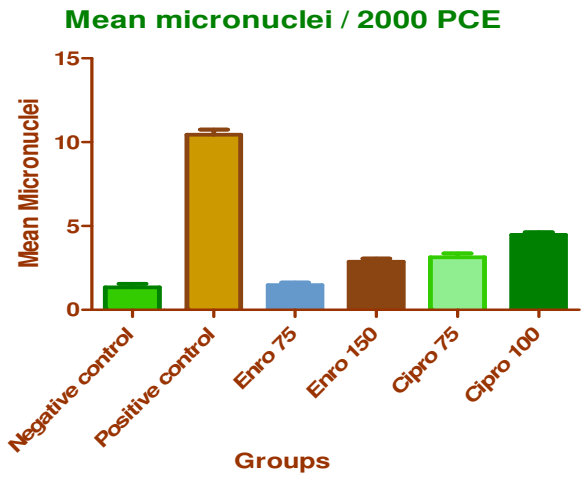


Figure 4.29 Micronuclei/2000 polychromatic erythrocytes (PCE) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

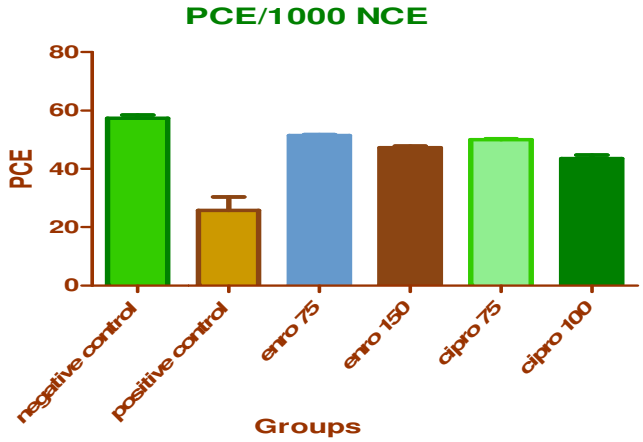


Figure 4.30 Polychromatic erythrocytes (PCE) /1000 total erythrocytes (TE) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

therapeutic dose of enrofloxacin @ 50mg/L drinking water for 5 days without withdrawal.

In this study, the cyclophosphamide induced maximum oxidative stress in the cells. Treatment groups treated with enrofloxacin and ciprofloxacin with varying doses also induced oxidative stress in dose dependent manner. Findings of this study may be correlate with the findings of **Rawi *et al.* (2011)** where ciprofloxacin induced dose dependent oxidative stress in different tissues in albino rats. The possible mechanism of induction of oxidative stress by enrofloxacin and ciprofloxacin might be due to t production of hydroxyl radicals in both gram negative and gram positive organism(**Kohanski *et al.*, 2007)** and in host cells **(Rawi *et al*., 2011).**

Ibrahim *at al.* (2011) also reported an increase in LPO levels as well as catalase and GSH levels in chickens following administration of enrofloxacin @ 10mg/kg b wt.

4.6 Genotoxicity assessment

4.6.1 Micronucleus assay

Micronuclei assay was done to assess the genotoxic effects following sub acute toxicity of enrofloxacin @ 75mg/kg b wt and 150 mg/kg b wt and Ciprofloxacin @ 50mg/kg b wt and 100 mg/kg b wt in rats for 28 days. The mean number of micronuclei/2000 polychromatic erythrocytes (PCE) in bone marrow, in group I (negative control), group II (positive control, Cyclophosphamide @ 20 mg/kg BW) group III (enrofloxacin @ 75mg/kg BW), group IV(enrofloxacin @150mg/kg BW) group III (ciprofloxacin @50mg/kg B.W) and group IV (ciprofloxacin @ 100mg/kg B.W) are shown in the Table 4.32 and Figure 4.29.

Enrofloxacin at a dose rate of 75mg/kg did not cause significant ($P<0.05$) change in the formation of micronuclei as compared to the negative control group I whereas @150mg/kg caused significantly higher damage as shown from the formation of micronuclei. Ciprofloxacin at a dose rate of 50mg/kg and 100 mg/kg caused significantly ($P<0.05$) higher damage as shown from the formation of micronuclei (Plate 4.1 and 4.2) Positive control group II treated with cyclophosphamide at the dose of 20mg/kg caused significant ($P<0.05$) damage to erythrocytes as shown from formation of micronuclei.

The mean number of polychromatic erythrocytes (PCE) /2000 total erythrocytes (TE) in bone marrow of different groups is given in the Table 4.33 and Figure 4.30.

Enrofloxacin at a dose rate of 75mg/kg did not cause any significant ($P<0.05$) change in the formation of polychromatic erythrocytes(PCEs) as compared to the negative control whereas at a dose rate of 150mg/kg it caused significantly higher damage than control. Ciprofloxacin at a dose rate 100mg/kg caused significantly ($P<0.05$) more damage than the control. Formation of polychromatic erythrocytes caused by cyclophosphamide at the dose of 20mg/kg was significantly higher than the treatment groups.

However, **Herbold (2001)** reported in his studies that there was no significant ($P<0.05$) increase in the micronucleated polychromatic or micronucleated normochromatic erythrocyte counts after treatment with 4000mg/kg b wt of enrofloxacin after first test of 24, 48 or 72 h in mice, but a statistical significance ($P<0.05$) change of the ratio of normochromatic to polychromatic erythrocytes was observed for animals sacrificed 72 h after treatment.

Gürbay *et al*. (2002) showed that ciprofloxacin was not cytotoxic to human fibroblasts at any of the concentrations tested when the cells were incubated with ciprofloxacin @ 0.0129-0.387 mM concentration after 24 h. However, significant level of cytotoxicity was observed at concentrations 0.129 and 0.194 mM and >0.129 mM, following 48 and 72 hours of exposure, respectively. In contrast, **Jun *et al*. (2003)** identified apoptotic effects in Jurkat T cells at concentration levels as low as 2.5 µg/ ml. The in vitro micronucleus test is a genotoxicity test for the detection of micronuclei in the cytoplasm of interphase cells. Micronucleus may originate from acentric chromosome fragments (i.e. lacking a centromere) or whole chromosomes that are unable to migrate to the poles during the anaphase stage of cell division **(OECD 2010).** Thus, genetic damage becomes manifested in the newly formed daughter cell **(Reifferscheid *et al*., 2008).**

4.6.2 Chromosomal aberration assay

Chromosomal aberration assay was performed in bone marrow cells of colchicine pre-treated rats after sacrifice (as it arrests the cell cycle in metaphase). The structural chromosomal aberration like breaks (Fig.4.3), gaps (Fig.4.4), fragments, ring

Table 4.32 : Micronuclei/2000 polychromatic erythrocytes (PCE) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	Mean micronuclei/2000 PCE
			28^{th} day
I.	Control	1 ml of distilled water, po	1.33±0.21
II.	CYP	20 mg/kg b wt, ip	10.44 ± 1.14^{a}
III.	Enrofloxacin	75 mg/kg b wt, po	1.46 ± 0.33^{b}
IV	Enrofloxacin	150 mg/kg b wt, po	2.83 ± 0.37^{abc}
V.	Ciprofloxacin	50 mg/kg b wt, po	3.13 ± 0.16^{abc}
VI.	Ciprofloxacin	100 mg/kg b wt, po	4.46 ± 0.307^{abde}

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28^{th} day only.
a =significant(P<0.05)difference as compared to group I; b =significant(P<0.05)difference as compared to group II;c =significant(P<0.05)difference as compared to group III; d =significant(P<0.05)difference as compared to group IV;e =significant(P<0.05)difference as compared to group V;

ANOVA Table	SS	df	MS
Treatment (between columns)	343.6	5	68.73
Residual (within columns)	8.138	30	0.2713
Total	351.8	35	

Table-4.33 Polychromatic erythrocytes (PCE) /1000 total erythrocytes (TE) following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	PCE/1000 NCE
			28th day
I.	Control	1 ml of distilled water, po	57.38±1.29
II.	CYP	20 mg/kg b wt, ip	25.78±4.14[a]
III.	Enrofloxacin	75 mg/kg b wt, po	51.45±0.32[b]
IV	Enrofloxacin	150 mg/kg b wt, po	47.28±0.53[ab]
V.	Ciprofloxacin	50 mg/kg b wt, po	49.97±0.40[b]
VI.	Ciprofloxacin	100 mg/kg b wt, po	43.52±1.17[ab]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant(P<0.05)difference as compared to group I; b =significant(P<0.05)difference as compared to group II;

ANOVA Table	SS	df	MS
Treatment (between columns)	3550	5	709.9
Residual (within columns)	715.4	30	23.85
Total	4265	35	

Table- 4.34 Structural chromosomal aberration following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	structural chromosomal aberrations (%)
			28th day
I.	Control	1 ml of distilled water, po	1.34±0.09
II.	CYP	20 mg/kg b wt, ip	10.57 ±1.24[a]
III.	Enrofloxacin	75 mg/kg b wt, po	1.98±0.45[ab]
IV	Enrofloxacin	150 mg/kg b wt, po	2.33±0.05[ab]
V.	Ciprofloxacin	50 mg/kg b wt, po	2.97 ±0. 66[abcd]
VI.	Ciprofloxacin	100 mg/kg b wt, po	4.54±0. 21[abcde]

Values in the Table are mean ±S.E. (n=6); CYP= Cyclophosphamide, and it was administered on 28th day only.
a =significant(P<0.05)difference as compared to group I; b =significant(P<0.05)difference as compared to group II;
c =significant(P<0.05)difference as compared to group III;d =significant(P<0.05)difference as compared to group IV;
e =significant(P<0.05)difference as compared to group V.;

ANOVA Table	SS	df	MS
Treatment (between columns)	351.0	5	70.19
Residual (within columns)	2.844	30	0.09480
Total	353.8	35	

Table- 4.35 Olive tail movement following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

Groups	Treatments	Dose and route	OTM
			28^{th} day
I.	Control	1 ml of distilled water, po	0.0352±0.00313
II.	CYP	20 mg/kg b wt, ip	$2.138±0.136^{a}$
III.	Enrofloxacin	75 mg/kg b wt, po	$0.155±0.056^{ab}$
IV	Enrofloxacin	150 mg/kg b wt, po	$0.359±0.04^{abc}$
V.	Ciprofloxacin	50 mg/kg b wt, po	$0401±0.42^{abcd}$
VI.	Ciprofloxacin	100 mg/kg b wt, po	$0.718±0.182^{abcde}$

Values in the Table are mean ±S.E. (n=6); CYP= cyclophosphamide, and it was administered on 28^{th} day only.
a =significant(P<0.05)difference as compared to group I; b =significant(P<0.05)difference as compared to group II;
c =significant(P<0.05)difference as compared to group III;d =significant(P<0.05)difference as compared to group IV;
e =significant(P<0.05)difference as compared to group V.;

ANOVA Table	SS	df	MS
Treatment (between columns)	17.93	5	3.585
Residual (within columns)	0.1387	30	0.004624
Total	18.06	35	

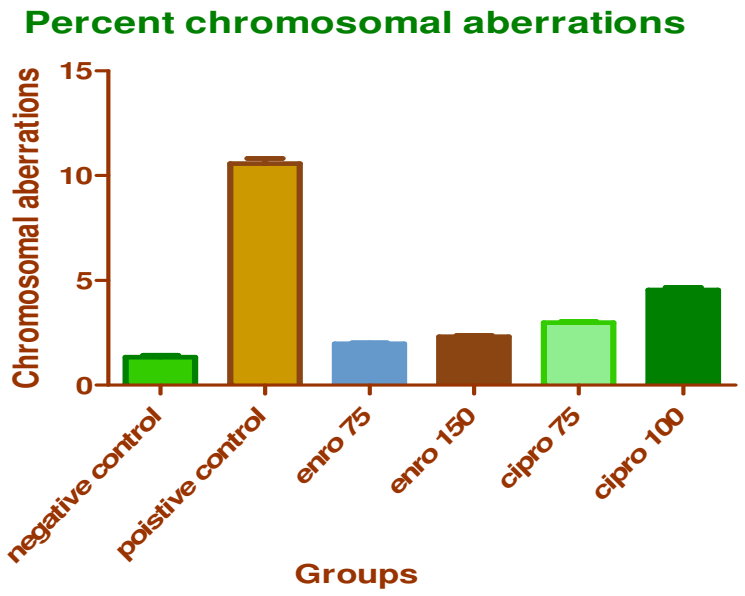


Figure 4.31 Structural chromosomal aberration following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

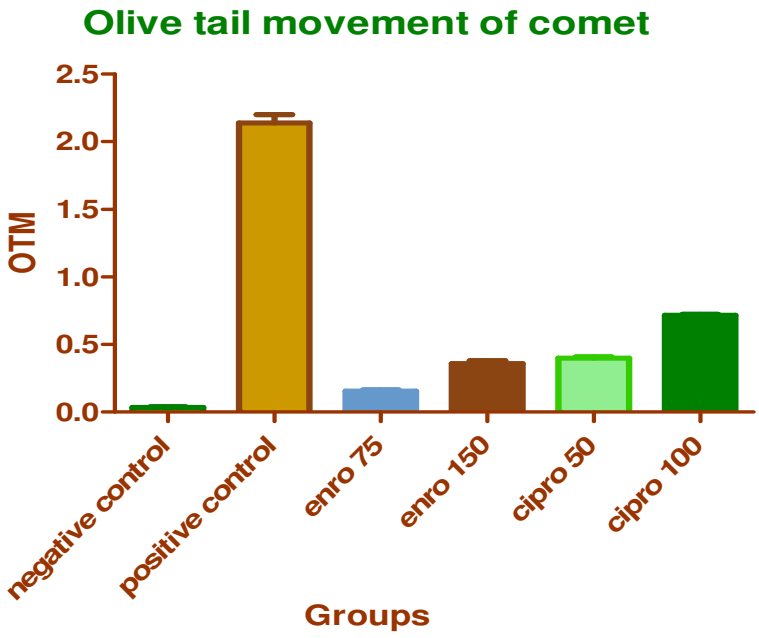


Figure 4.31 Olive tail movement following administration of enrofloxacin and ciprofloxacin for 28 days in rats.

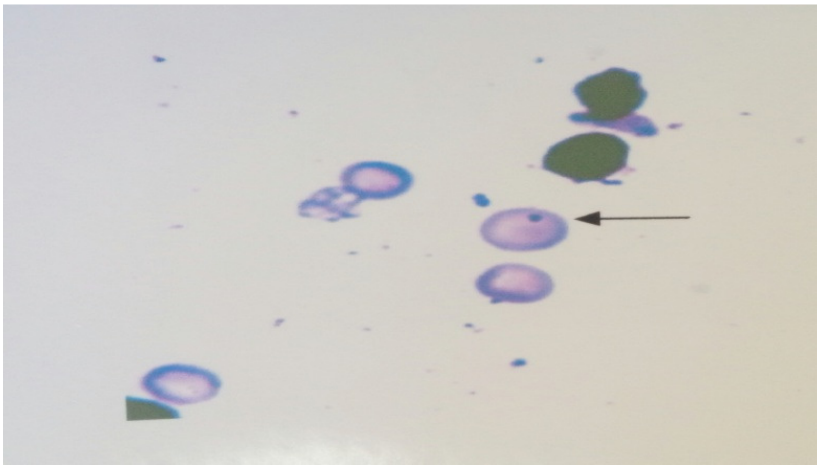


Plate 4.1: Photomicrograph of RBC of group IV showing micronucleus formation, MayGruenwald stain 400X

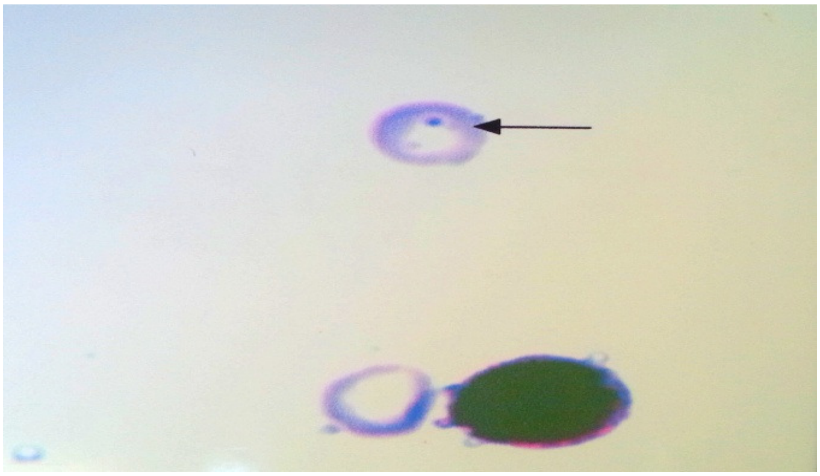


Plate 4.2: Photomicrograph of RBC of group IV showing micronucleus formation, MayGruenwald stain 400X

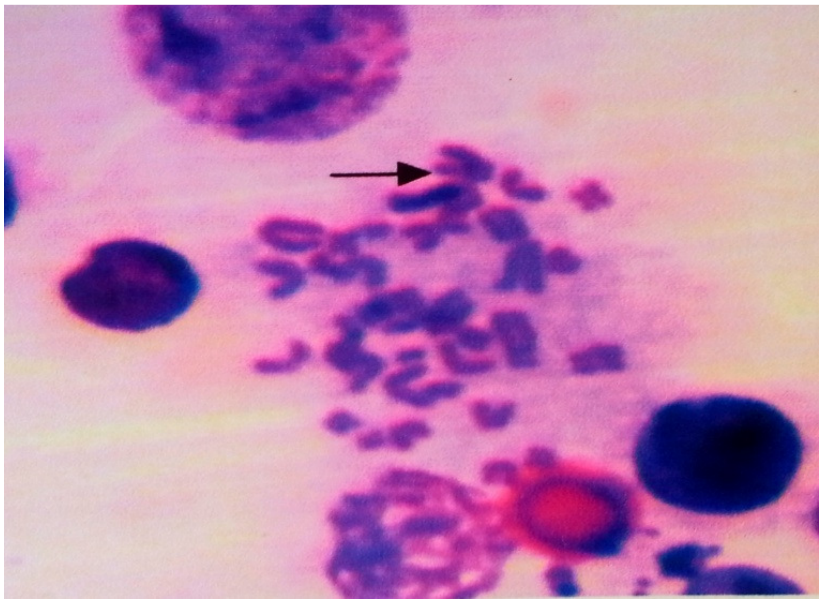


Plate 4.3: Photomicrograph of group VI showing chromosomal spread indicating chromosomal break (arrow). Giemsa 400X

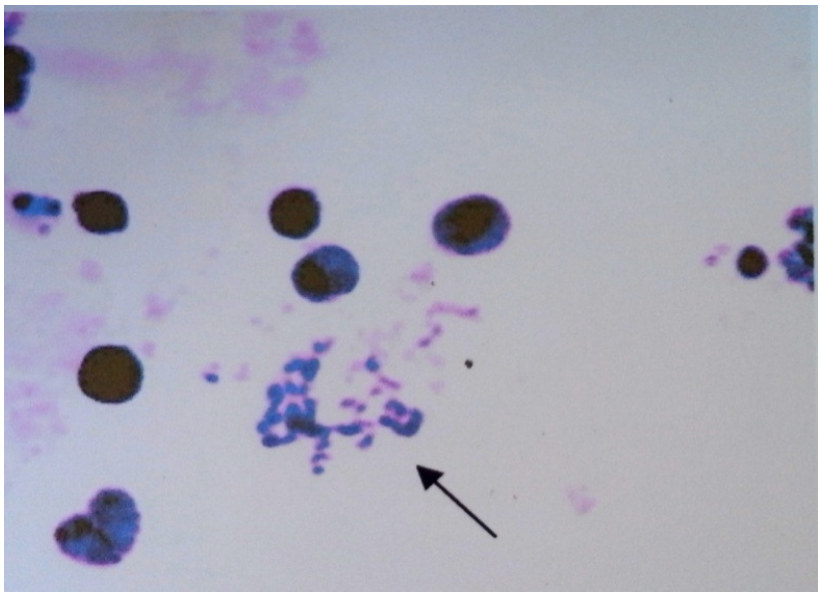


Plate 4.4: Photomicrograph of group VI showing chromosomal spread indicating pulverization (arrow). Giemsa 400X

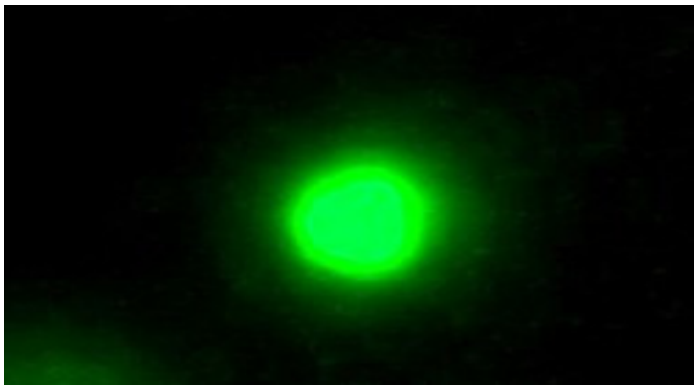


Plate 4.5: Photomicrograph showing of group I showing normal Comet indicating no DNA damage (SYBER green staining) 400X

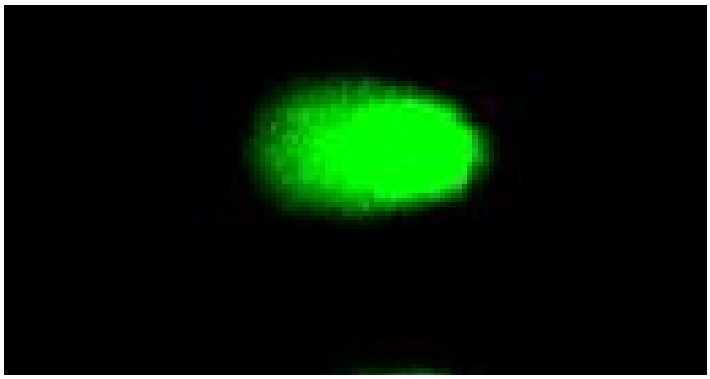


Plate 4.6: Photomicrograph of group VI showing Comet indicating DNA damage (SYBER green staining) 400X

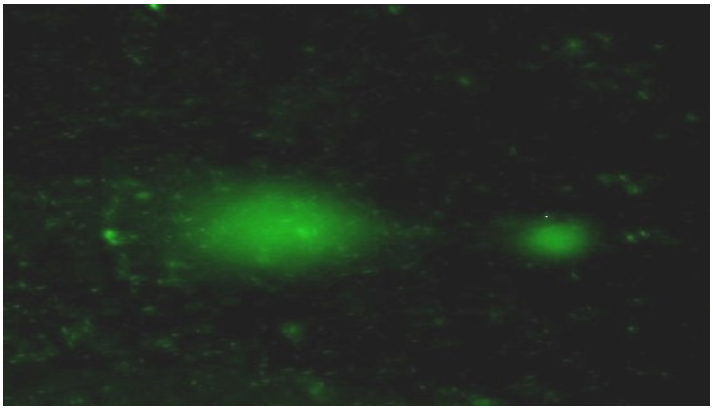


Plate 4.7: Photomicrograph of group II showing Comet indicating extensive DNA damage(SYBER green staining) 400X

and pulverizations chromosomes were observed in bone marrow cells of different treatment groups II to VI is shown in Table 4.34 and Figure 4.31.

Enrofloxacin at a dose rate of 75mg/kg b wt and 150mg/kg b wt caused significant (P<0.05) change in the formation of chromosomal aberrations as compared to the negative control, Ciprofloxacin at a dose rate of 50mg/kg and 100 mg/kg caused significant (P<0.05) damage as shown from the formation of chromosomal aberrations. Whereas, formation of chromosomal aberrations caused by cyclophosphamide at the dose of 20mg/kg was significantly (P<0.05) high than damage caused by the treatment groups of enrofloxacin and ciprofloxacin.

Enrofloxacin induced chromosomal aberrations at concentrations varying between 5 to 50mg/l in human lymphocytes culture, reduction in the mitotic index and fuzzy metaphases were observed at 50 mg/l of ciprofloxacin, indicating a cytotoxic effect of this compound. The chromosome aberrations range in control was 1.0-2.0% and experiment group 7.0-12.0% on human lymphocytes culture *in vitro* after administrating 40 µg/ml concentration of ciprofloxacin. **(Gorla *et al.*, 1999 and Ambulkar *et al.,* 2009).** These findings are in agreement with the current findings of the study where chromosomal aberrations were observed in high dose groups.

4.6.3 Comet assay

Comet assay was performed with the rat blood immediately after the sacrifice of animals. Comets were scored and OTM (olive tail movement) cell was calculated. The mean tail moment is shown in Table 4.35 and Figure 4.32.

Enrofloxacin @ 75mg/kg b wt and 150mg/kg b wt cause significant (P<0.05) change in the olive tail moment (OTM) as compared to the negative control. Ciprofloxacin @ 50mg/kg and 100 mg/kg B.W caused significant (P<0.05) damage as shown from increase in the OTM. Whereas olive tail moment (OTM) caused by cyclophosphamide at the dose of 20mg/kg was significantly (P<0.05) higher than both groups of enrofloxacin and ciprofloxacin in this study (Plate 4.5 to 4.7).

In a study, enrofloxacin and ciprofloxacin showed significant increase in DNA migration after 4 and 20 h treatment, @ 125 and 250 µg/mL in WTK-1 human lymphoma cell lines and mean migration of 50 nuclei increased significantly, after treating them with ciprofloxacin @ 1000 µg/mL. Genotoxic activity was found to be

concentration- and time-dependent in comet assay and activity was increased with the length of the treatment period **(Itoh *et al*., 2006).**

4.7 Pathological studies

4.7.1 Gross pathology

No gross lesions could be recorded in liver of control group I. In group II rats, liver was reddish in colour and slightly increased in size. In groups IV and VI rats there was a nodule formation in liver (Plate 4.9). Kidneys of groups II and VI were swollen, slightly reddish in coloration and congested (plate 4.8). Kidneys of group IV and VI showed dark colour changes. Kidneys of rats of other groups showed no significant ($P<0.05$) change. Spleen of group II rat were and reddish in colour. Spleen of all other groups did not reveal any untoward change. No gross lesions could be observed in brain of rats any of the groups.

4.7.2 Histopathological examination

No lesions could be recorded in the liver of groups I, III and V. Liver of group II rats showed mild congestion of large and small vessels, sinusoidal congestion leading to loss of sinusoidal spaces. Group IV and VI rats showed severe degeneration and swelling of hepatocytes throughout the parenchyma. Lesions in liver were severest in group VI followed by groups IV, II (Plates 4.10 and 4.11). These findings suggest thet these antibiotics have hepatotoxic action on higher doses.

No lesions could be recorded in kidneys of groups I, II, III, and V rats. Kidneys of group VI rats revealed congestion of large blood vessels and interstitial hemorrhages, vacuolation of glomeruli, coagulative necrosis of varying degrees in many of the kidney tubular epithelial (KTE) cells in many of tubules and infiltration of mononuclear cells in interstitium. Lesions were similar and of varying intensity in group IV rats. Lesions in kidneys were mainly seen in higher dose group rats (Plates 4.12 and 4.13). These changes evidenced some nephrotoxic effects in rats after 28 days.

Sureshkumar *et al*. (2013) observed degeneration of hepatocytes on day 1 post treatment and vacuolar degeneration on day 3, they also reported sinusoidal dilatation in poultry following administration of enrofloxacin @ 10mg/kg b wt for 5 days. He also

reported necrosis and degeneration of tubular epithelium with infiltration of esinophils in kidney after treatment with enrofloxacin.

Amal (2011) observed necrotic, central vein with perivenous leucocytic infiltration and congested blood sinusoids, ballooning of hepatocytes due to hydropic degeneration and focal necrosis in the course of the hepatic lobules and some degree of hepatic hyperatrophy following administration of enrofloxacin @ 75mg/kg b wt in rats for 10 days. The findings of the present study are in agreement with the above findings indicating enrofloxacin and ciprofloxacin induced hepatic toxicity at high doses.

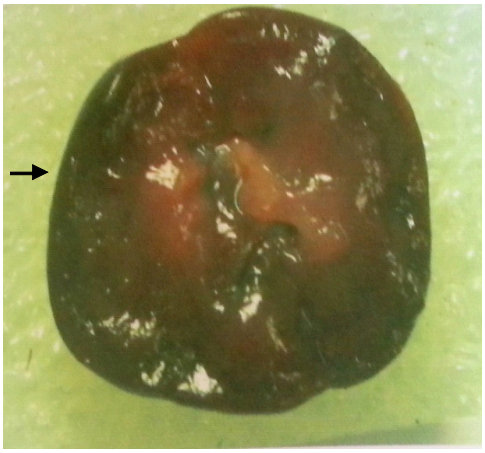


Plate 4.8: Photograph of kidney of group VI showing congestion of kidney cortex.

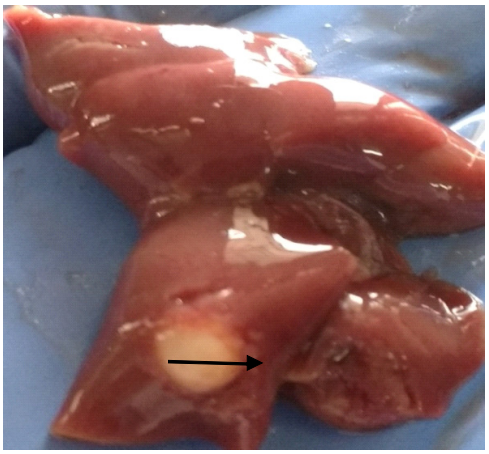


Plate 4.9: Photograph of liver of group VI showing nodule formation in liver.

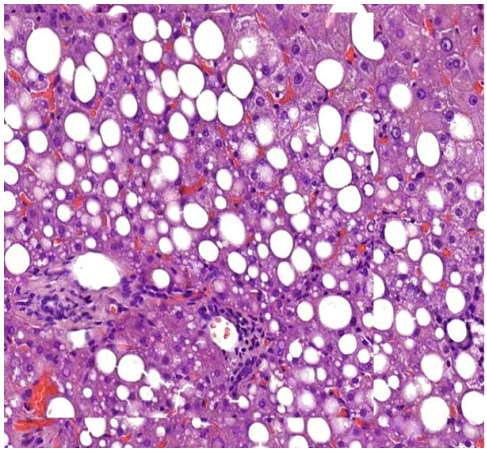


Plate 4.10: Photomicrograph of liver of group VI showing vacuolar degeneration of hepatocytes. H&E 400X

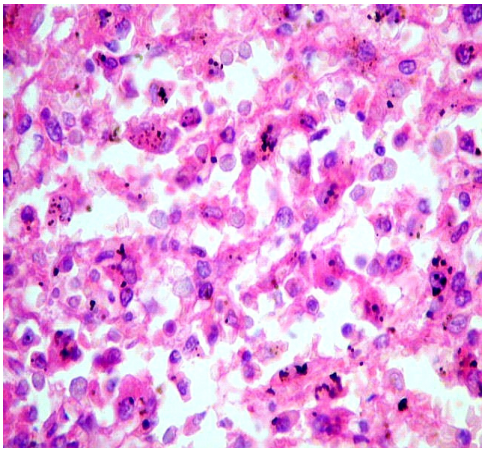


Plate 4.11: Photomicrograph of liver of group VI showing sinusoidal dilatation. H&E 400X

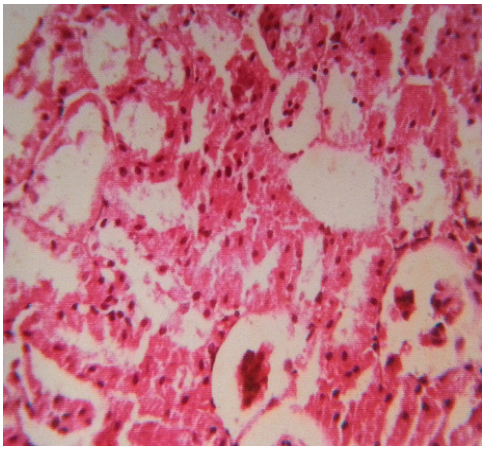


Plate 4.12: Photomicrograph of kidney of group VI showing necrotic epithelial lining in kidney. H&E 400X

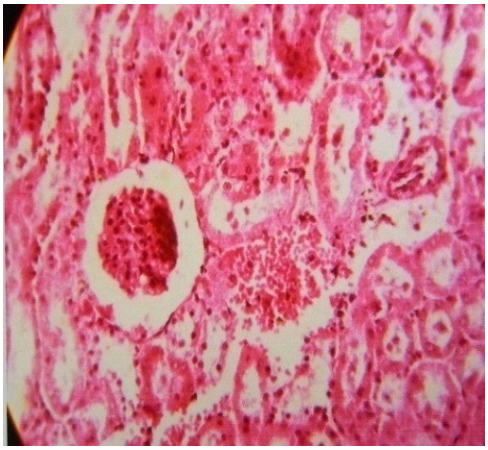


Plate 4.13: Photomicrograph of kidney of group VI showing degeneration and necrosis with condensation of glomerular tuft. H&E

Summary and Conclusions

Chapter 5 SUMMARY AND CONCLUSION

This study was undertaken to investigate the toxicological evaluation of enrofloxacin and ciprofloxacin with special reference to genotoxicity potential following administration of enrof1oxacin (75 mg/kg and 150 mg/kg b wt) and ciprofloxacin (50mg/kg and 100 mg/kg b wt) for 28 days and cyclophosphamide (20mg/kg b wt 24h prior to sacrifice) as a standard genotoxic drug in rats.

Toxicological evaluation of enrofloxacin and ciprofloxacin was carried out by estimating hematological and biochemical parameters whereas gentotoxic effects were determined by recording mincronucleus assay, chromosomal aberration assay and comet assay tests.

There was a significant ($P<0.05$) reduction in Hb concentration in the treatment groups from III to VI administered with enrofloxacin and ciprofloxacin at high and low doses in comparison to control after 28 days. However, cylcophosphamide treated group II showed non significant decrease in Hb level. A significant ($P<0.05$) reduction in PCV values was observed in the treatment groups from III to VI as compared to control group 28 days. The high dose groups showed significant ($P<0.05$) decline in PCV values as compared with low dose groups. A significant ($P<0.05$) reduction in TEC values was observed in the treatment groups from II to VI except in group III as compared to control group I 28 days. The low dose groups showed significant ($P<0.05$) increase in TEC values as compared with cyclophosphamide treated positive group II. A significant ($P<0.05$) reduction in MCV values was observed in the treatment groups from II to VI as compared to control group 28 days. A significant ($P<0.05$) reduction in MCH and MCHC value was observed in the treated groups from II to VI as compared to control group I after 28 days. There was a significant ($P<0.05$) reduction in TLC in all the treatment groups II to VI as compared to control group I after 28 days.

There was a significant ($P<0.05$) reduction in total serum proteins in all the treatment groups except group III after 28 days as compared to control group I. A significant ($P<0.05$) decrease in albumin level was observed in all the treatment groups as compared to control group I after 28 days except in group III. There was significant ($P<0.05$) reduction in the globulin values were observed in all the groups II

to VI. A significant (P<0.05) increase in A: G was observed in all the groups II to VI after 28 days.

A significant (P<0.05) decrease in blood glucose level was observed in all the treatment groups III to VI as compared to control group I. There was no significant (P<0.05) change was observed in lipid profile which included triglycerides, cholesterol in the treatment groups as compared to control group I after 28 days, however triglyceride and cholesterol values in treatment group increased non significantly as compared to control group.

Serum levels of BUN and creatinine did not vary significantly (P<0.05) in the treated groups except group V and VI this shows renal system was not affected much by these antibiotics. Group V and VI administered with ciprofloxacin @ 75mg/kg b wt and 150mg/kg b wt showed significant increase in BUN values. Significant (P<0.05) increase in creatinine values were observed in high dose groups IV and VI as compared to control group I.

Significant (P<0.05) increase in the levels of ALT and AST was observed in groups II and VI as compared to control group I after 28 days, however other groups did not showed any significant (P<0.05) change in values. ALP levels in serum did not vary significantly (P<0.05) in all the treatment groups as compared to control group I.

There was significant (P<0.05) increase in lipid peroxidation of RBCs in cyclophosphamide treated group II and low dose treated groups III and V was observed as compared to control group I.

There was significant (P<0.05) increase in GSH, SOD and catalase levels of RBCs in cyclophosphamide treated group II in comparison to group I. Low dose groups III and V showed significant (P<0.05) increase in GSH, SOD and catalase values than the higher dose groups IV and VI. A significant (P<0.05) increase in LPO status of liver, kidney, spleen and heart was observed by the treatment of cyclophosphamide group II as compared to control group I. Low dose groups III and V showed significant (P<0.05) increase in GSH, SOD and catalase values as compared to the higher dose groups IV and VI in liver, kidney, spleen and heart following 28 days of oral administration of enrofloxacin and ciprofloxacin in rats.

Assessment of genotoxic effect of enrofloxacin and ciprofloxacin in Wistar rats was conducted by employing micronucleus assay, chromosomal aberration and comet assay tests. In micronucleus assay the high dose groups IV and VI showed significant increase in the formation of micronuclei than the low dose groups III and V. Group II showed significant ($P<0.05$) increase in micronuclei count as compared to other groups. There was significant ($P<0.05$) decline in the formation of polychromatic erythrocyte count as compared to low dose groups. There was significant ($P<0.05$) increase in the in group II as compared to other groups III to VI. High dose group of ciprofloxacin induced significant ($P<0.05$) increase in chromosomal aberrations as compared to group V. The mean value of OTM/cell of comet assay increased in a dose dependent manner with a significantly ($P<0.05$) higher value in ciprofloxacin treated group VI. Groups III and V given with the low doses of enrofloxacin and ciprofloxacin did not show genotoxic effects but high dose groups IV and VI showed genotoxic effects but not up to the extent of positive control.

Grossly there were no apparent lesions observed in liver, kidney and spleen rat of control groups I and II, however, liver of group IV and VI rats revealed nodule formation. Liver showed mild congestion. In kidney there was mild interstitial congestion and few of the glomerular tufts were observed in low dose groups III and V. Groups IV and VI with high doses showed interstitial congestion. Glomeruli also showing degeneration and necrosis with condensation of the glomerular tuft and increased Bowman's space in the kidney. Liver showed marked congestion with degeneration of hepatocytes and sinusoidal dilatations.

Thus concluded from the present study that:

1. Enrofloxacin and ciprofloxacin altered hematological parameters like Hb, PCV, MCH, MCHC and TLC but not significantly as compared to cyclophosphamide after 28 days in rats.
2. Enrofloxacin and ciprofloxacin at high doses induced hepatic damage as evidenced by reduction in serum proteins and increase in enzymatic activity of ALT and AST.

3. At high doses enrofloxacin and ciprofloxacin induced mild nephrotoxicity as evidenced by increased BUN and creatinine values in high dose treatment groups.
4. High dose of enrofloxacin and ciprofloxacin induced oxidative stress in rats which was more in low dose groups than in high dose groups.
5. Enrofloxacin and ciprofloxacin induced genetic damage at high doses in rats.

Literature Cited

LITERATURE CITED

Aebi, H.E. 1988. Catalase. In: Bergemeyer H.U., Bergemeyer J., Garibi, M. (Ed), Methods of enzymatic analysis, Verlag Chemie, Weinhein. **3**(3):273-286.

Al-Ahmad, A., Daschner, F.D. and Kümmerer, K. 1999. Biodegradability of cefotiam, ciprofloxacin, meropenem, penicillin G, and sulfamethoxazole and inhibition of waste water bacteria. *Arch Environ Con Tox* **37**:158–163.

Albertini, S., Chetelat, A.-A., Miller.B., Muster, W., Pudjadas, E., Strobel, R. and Gocke, E.1995. Genotoxicity of 17 gyrase- and four mammalian topoisomerase II-poisons in prokaryotic and eukaryotic test systems. *Mutagenesis*, **10**:343-351.

Alberti, S., Schuster, G., Parini, P., Feltkamp, D., Diczfalusy, U., Rudling, M. and Gustafsson, J. Å. 2001. Hepatic cholesterol metabolism and resistance to dietary cholesterol in LXRβ-deficient mice. *The Journal of clinical investigation*, **107**(5):565-573.

Albesa, I., Becerra, M.C., Battan, P.C. and Paez, P.L. 2004. Oxidative stress involved in the antibacterial action of different antibiotics. *Biochem. Biophys. Res. Commun.* **317:** 605-609.

Alcalde, M., Donoso, M. S., Carda Diaz, M. and Puscosio Narvaezi, J. M. 1995. Liver Dysfunction Due to Ciprofloxacin, *Acta Gastroenterologica Belgica*, **58**(5): 475-476.

Al-Nazawi, M.H. 2008. Effects of Enrofloxacin and Marbofloxacin Administration on Some Fertility Parameters of Male Chicken. *Journal of Taibah University for Science*, **1**:1-4.

Altreuther, P. 1987. Data on chemistry and toxicology of Baytril. *Vet. Med. Rev*, **2**: 87-89.

Amacher, D.E. 2002. A toxicologist's guide to biomarkers of hepatic response. *Hum. Exp. Toxicol.* **21**: 253-262.

Amal, A. A. El, Daly. 2011. The Protective Effect of Green Tea Extract against Enrofloxacin Action on the Rat Liver; Histological, Histochemical and Ultrastructural studies. *Journal of American Science*, **7**(4):669-679

Ambulkar, P.S., Ghosh, S.K., Ingole, I.V. and Pal, A.K. 2009. Genotoxic and cytotoxic effects of antibacterial drug, ciprofloxacin, on human lymphocytes in vitro. *Nepal Med. Coll. J.* **11**(3): 147-151.

Anderson, D., Bishop, J.B., Garner, R.C., Ostrosky-Wegman. And Selby, P.B. 1995. Cyclophosphamide: review of its mutagenicity for a assessment of potential germ cell risks, *Mutat. Res*. **330**:115–81.

Anon. 1991. The Merck veterinary mannual. 7th edition. Mecrk & Co INC. Rahway, USA.1457-1460.

Anselmi, C., Bocchinfuso, G., De Santis, P. and Fuà, M. 1998. Statistical Thermodynamic Approach for Evaluating the Writhe Transformations in Circular DNAs. *The Journal of Physical Chemistry B*.**102**:5704-5714.

Aral, F., Karaçal, F. and Baba, F. 2008. The Effect of Enrofloxacin on Sperm Quality in Male Mice. *Research in Veterinary Science*. **84**:95-99.

Arivuchelvan, S., Murugesan. and Mekala, P.2013. Immunomodulatory effect of Withania somnifera in broilers treated with high doses of enrofloxacin. Indian *Journal of Drugs and Diseases*.**2** (4):278–294.

Aziz, A. A. 2005. The influence of enrofloxacin on hematological features, total cholesterol, blood glucose and body weight of broilers. *Alqadissyia Journal of Veterinary Science* **4**:29-33.

Banu, B., Danadevi, K., Jamil, K., Ahuja, Y.R., Rao, V.K. and Ishaq, M. 2001. In vivo genotoxic effect of arsenic trioxide in mice using comet assay, *Toxicology,* **162**(3):171-7.

Barski, D., Spodniewska, A. and Zasadowski, A. 2011. Activity of superoxide dysmutase, catalase and glutathione peroxidase in rats exposed to chlorpyrifos and enrofloxacin *Polish Journal of Veterinary Sciences,* **14**(4):523-529.

Basaran, A., Erol, K., Basaran, N., Güneş, H.V., Açikalin, E., Timuralp, G., Değirmenci, I., Cakmak, E.A. and Tomatir, A.G. 1993. Effects of ciprofloxacin on chromosomes, and hepatic and renal functions in rats. *Exper Chemother* **39**: 182-188.

Bauditz, R. 1987. The results of clinical testing with Baytril in dogs and cats. *Med. Vet.* N., **2**: 137-140.

Becerra, M.C. and Albesa, I. 2002. Oxidative stress induced by ciprofloxacin in *Staphylococcus aureus*. *Biochem. Biophys. Res. Commun.*, **297**: 1003-1007.

Belal, F., Al-Majed, A.A. and Al-Obaid, A.M.1999. Methods of analysis of 4-quinolone antibacterials. *Talanta*, **50**:765-786.

Benzer, F., Kılıc¸, A., Yılmaz, S., Eris, ¸ir, M., Timurkaan, N. and Ertas, ¸ H.B. 2009. Influence of enrofloxacin administration on oxidative stress and antioxidant enzyme activities of experimentally infected broilers with *Salmonella enteric serovar enteritidis.* E-Journal of New World Sciences Academy, *Veterinary Sciences*, **4**: 24-33.

Blum, Michael, D., David, J., Graham. and Carolyn, A. McCloskey. 1994.Temafloxacin syndrome: review of 95 cases. *Clinical Infectious Diseases,* **18**(6):946-950.

Boddy, A.V. and Yule, S.M. 2000. Metabolism and pharmacokinetics of oxazaphosphorines. *Clinical Pharmacokinetics*, **38**(4):291-304.

Bregante, M.A., Saez, P., Aramayona, J.J., Fraile, L., Garcia, M.A. and Solans, C., 1999. Comparative pharmacokinetics of enrofloxacin in mice, rats, rabbits, sheep, and cows. *American journal of veterinary research*, **60**(9):1111-1116.

Brown, S.A. 1996. Fluoroquinolones in animal health. *J. Vet. Pharmacol. Ther,* **19**: 1-14.

Caesar, M. and Stille, W.1984.The Chemotherapeutic Agents of the Nalidixic Acid Group, Munich: W. *Zuckschwerdt Verlag*.

Carreras, I., Castellari, M., GarciaRequeiro, J.A., Guerrero, L., Esteve-Garcia, E. and Sarraga, C. 2004. Influence of Enrofloxacin Administration and Alpha-Tocopheryl Acetate Supplemented Diets on Oxidative Stability of Broiler Tissues. Poult Sci, **83**(5): 796-802

Cester, C.C. and Toutain, P.L. 1997. A Comprehensive Model for Enrofloxacin to Ciprofloxacin Transformation and Disposition in Dog. *Journal of Pharmaceutical Sciences*, **86**:1148-1155.

Channa, M. A. and Janjua, M. Z. 2003. Effects of Ciprofloxacin on Fetal Hepatocytes, " *Journal of Pakistan Medical Association*, **53**(10): 448-450.

Chauhan, L.K.S., Pant, N., Gupta, S.K. and Srivastava, S.P. 2000. Induction of chromosome aberrations, micronucleus formation and sperm abnormalities in mouse following carboturan exposure, *Mutat. Res,* **465**: 123-129.

Cholongitas, E., Georgousaki, C., Spyrou, S. and Deisenaki, M. 2001. Ciprofloxacin Induce Acute Cholestatic Hepatic Jaundice, *Annuals of Hepatology*, **83**(4): 400-401.

Chong, S., Chen, C., Ge, H. and Xie, X.S. 2014. Mechanism of Transcriptional Bursting in Bacteria. *Cell*, **158**:314-326.

Christ, W., Lehnert, T. and Ulbrich, B. 1988. Specific toxicologic aspects of the quinolone. *Rev Infect Dis* **10**:603–606.

Ciaravino, V., Suto, M.J. and Theiss, J.C. 1993. High capacity in vitro micronu-cleus assay for the assessment of chromosome damage: Results with quinolone/naphthyridone antibacterials. *Mutat Res,* **298**:227–236.

Cohen, J.T. and Jao, J.Y. 1970. Enzymatic basis of cyclophosphamide activation by hepatic microsomes of the rat. *Journal of Pharmacology and Experimental Therapeutics*, **174**(2):206-210.

Committee for Medicinal Products for Veterinary Use (CVMP). 2007. Public Statement on the Use of (Fluoro) Quinolones in Food-Producing Animals in the European Union: Development of Resistance and Impact on Human and Animal Health.

Coulet, M., Morello, C., Cox, P. and Lohuis, J. 2005. Pharmacokinetics of Ibafloxacin in Healthy Cats. *Journal of Veterinary Pharmacology and Therapeutics*, **28**:37-44.

Curry, P.T., Kropko, M.L., Garvin, J.R., Fiedler, F.D. and Theiss, J.C. 1996. *In vitro* induction of micronuclei and chromosome aberrations by quinolones: Possible mechanisms. *Mutat Res* **352**:143–150.

Dautrey, S., Felice, K., Petiet, A., Lacour, B., Carbon, C. and Farinotti, R. 1999. Active Intestinal Elimination of Ciprofloxacin in Rats: Modulation by Different Substrates, *British Journal of Pharmacology*, **127**:1728-1734.

Demirer, T., Buckner, C.D. and Bensinger, W.I. 1996. Optimization of peripheral blood stem cell mobilization. *Stem Cells*, **14**(1):106-116.

Demuth, T.P., White, R.E., Tietjen, R.A., Storrin, R.J., Skuster, J.R. and Andersen, J.A. 1991. Synthesis and antibacterial activity of new C-10 quinolonyl-cephem esters. *J. Antibiot*. **44**: 200–209.

Dhinaa, A. N. and Palanisamy, P. K. J. 2010. Z-Scan technique: To measure the total protein and albumin in blood *Biomedical Science and Engineering.* **3**: 285-290.

Dichiara, A.J., Atkinson, M., Goodman, Z. and Sherman, K.E.2008. Ciprofloxacin-induced acute cholestatic liver injury and associated kidney injury. Case report and review. *Minerva Gastroenterol Dietol* **54**:307–15.

Domagala, J.M. 1994. Structure-activity and structure-side-effect relationships for the quinolone antibacterials. *J Antimicrob Chemo ther* **33**:685–706.

Donelli, M.G., Bartosek, I., Guaitani, A., Martini, A., Colombo, T., Pacciarini, M.A. and Modica, R.1976. Importance of pharmacokinetic studies on cyclophosphamide (NSC-26271) in understanding its cytotoxic effect. *Cancer Treatment Reports*, **60**(4):395-401.

Drlica, K., Engle, E.C., Manes, S.H. 1980.DNA gyrase on the bacterial chromosome: possibility of two levels of action, *Proc Natl. Acad. Sci. USA*. **77**:6879-83.

Dufour, D.R., Lott, J.A., Nolte, F.S., Gretch, D.R. and Koff, R.S. 2001. Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory tests in screening, diagnosis and monitoring. *Clin. Chem.* **47**: 1133-1135.

Durgut, Ramazan., Oktay, Hasan öztürk., Ahmet, Nacar., Aliye. and Sağkan-öztürk.2016. Cardiotoxic effects of enrofloxacin on electrophysiological activity, cardiac markers, oxidative stress, and haematological findings in rabbits. *Turkish Journal of Zoology*. **40**: 513-521.

Emadi, A., Jones, R.J. and Brodsky, R.A., 2009. Cyclophosphamide and cancer: golden anniversary. *Nature Reviews Clinical Oncology*, **6**(11):638-647.

Enckson, L.C., Bradley, M.O., Ducore, J.M., Ewig, R.A.G. and Kohn, K.W. 1980. DNA crosslinkig and cyotoxicity in normal and transformed human cells treated with antitumor nitrosoureas. *Proc. Natl. Acad. Sei.* USA, **77**:467-471.

Fass, D., Bogden, C.E., Berger, J.M. 1999. Quaternary changes in topoisomerase II may direct orthogonal movement of two DNA strands, *Nat. Struct. Biol*. 6:322-326.

Fauchier, N. 2013. The collection of specialties for veterinary use. Med'Vet, 2014th Edition, Med'com, Paris.

Ferguson, L.R. and Baguley, B. 1994.Topoisomerase II enzymes and muta-genicity. *Environ Mol Mutagen* **24**:245–261.

Ferguson, L.R. and Whiteside, G. 1995. Detection of aneuploidy after treatment by amsacrine and other topoisomerase II poisons. In Abbondandolo A, Vig BK, Roi R (eds): ‘‘Proceedings on Chromosome Segregation and Aneuploidy.’’ IST (Instituto Nazionale per la Ricerca sul Cancro) Largo Rosanna Benzi, 10 Genova Edizi one 1996.

Frank, S. I. R., Pra, D., Erdtmann, B., Henriques, A. P., and Silva, J. D.2005. Influence of orange juice over the genotoxicity induced by alkylating agents: an *in vivo* analysis. *Mutagenesis,* **20**:279-283.

Fraser, A.G. and Harrower, A.D.B. 1977. Convulsions and hyperglycaemia associated with nalidixic acid. *Br. Med. J.* **2**:1518.

Gelatt, K.N., Van Der Woerdt, A., Ketring, K.L., Andrew, S.E., Brooks, D.E. and Biros, D.J. 2001. Enrofloxacin- Associated Retinal Degeneration in Cats. *Veterinary Ophthalmology*, **4**:99-106.

Gellert, M., Mizuuchi, K., O’Dea, M.H. and Nash, H.A. 1976. DNA Gyrase: An Enzyme that Introduces Superhelical Turns into DNA. *Proceedings of the National Academy of Sciences of the United States of America*, **73**:3872-3876.

Ghaly, F. M., Dina, M., Baraka., Shahira, H., Mahmoud., ohair, S., Abd EL-Salam., Somayah, M. Awad. and Abeer, A. EL-Makhzangy.2015. Efficacy of Ciprofloxacin and Clove Extract on Bacterial Infection of Clarias gariepinus. *Middle East Journal of Applied Sciences* **5**(1): 01-09.

Gibson, D.P., Mo, X., Switzer, A.G., Murphy, V.A. and Ardema, M.J.1998. Comparative genotoxicity of quinolone and quinolonyl-lactam antibacterials in the in vitro micronucleus assay in Chinese hamster ovary cells. *Environ. Mol. Mutagenesis* **31**:345–351.

Golet, E.M., Alder, A.C., Hartmann, A., Ternes, T.A. and Giger, W. 2001. Trace determination of fluoroquinolone antibacterial agents in urban wastewater by solid-phase extraction and liquid chromatography with fluorescence detection. *Anal. Chem.,* **73**:3632–3638.

Golet, E.M., Strehler, A., Alder, A.C. and Giger, W. 2002. Determination of fluoroquinolone antibacterial agents in sewage sludge and sludge treated soil using accelerated solvent extraction followed by solid phase extraction. *Anal. Chem.* **74**:5455–5462.

Golet, E.M., Xifra, I., Siegrist, H., Alder, A.C. and Giger, W. 2003. Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. *Environ. Sci. Technol.* **37**:3243–3249.

Gorla, N.B., Ledesma, O.S., Barbieri, G.P.1989. Thirteenfold increase of chromosomal aberrations non-randomly distrubuted in chagasic children treated with nifurtimox. *Mutat. Res.* **224**: 263-267.

Gorla, N., Garcia Ovendo, H. and Larripa, I.1999. Chromosomal aberrations in human lymphocytes exposed in vitro to enrofloxacin and ciprofloxacin. *Toxicol. Lett*. **104**:43–48.

Goswami, M., Mangoli, S.H. and Jawali, N. 2006.Involvement of Reactive Oxygen Species in the Action of Ciprofloxacin against Escherichia coli." *Antimicrobial Agents and Chemotherapy* **50** (3): 949-954.

Gough, A.W., Barsoum, N.J., Renlund, R.C., Sturgess, J.M. and De, La, Iglesia, F.A.1985. Fine structural changes during reparative phase of canine drug-induced arthropathy. *Vet. Pathol.* **22**:82-84.

Gowda, S., Desai, P. B., Kulkarni, S. S., Hull, V. V., Math, A. A. and Vernekar, S. N. 2010. Markers of renal function tests. *N. Am. J. Med. Sci.*, **2**(4): 170-173.

Greene, C.E., Brockus, C.W., Currin, M.P. and Jones, C.J. 2002. Infection with Basidiobolus ranarum in two dogs. *Journal of the American Veterinary Medical Association*, **221**(4):528-532.

Gubaev, A. and Klostermeier, D. 2014. The Mechanism of Negative DNA Supercoiling: A Cascade of DNA-Induced Conformational Changes Prepares Gyrase for Strand Passage. *DNA Repair*, **16**:23-34.

Gürbay, A., Garrel, D., Osman, M., Richard, M.J., Favier, A. and Hincal, F. 2002. Cytotoxicity in ciprofloxacin-treated human fibroblast cells and protection by vitamin E. *Hum Exp Toxicol*. **21**:635.

Hall, A.G. and Tilby, M.J.1992. Mechanisms of action of, and modes of resistance to, alkylating agents used in the treatment of haematological malignancies. *Blood Rev.* **6**: 163–173.

Halling-Sørensen, B., Holten, H.C., Lützhøft, A.H.R. and Ingerslev, F. 2000. Environmental risk assessment of antibiotics: comparison of mecillinam, trimethoprim and ciprofloxacin. *J. Antimicrob. Chemoth.* **46**:53–58.

Halliwell, B. 1992. Reactive oxygen species and the central nervous system. *J. Neurochem.*, **59**:1609-1623.

Hamilton-Miller, J.M.T. 1994. Dual-action antibiotic hybrids. *J. Antimicrob Chemother.* **33**:197–200.

Hane, M.W., Wood, T.H. 1969.*Escherichia coli* K-12 mutants resistant to nalidixic acid: genetic mapping and dominance studies. *J. Bacteriol.* **99**:238-41.

Hartmann, A., Golet, E.M., Gartiser, S., Alder, A.C., Koller, T. and Widmer, R.M. 1999. Primary DNA damage but not mutagenicity correlates with ciprofloxacin concentrations in German hospital wastewaters. *Arch Environ Con Tox* **36**: 115–119.

Haubitz, M., Bohnenstengel, F., Brunkhorst, R., Schwab, M., Hofmann, U. and Busse, D.2002. Cyclophosphamide pharmacokinetics and dose requirements in patients with renal insufficiency. *Kidney International*, **61**(4):1495-1501.

Hayashi, M., Sofuni and lshidate. 1983. An application of acridine orange fluorescent staining to the micronucleus test. *Mutat. Res.,* **120**: 241-247.

Hayem, G., Petit, P.X., Levacher, M., Gaudin, C., Kahn, M.F. and Pocidalo, J.J. 1994. Cytofluorometric Analysis of Chondrotoxicity of Fluoroquinolone Antimicrobial Agents. *Antimicrobial Agents and Chemotherapy*, **38**:243-247.

Heddle, J.A. and Salamone, M.F. 1981. Chromosomal Aberrations and Bone Marrow Toxicity. *Environmental Health Perspectives,* **39**:23-27.

Herbold,.B.A., Brendler-Schwaab, S.Y., Ahr, H.J. 2001. Ciprofloxacin: in vivo genotoxicity studies. *Mutat. Res*. **498**: 193-205.

Hooper, C. D. 2001. Mechanisms of Action of Antimicrobials Focus on Fluoroquinolones. *Clin Infect Dis*. **32**: 9-15.

Horowitz, D.S. and Wang, J.C. 1987. Mapping the Active Site Tyrosine of *Escherichia coli* DNA Gyrase. *The Journal of Biological Chemistry*, **262**: 5339-5344.

Hosseinimehr, S. J., Azadbakht, M., and Jahanabadi, J.2008. Protective effect of hawthorn extract against genotoxicity induced by cyclophosphamide in mouse bone marrow cells. *Environmental Toxicology and Pharmacology,* **25**:51-56.

Hwang, Y.H., Kim, M.S., Song, I.B., Lim, J.H., Park, B.K. and Yun, H.I. 2009. Altered Pharmacokinetics of Enrofloxacin in Experimental Models of Hepatic and Renal Impairment. *Veterinary Research Communications*, **33**:481-487.

Ibrahim, I.A., Abd-El-Rehim, A., Ali, A.G. and Dalia, M.2011. Biochemical Study on the Effect of Some Antibiotics in Blood of Chicken. *SCMVJ* **16**(2):69-74.

Idowu, O.R., Peggins, J.O., Cullison, R. and von Bredow, J. 2010. Comparative Pharmacokinetics of Enrofloxacin and Ciprofloxacin in Lactating Dairy Cows and Beef Steers Following Intravenous Administration of Enrofloxacin. *Research in Veterinary Science*, **89**:230-235.

Ingham, B., Brentnall, D.W., Dale, E.A. and McFadzean, J.A. 1977. Arthropathy induced by antibacterial fused N-alkyl-4-pyridone-3carboxylic acids. *Toxicol. Lett.* **1**:21-26.

Isabella, L. Karle., Jean, M. Karle., William, Egan., Gerald, Zon. And Joan, A. Brandt.1977. Absolute configuration of (+)-cyclophosphamide. A crystal and molecular structure determination by x-ray diffraction. *J. Am. Chem. Soc*., **99** (14):4803–07.

Itoh, T., Mitsumori, K., Kawaguchi, S. and Sasaki, Y.F. 2006. Genotoxic potential of quinolone antimicrobials in the in vitro comet assay and micronucleus test. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, **603**(2):135-144.

Jena, G.B., Kaul, C.L. and Ramarao, P. 2002. Genotoxicity testing, a regulatory requirement for drug discovery and development: Impact of ICH guidelines. *Indian Journal of Pharmacology,* **34**: 86-99.

Johnson, G.M., Upman, P.J. and Wailin, R.F. 1998. A Practical Guide to ISO 10993-3: Genotoxicity. *Medical Device & Diagnostic Industry Magazine*.

Jun, Y.T., Kim, H.J., Song, M.J., Lim, J.H., Lee, D.G., Han, K.J., Choi, S.M., Yoo, J.H., Shin, W.S. and Choi, J.H. 2003. In vitro effects of ciprofloxacin and roxithromycin on apoptosis of Jurkat T lymphocytes. *Antimicrob Agents Chemother.* **47(3):**1161–1164.

Kaartinen, L., Salonen, M., Alli, L. and Payorala, S. 1995. Pharmacokinetics of enrofloxacin after single intravenous, intramuscular and subcutaneous injection in lactating cows. *J. vet. Pharma. Therpaeutics.***18**:357-362.

Kashida, Yoko., Yu, F., Sasaki, Koh-ichi, Ohsawa, Natsue, Y., okohama, Akiko, Takahashi., Takao, Watanabe. and Kunitoshi, Mitsumori.2002. Mechanistic Study on Flumequine Hepatocarcinogenicity Focusing on DNA Damage in Mice. *Toxicol. Sci.,* **69** (2): 317-321.

Kato, J., Nishimura, Y., Imamura, R., Niki, H., Hiraga, S. and Suzuki, H. 1990. New Topoisomerase Essential for Chromosome Segregation in *E. coli*. *Cell*. **63**:393-404.

Kirsch-Volders, M. 1997. Towards a validation on the micronucleus test. *Mutat Res* **392**:1–4.

Klecak, G., Urbach, F. and Urwyler, H. 1997. Fluoroquinolone antibacterials enhance UVA-induced skin tumors. *J Photochem Photobiol B* **37 (3):**174–181.

Kohanski, M.A., Dwyer, D.J., Hayete, B., Lawrence, C.A. and Collins, J.J. 2007. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell*, **130**: 797-810.

Kovarsky, J. 1983. Clinical pharmacology and toxicology of cyclophosphamide: emphasis on use in rheumatic diseases. In *Seminars in arthritis and rheumatism* WB Saunders. **12**(4):359-372.

Kümmerer, K. 2001. Drugs in the environment: emission of drugs, diagnostic aids, and disinfectants into wastewater by hospitals in relation to other sources—a review. *Chemosphere,* **45**:957–969.

Lemus, J.A., Blanco, G., Arroyo, B., Martinez, F. and Grande, J. 2009. Fatal embryo chondral damage associated with fluoroquinolones in eggs of threatened avian scavengers. *Environ. Pollut.* **157**:2421–2427.

Levine, C., Hiasa, H. and Marians, K.J. 1998. DNA Gyrase and Topoisomerase IV: Biochemical Activities, Physiological Roles during Chromosome Replication, and Drug Sensitivities. *Biochimica et Biophysica Acta* (*BBA*)-*Gene Structure and Expression*. **1400**:29-43.

Lillie, R.D. 1965. *Histopathological Technique and Practical Histochemistry. New York, USA. The Blackinton Co. Inc.*

Lindberg, R.H., Olofsson, U., Rendahl, P., Johansson, M.I., Tysklind, M. and Andersson, B.A.V. 2006. Behavior of fluoroquinolones and thrimethoprim during mechanical, chemical, and active sludge treatment of sewage water and digestion of sludge. *Environ. Sci. Technol.*, **40**:1042–1048.

Lykkesfeldt, J. and Svendsen, O. 2007. Oxidants and antioxidants in disease: oxidative stress in farm animals. *Vet. J.* **173**: 502-511.

Madesh, M. and Balasubramanian, K.A. 1998. Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. *Indian. J. Biochem. Biophys*. **35**: 184-88.

Malhi, P.K. and Grover, L.S. 1987. Genotoxic effects of some organophosphorus pesticides II *in vivo* chromosomal aberration bioassay in bone marrow cells in rat. *Mutat. Res.,* **188**: 45-51.

Mamber, S.W., Kolek, B., Brookshire, K.W., Bonner, D.P. and Fung-Tomc, J. 1993. Activity of quinolones in the Ames *Salmonella* TA 102 mutagenicity test and other bacterial Genotoxicity assays, *Antimicrob. Agents Chemother*. **37** : 213–217.

Mann, F. C. and Bollman, J. L. 1933. Studies on the physiology of the liver. 24. The effect of insulin on the blood sugar following total removal of the pancreas and liver. *American Journal of Physiology*, **103**: 45-47.

Martinez, M., McDermott, P. and Walker, R. 2006. Pharmacology of the Fluoroquinolones: A Perspective for the Use in Domestic Animals. *The Veterinary Journal.* **172:**10-28.

Marzin, D. 1997. The position of the in vitro micronucleus test within the battery of screening for genotoxic potential determination and regulatory guidelines. *Mutat Res,* **392**:175–181.

Maślanka, T., Jaroszewski, J.J., Mikołajczyk, A. and Rotkiewicz, T. 2009. Effect of Increasing Doses of Enrofloxacin on Chicken Articular Cartilage. *Polish Journal of Veterinary Sciences*, **12**:21-33.

Merschsundermann, V., Hauff, K., Braun, P., Lu, W. and Hof, H.1994.DNA-damage caused by antibiotic drugs - quinolones. *International Journal of Oncology*, **5**: 855-859.

Miller, B., Albertini, S., Locher, F., Thybaud, V. and Lorge, E. 1997.Comparative evaluation of the in vitro micronucleus test and the in vitro chromosome aberration test: Industrial experience. *Mutat. Res.* **392**:45–49.

Mitelman, F., Kolnig, A.M. and Str.mbeck, B.1988. No cytogenetic effects of quinolone treatment in humans. *Antimicrob Agents Chemother.* **32**: 936-937.

Moein, S., Farzami, B., Khaghani, S., Moein, M.R. and Larijani, B. 2007. Antioxidant properties and prevention of cell cytotoxicity of Phlomis persica Boiss. *DARU*. **15**(2): 83-88.

Morais, M.M., Belarmino-Filho, J.N., Brito, G.A.C. and Ribeiro, R.A. 1999. Pharmacological and histopathological study of cyclophosphamide-induced hemorrhagic cystitis-comparison of the effects of dexamethasone and Mesna. *Brazilian Journal of Medical and Biological Research*, **32**(10):1211-1215.

Mukherjee, A., Sen, S. and Agarwal, K. 1993. Ciprofloxacin: mammalian DNA topoisomerase type-II poison *in vivo*. *Mut. Res*. **301:**87–92.

Mulligan, L.T.1997. Toxicological evaluation of certain veterinary drug residues in food WHO food additives series 39, 889. Center for Veterinary Medicine, Food and Drug administration, Rockville, Maryland, USA.

Murata, M., Suzuki, T., and Midorikawa, K.2004. Oxidative DNA damage by a hydroperoxide derivative of cyclophosphamide. *Free Radical Biology and Medicine*, **37**:793-802.

Nathwani, R.A., Pais, S., Reynolds, T.B. and Kaplowitz, N. 2005. Serum alanine aminotransferase in skeletal muscle diseases. *Hepatology.* **41**: 380-382.

Neer, T. M. 1988. Clinical pharmacologic features ¯uoroquinolone antimicrobial drugs. *J. Am. Vet. Med. Assoc.* **5**:577-580.

Neuman, M. 1986. Comparative Pharmacokinetic Parameters of New Systemic Fluoroquinolones. *International Journal of Clinical Pharmacology Research*, **7**:173-179.

Nielsen, P. and Gyrd-Hansen, N. 1997. Bioavailability of Enrofloxacin after Oral Administration to Fed and Fasted Pigs. *Pharmacology & Pharmaceutical Medicine*, **80**:246-250.

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. *Veterinary Quarterly*, **10**:156-163.

OECD. 2010. OECD guidelines for the testing of chemicals, section 4: Health effects. Test No. 487: In Vitro Mammalian Cell Micronucleus Test,1-23.

Ogino, T., Mizuno, Y., Ogata, T. and Takahashi, Y. 2005. Pharmacokinetic Interactions of Flunixin Meglumine and Enrofloxacin in Dogs. *American Journal of Veterinary Research*, **66:**1209-1213.

Olive P.L., Banath, J.P. and Durand, R.E.1990. Heterogeniety in radiation-induced DNA damage and repair in tumour and normal cells measured using the "Comet" assay. *Radiat. Res*., **122**:86-94.

Oridupa, A.O., Omobowale, T.O., Abiola, J.O., Azeez, I. O. and Ajibade, T.O. 2013. Effect of Ciprofloxacin and Levofloxacin on haematological parameters of dogs. *Afr. J. Biomed. Res.* **16** :25-*29*

Packman, C.H. 2001. Drug-related immune haemolytic anemia. In: Williams Hematology, Ernest Beutler, Marshall A. Lichtman, Barry S. Coller, Thomas J. Kipps, and Uri Seligsohn (eds), Chapter 57, McGraw-Hill Publishers.

Palma-Carlos, A. G., Palma-Carlos, M. L. and Martins-Silva, J. A. 1971. Effect of alkylating agents on haem metabolism. *South African medical journal. Suid-Afrikaanse Tydskrif vir Geneeskunde*, 81-84.

Papich, M.G. and Riviere, J.E. 2009. Fluoroquinolone antibacterialdrugs. *J.Veterinary Pharmacology and Therapeutics.* **9**:983-1011.

Pasquali, F. and Manfreda, G. 2007. Mutant Prevention Concentration of Ciprofloxacin and Enrofloxacin against *Escherichia coli*, Salmonella Typhimurium and Pseudomonas Aeruginosa. *Veterinary Microbiology*, **119**:304-310.

Peng, H. and Marians, K.J. 1993. Decatenation Activity of Topoisomerase IV during oriC and pBR322 DNA Replication *in Vitro*. *Proceedings of the National Academy of Sciences of the United States of America*, **90**:8571-8575.

Peng, H. and Marians, K.J. 1995. The Interaction of *Escherichia coli* Topoisomerase IV with DNA. *The Journal of Biological Chemistry*, **270**: 25286-25290.

Pierce, A. and Nester, T. 2011. Pathology consultation on drug-induced hemolytic anemia. *Am. J. Clin. Path.* **136**:7-12.

Pino, A. 1995. Induction of sperm abnormalities and dominant lethal effects in mice treated with ciprofloxacin. *Med. Sci. Res.* **23**: 321- 322.

Pourabbas, Saeed. and Adel, Feizi. 2015. Clinical investigation, autopsy and some biochemical indices in broiler chickens following treatment with enrofloxacin angoin study by colibacillosis. *Inter.journal of biology pharm. Allied Sci*., **4(2):** 719-727.

Prins, H.K. and Loos, J.A. 1969. Glutathione. In J. J. Yunis, editor. Biochemical methods in Red cell Genetics. *Academic Press, New York.* pp.115-137.

Priyadharshini, K.M. 2013. Ciprofloxacin induced body weight and haemotological changes in rats and anti-oxidant vitamin a, c and e as rescue agents. *International Journal of Engineering Science Invention.* **2**(2):*21-31.*

Rahal, A., Kumar, A., Ahmad, A.H., Malik, J.K. and Ahuja, V. 2006. Pharmacokinetics of enrofloxacin in sheep following intravenous and subcutaneous administration. *Journal of Veterinary Pharmacology and Therapeutics*, **29**(4): 321-324.

Ralph, R.K., Judd, W., Pommie, r Y. and Kohn, K.W.1994. DNA topoisomerases, in: S. Neidle and M. Waring (Eds.), Topics in Molecular and Structural Biology. *Molecular Aspects of Anticancer Drug-DNA Interactions,* **2(1):**1–95.

Rao, G.S., Ramesh, S., Ahmad, A.H., Tripathi, H.C., Sharma, L.D. and Malik, J.K. 2002. Pharmacokinetics of Enrofloxacin and Its Metabolite Ciprofloxacin in Goats Given Enrofloxacin Alone and in Combination with Probenecid. *The Veterinary Journal*, **163**:85-93.

Rao, K.P.S. and Narayana, K. 2005. In vivo chromosome damaging effects of an inosin monophosphate dehydrogenase inhibitor : Rivavirin in mice. *Indian J. Pharmacol,* **37**(2): 90-95.

Rehman, S.U. 1984. Lead induced regional lipid peroxidation in brain. *Toxicol. Lett.* **21**: 333-337.

Reifferscheid, G., Ziemann, C., Fieblinger, D., Dill, F., Gminski, R., Grummt, H.J., Hafner, C., Hollert, H., Kunz, S., Rodrigo, G., Stopper, H. and Selke, D.2008. Measurement of genotoxicity in wastewater samples with the in vitro micronucleus test—results of a round-robin study in the context of standardisation according to ISO. *Mutat Res* **649**:15–27.

Rishi, K.K. and Grewal, S. 1995. Chromosome aberration test for the insecticide dichlorvos, on fish chromosomes. *Mutation Research/ Genetic Toxicology,* **344**:1-2.

Rosner, M. H., & Bolton, W. K. 2006. Renal function testing. *American Journal of Kidney Diseases*, **47**(1): 174-183.

Saukkonen, J. J., Cohn, D. L., Jasmer, R. M., Schenker, S., Jereb, J. A., Nolan, C. M., and Bernardo, J. (2006). An official ATS statement: hepatotoxicity of antituberculosis therapy. *American Journal of Respiratory and Critical Care Medicine*, **174**(8): 935-952.

Sayed, M. Rawi., Iman, M. Mourad., Nadia, M. S., Arafa. and Naema, I. Alazabi. 2011. Effect of ciprofloxacin and levofloxacin on some oxidative stress parameters in brain regions of male albino rats. *African Journal of Pharmacy and Pharmacology* **5(16)**: 1888-1897.

Scandalios, J. G. 1993. Oxygen stress and superoxide dismutases. *Plant Physiology*, **101**(1): 7.

Sedalk, J. and Lindsay, R. H. 1968. Estimation of total protein bound and non protein bound sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem*. **25**: 192-205.

Seedher, N. and Agarwal, P. 2009. Various Solvent Systems for Solubility Enhancement of Enrofloxacin. *Indian Journal of Pharmaceutical Sciences*, ***71***(1), 82–87.

Selvakumar, E., Prahalathan, C., Mythili, Y. and Varalakshmi, P. 2005. Mitigation of oxidative stress in cyclophosphamide-challenged hepatic tissue by DL-alpha-lipoic acid. *Mol. Cell. Biochem.* **272**: 179-185.

Senthilkumar, S., Devaki, T., Manohar, B. M., & Babu, M. S. 2006. Effect of squalene on cyclophosphamide-induced toxicity. *Clinica Chimica Acta*, **364**(1): 335-342.

Shailer, P.A., Randinitis, E. and Rausch, G.1997. Effect of clinafloxacin on serum insulin and glucose levels in healthy subjects, *Clin. Pharm. Ther.*, **61**:148.

Shaki, F., Ashari, S. and Ahangar, N. 2016. Melatonin can attenuate ciprofloxacin induced nephrotoxicity: Involvement of nitric oxide and TNF-α. *Biomedicine & Pharmacotherapy*, **84**:1172-1178.

Shayne, C. Gad and Marian, G. McCord 2008. Safety Evaluation in the Development of Medical Devices and Combination Products. Third Edition ISBN: 9781420071641. p 218.

Shoorijeh, S.J., Tamadon, A., Vahedi, M. and Behzadi, M.A.2012. Hematological and biochemical alterations due to over dosage of enrofloxacin in cats. *Pak. Vet. J.*, **32**(1):73-76.

Sies, H. 1991. Oxidative stress: from basic research to clinical application. *Am. J. Med*., **30**: 31-38.

Simon, C. and Stille, W. 1993. Antibiotic therapy in Clinic and 8. Auage, Schlattauer, Stuttgart New York.

Singh, N.P., McCoy, M.T., Tice, R.R. and Schneidez, E.L. 1988. A simple technique for quanlitation of low levels of DNA damage in individual cells. *Exp. Cell Res,* **175**: 184- 191.

Sisca, T.S., Heel, R.C. and Romankiewicz, J.A.1984. Cinoxacin: a review of its pharmacological properties and therapeutic efficacy in the treatment of urinary tract infections. *Drugs* **25**:544-69.

Snover, D.C., Weisdorr, S. Bloomer, J., McGlave, P. and Weisdorr, D. 1989. Nodular regenerative hyperp lasia of the liver following bone marrow transplantation. *Hepatology.* **9**: 443-448.

Speit, G. and Hartman, A. 2005. The Comet Assay: A Sensitive Genotoxicity Test for the Detection of DNA Damage and Repair, DNA Repair Protocols: Mammalian Systems. *Methods in Molecular Biology,* **314**: 275-286.

Stahlmann, R. 2003. Children as a special population at risk—quinolones as an example for xenobiotics exhibiting skeletal toxicity. *Arch. Toxicol*. **77**:7–11.

Stahlmann, R. and Lode, H. 1998. Safety of quinolones. *Lancet* **352**:1313.

Steinman, A., Britzi, M., Levi, O., Lavy, E., Lichter, A. and Soback, S. 2006 Lack of Effect of Diet on the Pharmacokinetics of Enrofloxacin in Horses. *Journal of Veterinary Pharmacology and Therapeutics*, **29**:67-70.

Storms, R. W., Trujillo, A. P., Springer, J. B., Shah, L., Colvin, O. M., Ludeman, S. M. and Smith, C. 1999. Isolation of primitive human hematopoietic progenitors on the basis of aldehyde dehydrogenase activity. *Proceedings of the National Academy of Sciences*, **96**(16): 9118-9123.

Sumano, L.H., Gutierrez, O.L., Aguilera, R., Rosiles, M.R., Bernard, B.M.J. and Gracia, M.J. 2004. Influence of Hard Water on the Bioavailability of Enrofloxacin in Broilers. *Poultry Science*, **83**:726-731.

Sureshkumar, V., Sarathchandra, G. and Ramesh, J. 2013. Biochemical, histopathological and ultra structural profile after pulsed water medication of enrofloxacin in broiler chickens, *Vet World* **6**(9): 668-673.

Swanson, B. N., V. K. Boppana, P. H. Vlasses, H. H. Rotmensch, and B. K. Ferguson. 1983. Norfloxacin disposition after sequentially increasing oral doses. *Antimicrob. Agents Chemother.* **23**:284-288.

Takayama, S., Hirohashi, M., Kato, M. and Shimada, H. 1995. Toxicity of quinolone antimicrobial agents. *J. Toxicol. Environ. Health.* **45**: 1-45.

Veerareddy, Talla, V. 2011. Oxidative stress induced by fluoroquinolones on treatment for complicated urinary tract infections in Indian patients. *J. Young Pharmacists*. **3**:304-9.

Tessa, Trouchon., Sébastien, Lefebvre. 2016. A Review of Enrofloxacin for Veterinary Use. *Journal of Veterinary Medicine.* **6**:40-58.

Thorsteinsson, S.B., Bergan, T., Oddsdottir, S., Rohwedder, R. and Holm, R. 1986. Crystalluria and ciprofloxacin, influence of urinary pH and hydration. *Chemotherapy, 32*:408–17.

Tice, R.R. Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, R., Miyamae, Y. and Rojas, E. 2000. Single cell gel/ comet assay: guideline for in vitro and in vivo genetic toxicology testing. *Environmental and Molecular Mutagenesis,* **35:** 206-221.

Traş, B., Maden, M., Baş, A. L., Elmas, M., Yazar, E. and Civelek, T. 2001. Investigation of Biochemical and Haematological Side-effects of Enrofloxacin in Dogs. *Journal of Veterinary Medicine Series A*, **48**: 59–63.

Tse, Y.C., Kirkegaard, K. and Wang, J.C. 1980. Covalent Bonds between Protein and DNA. Formation of Phosphotyrosine Linkage between Certain DNA Topoisomerases and DNA. *The Journal of Biological Chemistry*, **255**: 5560-5565.

Tyczkowska, K., Hedeen, K.M., Aucoin, D.P. and Aronson, A.L. 1989. High-Performance Liquid Chromatographic Method for the Simultaneous Determination of Enrofloxacin and Its Primary Metabolite Ciprofloxacin in Canine Serum and Prostatic Tissue. *Journal of Chromatography B*: *Biomedical Sciences and Applications*, **493**:337-346.

Umezawa, N., Arakane, K., Ryu, A., Mashiko, S., Hirobe, M. and Nagano, T. 1997. Participation of reactive oxygen species in phototoxicity induced by quinolone antibacterial agents. *Arch. Biochem. Biophys.* **342**: 275-281.

Von Rosenstiel, N. and Adam, D.1994. Quinolone antibacterials: An update of their pharmacology and therapeutic use. *Drugs.* **47(6):**872–901.

Wang, J.C.1998. Moving One DNA Double Helix through Another by a Type II DNA Topoisomerase: The Story of a Simple Molecular Machine. *Quarterly Reviews of Biophysics*, **31**:107-144.

Westropp, J.L., Sykes, J.E., Irom, S., Daniels, J.B., Smith, A. and Keil, D. 2012. Evaluation of the Efficacy and Safety of High Dose Short Duration Enrofloxacin Treatment Regimen for Uncomplicated Urinary Tract Infections in Dogs. *Journal of Veterinary Internal Medicine*. **26**:506-512.

Wiernik, P. and Duncan, J.H. 1971. Cyclophosphamide in human milk. *The Lancet*, **297**(7705):912.

Wiuff, C., Lykkesfeldt, J., Aarestrup, F.M. and Svendsen, O. 2002. Distribution of Enrofloxacin in Intestinal Tissue and Contents of Healthy Pigs after Oral and Intramuscular Administrations. *Journal of Veterinary Pharmacology and Therapeutics*, **25**:335-342.

Wolfson, I.S. and Hooper, D.C. 1985. The Fluoroquinolones: Structure, Mechanisms of Action and Resistance, and Spectra of Activity *in Vitro. Antimicrob. Ag. Chemother.*, **28**: 581- 586.

Wyckoff, E., Natalie, D.M., Nolan, J.M., Lee, M. and Hsieh, T.S. 1989. Structure of the *Drosophila* DNA topoisomerase-II gene: nucleotide sequence and homology among topoisomerase-II, *J. Mol. Biol*. **205(1):**1-13.

Yabe, k., Satoh, Y. and Iishi, T. 2004. Early pathophysiologic feature of arthropathy in juvenile dogs induced by ofloxacin, a quinolone antimicrobial agent. *Veterinary Pathology* **41**:673-681.

Yazar, E. and Tras, B. 2001. Effects of fluoroquinolone antibiotics on hepatic superoxide dismutase and glutathione peroxidase activities in healthy and experimentally induced peritonitis mice. *Revue Med Vet* **152**: 235-238.

Zechiedrich, E.L. and Cozzarelli, N.R. 1995. Roles of Topoisomerase IV and DNA Gyrase in DNA Unlinking during Replication in *Escherichia coli*. *Genes & Development*, **9**:2859-2869.

Ziółkowski, H., Jaroszewski, J.J., Maślanka, T., Grabowski, T., Katolik, K. and Pawęska, J. 2014. Influence of Oral Co-Administration of a Preparation Containing Calcium and Magnesium and Food on Enrofloxacin Pharmacokinetics. *Research in Veterinary Science*, **97**:99-104.

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ABSTRACT

Name	:	Srinivasu M	**Id.No.**	:	49574
Sem. & Yr of admn	:	I sem, 2015-16	**Degree**	:	M.V.Sc
Major	:	Veterinary Pharmacology & Toxicology	**Department**	:	Veterinary Pharmacology & Toxicology
Minor	:	Veterinary Physiology			
Thesis Title	:	**Toxicological evaluation of enrofloxacin and ciprofloxacin with special reference to genotoxicity in rats**			
Advisor	:	Dr. S.P. Singh			

This study was undertaken to investigate the sub acute toxicity including genotoxic potential of enrofloxacin and ciprofloxacin by estimating heamtobiochemical parametres, oxidant parameters following oral administration @75mg/kg b wt and 150mg/kg b wt of enrofloxacin and 50mg/kg b wt and 100mg/kg b wt of ciprofloxacin for 28 days. Cyclophosphamide (20mg/kg b wt 24h prior to sacrifice) was used as standard genotoxic compound in rats.

Thirty six Wistar rats of 8 weeks weighing 250-300gms were divided equally and randomly into six groups viz. I, II, III, IV, V and VI. Group I served as negative control and group II as positive control administered with cyclophosphamide @ 20mg/kg b wt i.p. 24 h prior to sacrifice. Other groups were administered with enrofloxacin @75mg/kg b wt in group III and @ 150mg/kg b wt group in IV. Ciprofloxacin was given @ 50mg/kg b wt in group V and @ 100mg/kg b wt group in VI p.o. for 28 days. A significant ($P<0.05$) decrease in absolute and relative liver weights were observed in group IV and VI as compared to control. A significant ($P<0.05$) reduction in Hb, PCV, MCH, MCHC, TEC and TLC was observed in treatments groups II to VI. There was significant ($P<0.05$) reduction in total serum protein and albumin in treatment groups II to VI. Significant ($P<0.05$) increase in globulin and A: G was observed in groups II to VI as compared to control group I. No significant ($P<0.05$) change was observed in triglycerides and bilirubin levels in the treatment groups. A significant ($P<0.05$) increase in BUN and creatinine values was observed in groups IV, V and VI. Significant ($P<0.05$) increase in AST and ALT activity was observed in groups II and VI as compared to control group I. A significant ($P<0.05$) increase in RBCs and tissue GSH, SOD and catalase activity and increase in LPO in RBCs and tissues were observed in groups II, III and V as compared with control group I. Histopathological changes such as severe congestion of large and small vessels, accumulation of mononuclear cells around many congested blood vessels, severe degeneration of hepatocytes and sinusoidal dilatation throughout the parenchyma in liver; severe congestion, interstitial hemorrhages, vacuolation of glomeruli and coagulative necrosis of varying degrees with condensation of glomerular tuft in many of the renal tubular epithelial cells in kidney were observed in higher dose groups of enrofloxacin and ciprofloxacin.

It is concluded from this study that enrofloxacin and ciprofloxacin at high doses produced hemotoxic, hepatotoxic, mild nephrotoxic, oxidative stress and effects genotoxic in rats after 28 days.

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सत्र एवं वर्ष : प्रथम, 2015–16 **उपाधि** : एम.वी.एस.सी.

प्रमुख विषय : पशु भेषज गुण एवं विष विज्ञान **विभाग** : पशु भेषज गुण एवं विष विज्ञान

गौण विषय : पशु शरीर क्रिया विज्ञान

शोध शीर्षक : ''चूहो में एनरोफ्लोक्सासिन और सिप्रोफ्लॉक्सासिन का अध्ययन जीन विषाक्तता के संदर्भ में''

सलाहकार : डा0 एस.पी. सिंह

इस अध्ययन में एनरोफ्लोक्सासिन और सिप्रोफ्लॉक्सासिन का अध्ययन जीन विषाक्तता के लिए किया गया है जिसमें रूधिर, जैव रसायनिक और आक्सीकारक मानको का अध्ययन किया गया है। इस अध्ययन में 75 मिलीग्राम तथा 150 मिलीग्राम प्रति किलो शारीरिक भार के अनुसार एनरोफ्लाक्सासिन और 50 मिलीग्राम तथा 100 मिलीग्राम प्रति किलो शारीरिक भार के अनुसार सिप्रोफ्लाक्सासिन 28 दिन तक मुख द्वारा चूहों में दिया गया। चूहों में प्रमाणिक जीन विषाक्तता के लिए साक्लोफासफामाइड (20 मिलीग्राम प्रति किलो ग्राम शारीरिक भार) के अनुसार अध्ययन पूर्ण होने के एक दिन पूर्व दिया गया। 8 सप्ताह की उम्र के 36 चूहे जिनका शारीरिक भार 250–300 ग्राम था, अक्रमतः 6 समूहों में रखा गया। पहला समूह निषेधात्मक जांच के लिए लिया गया, दूसरा समूह प्रभाव युक्त जांच के लिए लिया गया जिसमें साइक्लोफासफामाइड 20 मिलीग्राम प्रति किलोग्राम शारीरिक भार के अनुसार आई.पी. दिया गया। तृतीय और चतुर्थ समूह में एनरोफ्लाक्सासिन क्रमशः 75 मिलीग्राम प्रति किलोग्राम शारीरिक भार और 150 मिलीग्राम प्रति किलो ग्राम शारीरिक भार के अनुसार दिया गया। पंचम ओर छठवॉ समूह सिप्रोफ्लाक्सासिन क्रमशः 50 और 100 मिलीग्राम प्रति किलोग्राम शारीरिक भार के अनुसार 28 दिन तक दिया गया है। चतुर्थ और पंचम समूह के चूहों के यकृत का पूर्ण व सोपक्ष भार ($P<0.05$) निषोधात्मक समूह की तुलना में कम हुआ है। उपचार समूह द्वितीय व चतुर्थ में हिमोग्लोबिन, पी.सी.वी., एम.सी.वी., एस.सी.एच., टी.ई.सी. ओर टी.एल.सी. की महत्वपूर्ण कमी का पर्यवेक्षण किया गया। कुल सीरम प्रोटीन एवं एल्बुमिन में कोई महत्वपूर्ण परिवर्तन नही दिखा। नियंत्रण समूह 1 की तुलना में समूह द्वितीय व चतुर्थ में ग्लोबूलिन एवं एःजी की उल्लेखनीय वृद्धि का पर्यवेक्षण किया गया। नियंत्रण समूह 1 की तुलना में उपचार समूह द्वितीय व चतुर्थ में ट्राएग्लिस्राईड, बिलिरूिबन, ए.एल.टी, ए.एस.टी., बी.यू.एन. और ए.एल.पी. में कोई महत्वपूर्ण बदलाव उल्लेखनीय नहीं रहे। नियंत्रण समूह प्रथम की तुलना में समूह द्वितीय, तृतीय, पंचम में लाल रक्त कोशिकाऐं, ऊतक जी.एस.एच., एस.ओ.डी. एवं केटालेज गतिविधियों में महत्वपूर्ण कमी पाई गई एवं लाल रक्त कोशिकाओं, ऊतक में एल.पी.ओ. की वृद्धि देखी गई। ऊतकीय विकृती अध्ययन पर बदलाव जैसे बड़ी एवं छोटी रक्तवाहिनी में संकुलन, एक केन्द्रक कोशिकाओं का संचयन, यकृत की कोशिकाओं का क्षय होना, कोशिकाओं के मध्य रक्त का स्राव, कोशिकागुच्छीय के मध्य रिक्ति का बनना, गुर्दे की कोशिकाओं का क्षय होना अध्ययन में पाया गया। उच्च खुराक वाले इनरोफलीक्सासिन और सिप्रोफ्लोक्सासिन वाले समूह में वृक्कों की कोशिकाओं के मध्य एक केन्द्रक कोशिकाओं का संचयन हुआ।

अतः इस अध्ययन से निष्कर्ष निकला गया है कि एनरोफलोक्सासिन और सिप्रोफलोक्सासिन की अधिक खुराक रक्त विषाक्तता, यकृत विषाक्तता, अपचायक विषाक्तता एवं जीन की विषाक्तता करता है।

(एस.पी. सिंह)
सलाहकार

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लेखक