Assessment of Adverse Effects of Enrofloxacin in Goats

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

PUSA (SAMASTIPUR) BIHAR

(FACULTY OF POST-GRADUATE STUDIES)

In the partial fulfilment of the requirement

FOR THE DEGREE OF

Master of Veterinary Science

IN

VETERINARY PHARMACOLOGY AND TOXICOLOGY

By

Anuradha Kumari

Reg. No. - M/V.Phar/56/2002-2003

DEPARTMENT OF VETERINARY PHARMACOLOGY AND TOXICOLOGY

BIHAR VETERINARY COLLEGE

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BIHAR VETERINARY COLLEGE
PATNA – 800014
2004

Dedicated to

MY ADORABLE

Grand Parents
&
Grand Parents in-laws

DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY
Bihar Veterinary College, Patna-800014
Rajendra Agricultural University, Pusa, Bihar

Dr. S.D. Singh
Ph.D.
Associate Professor & Head
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CERTIFICATE - I

This is to certify that the thesis entitled **"ASSESSMENT OF ADVERSE EFFECTS OF ENROFLOXACIN IN GOATS"** submitted in partial fulfillment of the requirement for the degree of "**Master of Veterinary Science (Veterinary Pharmacology & Toxicology)**" of the faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar, is the record of bonafide research carried out by **DR. ANURADHA KUMARI**, under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that such help or information received during the course of this investigation and preparation of the thesis have been duly acknowledged.

(S.D. Singh)
Major Advisor

Endorsed :

(Chairman / Head of the Department)

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CERTIFICATE - II

We, the undersigned, members of the Advisory Committee of **DR. ANURADHA KUMARI**, a candidate for the degree of Master of Veterinary Science with Major in **Veterinary Pharmacology & Toxicology**, have gone through the manuscript of the thesis and agree that the thesis entitled **"ASSESSMENT OF ADVERSE EFFECTS OF ENROFLOXACIN IN GOATS"** may be submitted by **DR. ANURADHA KUMARI** in partial fulfillment of the requirements for the degree.

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CERTIFICATE - III

This is to certify that the thesis entitled **"ASSESSMENT OF ADVERSE EFFECTS OF ENROFLOXACIN IN GOATS"** submitted by **DR. ANURADHA KUMARI**, in partial fulfillment of the requirement for the degree of **Master of Veterinary Science (Veterinary Pharmacology & Toxicology)** of the faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar was examined and approved on .....30-10-2004.....

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(ii) **Dr. S. R. P. Sinha** 30/10/04

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ACKNOWLEDGEMENT

I wish to express my deep sense of gratitude and indebtedness to my Major Advisor ***Dr. S.D. Singh M.V.Sc., Ph.D., Associate Professor – cum-Head of the Department of Veterinary Pharmacology & Toxicology and Principal, Bihar Veterinary College, Patna*** *for his precious guidance, constructive suggestions, close supervision, constant encouragement, keen interest and healthy criticisms during the course of study as well as preparation of this manuscript, without which, this research programme would not have been completed.*

I am highly obliged to ***Dr. C. Jayachandran,*** *Ph. D., Associate Professor, Department of Pharmacology and Toxicology, Bihar Veterinary College for his active and inspiring guidance, meticulous planning, constant supervision, inspirational criticism and keen interest throughout the present investigation and during the preparation of this manuscript. I can never forget his obligation and constant encouragement which filled my heart with lot of confidence to reach against all the odds.*

I feel my immense pleasure to express my gratitude to the members of advisory committee, ex - member ***Dr. B.K, Sinha,*** *M.V.Sc., Ph.D. Retd. Associate Professor & Head, Department of Microbiology, and the present member* ***Dr. S.R.P. Sinha,*** *M.V. Sc., Ph. D. Associate Professor, Department of Parasitology,* ***Dr. J.N. Singh,*** *Associate Professor & Head, Department of Livestock Products Technology for their valuable suggestions and prudent guidance.*

My sincere thanks are also due to **Dr. H.N Jha**, *Retd. Associate Professor, Department of Pharmacology and Toxicology and* **Dr.S.P. Sinha**, *Retd. Associate Professor, Department of Pharmacology and Toxicology for their constant help and encouragement for the smooth running of the present research work.*

I am highly obliged to **Dr. K.G. Mandal**, *Sr. Assistant Professor, Animal Breeding and Genetics, for his unlimited help rendered in analyzing the data. I am also obliged to* **Dr. S.B. Verma**, *Associate Professor, Department of Animal Breeding and Genetics,* **Dr. B.K. Sinha**, *Retd. , Associate Professor & Head, Department of Pathology,* **Dr. S.S.Singh**, *Associate Professor & Head, Department of Livestock Production and Management,* **Dr. S.P. Sharma**, *Associate Professor & Head, Department of Surgery,* **Dr. S.R. Singh**, *Associate Professor & Head, Department of Animal Breeding and Genetics,* **Dr. J.N. Pandey**, *Retd. Associate Professor & Head, Department of Veterinary Public Health,* **Dr. S.P. Verma**, *Associate Professor & Head, Department of Veterinary Medicine and to all other teachers for their cooperation and showing intense interest in the present study.*

I am also thankful to my seniors, **Dr. Sanjeev Kumar, Dr. Mukesh Kumar, Dr. Nitesh Kumar** *and* **Dr. Sushma Lalita Baxla** *for their useful suggestions and cooperation.*

My warmest thanks also due to my friends **Dr. Archana, Dr. Shashi, Dr. Smita, Dr. Deepak Kumar Prasad, Dr. Nirbhay Kumar, Dr. Thakur Balwant Singh, Dr. Vijay** *and* **Dr. Praveen** *for their affection, generous help and co-operation shown to me during the present research work.*

The author is thankful to **Sri Vijay Kumar Singh**, *for rendering necessary help in all official works with keen interest without which, the preparation of this thesis is not possible. Thanks and appreciation are also due to* **Sri Nathun Pandit, Sri Ramashish Paswan** *and* **Sri Nayeem** *of the Department of Pharmacology and Toxicology for their constant help during research work.*

I express my sincere thanks and indebtedness to the **Rajendra Agricultural University,** *Pusa, Samastipur, Bihar for the generous help and opportunity by providing fellowship and other facilities during the tenure of my study.*

Gratitude alone fails to convey my feeling which cannot be expressed in words for the affectionate care, thought, moral support and encouragement constantly received from all members of my family specially my affectionate parents, elder brother **Sri Anshu Dhar Sharma**, *younger brother* **Bhim,** *sisters* **Dr. Arundhati, Dr. Anupama, Miss. Anamika, Miss. Khushi,** *Brother-in-law,* **Dr. Ashok**. *My warmest thanks are also extended to my lovely nieces,* **Arya Shankar, Apurva, Neha** *and* **Ashtha**, *Nephews,* **Kishlay** *and* **Suman Saurabh** *along with all well wishers for their good will conferred upon me.*

I have no words to express my gratitude towards the tremendous support and blessings that my father ***Sri Kripa Shankar Sharma*** *and my mother* ***Smt. Nilam Sharma****, father in law,* ***Sri Ram Lal Thakur*** *and mother in law* ***Smt. Indu Thakur*** *have bestowed on me. My sincere thanks are also extended to my husband* ***Mr. Maneesh Kumar*** *for constant inspiration during the entire course of study.*

I am thankful to ***Ranbaxy pharmaceuticals Ltd.*** *For providing* ***Enrofloxacin (Enrocin®)*** *as gift samples for conducting the present investigation.*

I am also thankful to ***Mr. Rajeev Prasad, Mr. Anit Prakash*** *and Mr.* ***Ajay Keshri*** *of* ***"Srishti Computer", Ashok Rajpath, Patna – 4*** *for their kind support in course of laser printing of this manuscript.*

Last, but not the least, I express my gratitude to the all pervading graciousness of the almighty God for giving me patience and strength to overcome the every hurdles which crossed my way in accomplishment of this endeavour.

Date : 22-05-04

Place : patna

Anuradha kumari

(Anuradha Kumari)

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Chapter - 1

Introduction

INTRODUCTION

Enrofloxacin, the latest among the fluroquinolone class of antimicrobials, was first synthesized in 1983 by Bayer Research Laboratory in Germany. It is a quinolone carboxylic acid derivative developed exclusively for veterinary use (Altreuther, 1987; Chu and Fernandes, 1989). It possesses broad-spectrum activities and is effective against both gram-negative bacteria such as *Escherichia coli, Salmonella, Klebsiella* and pseudomonas (Scheer, 1987) and gram-positive bacteria like *Streptococcus, Staphylococcus, Clostridium, Erysipelothrix rhusiopathiae* and *Mycoplasma* (Bauditiz, 1990). Enrofloxacin possesses excellent distribution in different tissues and body fluids and its distribution pattern is almost similar in all species. Enrofloxacin is suitable for the treatment of septicemia, gonorrhoea, respiratory infections, soft tissue infections and bone and joint infections as well. It is also active against several organisms, which are resistant to many other antimicrobials.

Goat is mainly reared in tropical countries including India. Goat is commonly called "poor man's cow" in India and "wet nurse of infants" in Europe. Goat, apart from its good quality of milk, contributes to the meat and leather industries. The animal is reared by sizeable population of poor rural folk, and hence, proper health

coverage of this poor species assume importance. Being small and popular species, goat has been considered useful for the present investigation.

Kinetic studies of enrofloxacin were conducted in rabbit (Scheer *et al.,* 1990), cow (Walser *et al.*, 1993), buffalo (Nitesh Kumar *et al.*, 2003), dog (Kung *et al.*,1993), pig (Anadon *et al.*, 1999), horse (Zehl, 1989), chicken (Soliman, 2000) etc. But, few toxicity studies only were conducted in animals. Though enrofloxacin possesses a wide margin of safety but animals like horse, dog, cattle and swine are prone to its toxicity. Toxicity of enrofloxacin shows a variable degree of clinical symptoms such as arthropathy and cartilaginous damage in juveniles (under eight months of pups), hepatic and renal toxicity, CNS toxicity like dullness, depression or excitement, in-coordination, convulsion etc. The drug is particularly toxic to muscle tissues which may be caused due to higher elevation in serum creatine phosphokinase (CPK) activity (Pyorala, 1994). Enrofloxacin associated retinal degeneration in cats was also demonstrated by Gelaltt *et al.* (2001). The available literatures do not report toxicity of enrofloxacin in goats.

Due to muscular damage caused by enrofloxacin, the preferred route of its administration in ruminants including goat is either i.v. or s.c. Currently in India, many pharmaceutical firms have introduced enrofloxacin for i.m. route also.

By taking into consideration of the above mentioned facts, the present investigation has been carried out in goats with the following objectives: –

(i) Toxicity study of enrofloxacin following a single higher therapeutic dose of 20 mg/kg by i.m. route.

(ii) Toxicity study of enrofloxacin following repeated daily i.m. administration of the drug at dose rate of 5 mg/kg for seven days.

Chapter - 2

Review of Literature

REVIEW OF LITERATURE

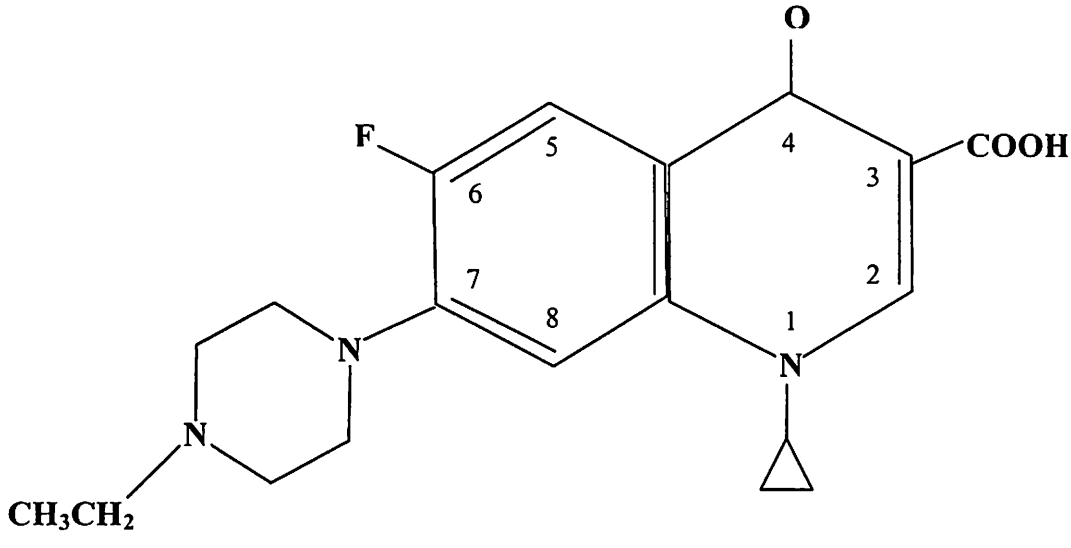
The quinolones are synthetic antimicrobial agents and nalidixic acid, the 1st member of this group was introduced in clinical practice in 1963. Nalidixic acid possesses narrow spectrum of activity (mainly gram-negative organisms) and mainly used for treating urinary tract infections caused by gram-negative organisms. Due to rapid development of resistance and narrow spectrum activity of nalidixic acid, systematic search was carried out to synthesize agents possessing wide spectrum of antimicrobial activity for systemic use. This led to the development of fluroquinolones, such as norfloxacin, ciprofloxacin, pefloxacin etc.

ENROFLOXACIN

Enrofloxacin, one of the fluroquinolones was synthesized in 1983 by Bayer Research Laboratory in Germany. It is exclusively used as a drug of choice for animal treatment only. Apart from its wide spectrum of antimicrobial activity, enrofloxacin possesses excellent distribution in different tissues and body fluids. Further, it has the additional benefit of being metabolized to ciprofloxacin, which also exerts potential antimicrobial activity.

Chemistry

Enrofloxacin is a crystalline, slightly yellowish powder with a slight bitter taste. The chemical structure of enrofloxacin is as follows.



Chemically it is 1- cyclopropyl – 7 – (4 ethyl – 1piperajinyl) – 6- Fluoro 1, 4 – dihydro – 4 – oxo – 3 – quinoline carboxylic acid.

Empirical formula = $C_{19}H_{22}FN_2O_3$

Molecular weight = 359.40

Antimicrobial Actions

Enrofloxacin is a broad spectrum antimicrobial with bactericidal action. It is effective against both gram-negative and gram-positive bacteria as well as mycoplasmas. In addition, some of the anaerobic pathogens are also susceptible. Development of

resistance is low with this drug. It does not exhibit cross resistance with other antimicrobials including quinolone drugs. Hence, it in effective against microorganisms that are resistant to β lactam antibiotics, tetracyclines, aminoglycosides or macrolides and has a special place in the therapy of multidrug resistant infections. The MIC values of enrofloxacin, for different species of microorganisms range between 0.01 to 2.0 μg/ml in veterinary practice (Prescott and yielding, 1990).

Mechanism of Action

All fluoroquinolones including enrofloxacin selectively inhibit bacterial DNA-gyrase enzyme, which is necessary for superhelical formation of DNA strands of chromosomes. The process of helical formation involves DNA strand breaking and resealing. Disruption of these mechanisms leads to degradation of DNA and lysis of bacterial cell.

TOXICITY STUDIES

Toxicity studies of Enrofloxacin have been mainly conducted in laboratory animals and pet animals like dog and cat. Few studies were conducted in domestic animals. The available literature reveal that enrofloxacin has minimal toxicity in ruminants except neuromuscular toxicity is seen after injection of enrofloxacin particularly by i.m. route. Hence, the preferred route of administration of enrofloxacin is either s.c. or i.v.

Seventeen cats were treated for certain medical disorders with enrofloxacin by parenteral and oral route in which retinal degeneration was developed soon after therapy. Clinical signs include mydriasis and acute blindness and all cats used had diffused retinal degeneration. Vision returned in a few cats but retinal degeneration persisted or even progressed (Gelatt *et al.*, 2001).

The i.m. and oral administration of enrofloxacin at the dose rate of 30 mg/kg every 12 hr for 10 days in African grey parrots showed no significant biochemical changes. However, polydipsia and polyuria occurred in treated birds, but resolved quickly upon discontinuation of enrofloxacin administration.

In a study conducted in cows by Pyorala (1994) regarding local tissue damage after i.m. injection of eight antimicrobial agents post 2 deep i.m. injections showed highest elevation of serum creatine phosphokinase (CPK) activity with enrofloxacin followed by tylosin and trimethoprim-sulfadoxine. Moderate elevations in CPK activity were seen with oxytetracycline, spiramycin and trimethoprim-sulfadiazine. No activity was noted with penicillin.

The original research work of Bayer in Germany on enrofloxacin under the trade name Baytril showed CNS effects of mild symptoms such as dizziness, headache and insomnia in man. Enrofloxacin competitively inhibits receptor binding of gamma-

aminobutyric acid (GABA), an inhibitory transmitter of CNS (Hooper and Wolfson, 1993). This effect is only seen in man, not in animals, particularly in ruminants.

As with all anti-infectives, enrofloxacin causes gastro intestinal disturbances such as nausea, vomiting or diarrhea may occasionally occur. In experimental studies on dogs (Altreuther, 1992), vomiting was induced only in doses far exceeding the therapeutic concentrations (>1000 mg/kg b.wt., orally). Enrofloxacin has only minimal effects on anaerobic organisms, which represents a considerable part of normal bowel flora, the incidence of intestinal side effects may be less frequent as compared to anti-infectives of other families (Hooper and Wolfson, 1993).

Altreuther (1992) reported that very young dogs between 1 and 4 weeks of age tolerate treatment with enrofloxacin (Baytril) from 5 to 25 mg/kg for upto 10 days without cartilage lesions. In animals older than 6 weeks, lesion occurred to a certain extent, depending on dosage and duration of treatment. There is, however, no evidence that treatment of pregnant of nursing dogs would have a very negative influence on the joint cartilage development of the offspring. In contrast to dogs, cartilage lesions could not be demonstrated in growing cats from 2 to 10 months of age when treated with Baytril doses of up to 25 mg/kg for upto 30 days. Thus

Altreuther (1992) concluded that cartilage tolerance towards enrofloxacin may be higher in cats than in dogs.

Studies on safety pharmacology revealed that oral enrofloxacin in doses upto 100 mg/kg b.wt., i.e., 20 times the recommended doses, showed no significant adverse effects on blood composition, blood coagulation or diuresis in laboratory animals. In the bronchoactivity test, no evidence of any effect of the drug on the smooth muscles of the respiratory system was found. Similarly, enrofloxacin (Baytril) did not elicit any allergic or pseudoallergic reactions. On local irritation test in guinea pig, enrofloxacin did not show any adverse effect and only it was noted to be slightly irritating to the eye (Altreuther, 1992). However, Baytril (0.5%) eye drop formulation did not produce any irritation after application to the rabbit eye.

For acute toxicity studies, the LD_{50} values were determined in different species by Altreuther (1987). All acute effects appeared at doses far exceeding the therapeutic range. Dogs vomited after oral application at doses above 1000 mg/kg b.wt., which is 200 times the recommended doses, such that LD_{50} could not be determined (Altreuther, 1992).

Sub chronic toxicity studies in rats, mice and adult dogs were conducted after feeding of enrofloxacin for a period of 13 weeks

and no effect level (NOEL), which is the dose that can be administered with food over prolonged periods without adverse effects, was determined (Altreuther, 1992). For rats, mice and adult dogs, general NOEL of 165, 550, 52 mg/kg b.wt., respectively were found. This means that at the recommended doses, adult dogs can be treated safely and for longer time without unwanted effects (Altreuther, 1992).

Embryotoxicity and Teratogenicity

Trials in rats daily treated with enrofloxacin (Baytril) 10, 50, 210 and 875 mg /kg b.wt. from the 6^{th} to the 15^{th} day of gestation produced no evidence of teratogenic effects from enrofloxacin, including the highest dosage group. Maternal toxic effects after 210 and 875 mg/kg, however, resulted in slightly reduced fetal weights and delayed ossification. In the highest dosage group, which received 175 times the dosage recommended for dogs and cats, smaller litter sizes were observed. A dosage of 50 mg enrofloxacin (Baytril) per kg b.wt. was tolerated without any adverse effect to mothers and offspring (Altreuther, 1987).

Mutagenicity

The point mutagenic effect of enrofloxacin (Baytril) was studied in the salmonella microsome test (Ames – Test) and in ovarian cells of the Chinese hamster (CHO-HGPRT forward mutation

assay). The unscheduled DNA synthesis test was performed to check for damage to the DNA. These test systems produced no evidence of any mutagenic effect (Altreuther, 1987).

Retinal toxicity in cats

It is well known that old quinolones, such as nalidixic acid, may affect the eye as a potential target organ (Takayama *et.al.*, 1995). In this connection, alterations of the lens or melanin-containing eye tissues (retina, iris, ciliary body) have been reported from human medicine. As a matter of precaution enrofloxacin (Baytril) therefore, has been evaluated in ophthalmoscopic and histopathological studies on laboratory animals, dogs and cats. These toxicological studies with repeated administration of high doses did not reveal any evidence of unwanted effects on the eyes of the animals examined.

Sporadic reports of blindness is cats previously treated with enrofloxacin (Baytril), however, have been received mainly from the US in the last few years. In an additional safety study on cats, enrofloxacin (Baytril) was given over 3 weeks at different doses (5, 20, 50 mg/kg b.wt.) once daily. No adverse effects were observed in cats that received 5 mg enrofloxacin (Baytril) per kg body weight. The administration of doses of 20 mg/kg body weight or greater caused salivation, vomition and depression and additionally resulted in mild

to severe fundic lesions. On ophthalmologic examination, changes in electro retina grams (including blindness) and diffuse light microscopic alterations. It has therefore been recommended to treat cats with enrofloxacin (Baytril) at daily doses not exceeding 5 mg/kg b. wt., which is the officially registered dose in most countries of the world. In contrast to cats, there is no evidence that visual alteration can also occur in other species, e.g. dogs.

Chapter – 3

Materials and Methods

MATERIALS AND METHODS

In the present study, five clinically healthy female goats of non-descript breed between 12–18 months of age and 16-22 Kg body weight were used. The goats were maintained on dry fodder, greens and cattle feed apart from 4 to 5 hours of routine grazing. Water was given *ad lib*.

EXPERIMENTAL DESIGN

Enrofloxacin was administered separately in each of five healthy goats by i.m. route at higher dose rate of 20 mg/kg for acute toxicity study and blood samples were collected at 0, 4, 8, 12 and 24 hours to know the adverse effect of this drug. An interval of 15 days was allowed to elapse before administration of next dose of the drug. For sub acute toxicity study, the drug was given by i.m. route at therapeutic dose of 5 mg/kg for 7 days and blood samples were collected at 0, 1, 2, 4 and 8 days to know the long term effect of the drug.

DRUG USED

Enrofloxacin was used in the present experiment. Enrocin® 10% - an injectable commercial preparation containing enrofloxacin in concentration of 100 mg/ml marketed by Ranbaxy Laboratories Limited, India supplied as gift sample was used.

COLLECTION OF BLOOD AND SERUM SAMPLES AND THEIR TIMINGS

Blood samples were collected at 0, 4, 8, 12 and 24 hours after i.m. administration of enrofloxacin in each of five healthy goats at the dose rate of 20 mg/kg body weight. After a washout period of 15 days, the blood samples were again collected at 0, 1, 2, 4 and 8 days i.m. administration of the drug at the dose rate of 5 mg/kg body weight daily for 7 days for sub acute toxicity study.

(A) Blood and Serum

Before collection of blood, the sites around the jugular vein on either side of the neck of animals were aseptically prepared. Blood samples were collected in distilled water washed centrifuge tubes containing appropriate amount of sodium oxalate (anticoagulant). For collection of serum, blood was collected in distilled water washed test tubes. Blood was allowed to clot in test tubes at room temperature. The serum was then centrifuged to collect clear serum. Blood was collected by vene-puncture with disposable 18 G needles at various above noted time intervals after drug administration. Blood and serum were transferred in different vials for different biochemical tests.

For total leucocyte count and differential leucocyte count blood was taken from ear vein.

The serum and blood samples were then kept in refrigerator until various haematological and biochemical tests were carried out.

Administration of drug

(i) Acute toxicity study

Enrofloxacin (Enrocin®) injection containing 100 mg of drug per ml was injected at the dose rate of 20 mg/kg body weight by i.m. route once only in each goat.

(ii) Subacute toxicity study

Enrofloxacin (Enrocin®) injection containing 100 mg of the drug per ml was injected at the dose rate of 5 mg/kg body weight once daily by i.m. route in each goat for 7 consecutive days.

PARAMETERS FOR TOXICITY STUDIES

For toxicity analysis, the following parameters were estimated.

(A) Clinical parameters :

(i) Toxicity signs and symptoms

(ii) Respiration rate/minutes.

(iii) Pulse rate/minute

(iv) Body temperature.

(v) Ruminal movement/5 minutes.

(B) Haematological parameters :

(i) Haemoglobin (gm %)

(ii) Total leucocyte count (TLC)

(iii) Differential leucocyte count (DLC)

(C) Biochemical parameters :

(i) Total plasma protein

(ii) Blood glucose

(iii) Serum cholesterol

(iv) Blood urea Nitrogen (BUN)

(v) Serum glutamate pyruvate transaminase (SGPT)

(vi) Serum Glutamate oxaloacetate transaminase (SGOT)

(D) Parameters for estimating muscle damage

(i) Creatine phosphokinase (CPK)

(A) Clinical parameters :

Respiration rate/minute, pulse rate/minute, Body temperature and ruminal movement/5 minute were noted in various above noted time internals in all the five healthy goats.

(B) Haematological parameters :

(i) Haemoglobin (Hb in g/dl)

The haemoglobin contents of blood samples were estimated by Acid Haematin method using Hellige and sahli's haemoglobinometer as per procedure described by Schalm *et al.* (1975).

Principle - When blood is added to 0.1 N HCl, Hb is converted to brown coloured acid haematin and the colour is matched with standards

Procedure-

1. 0.1 N HCl was added in the graduated tube upto the lowest mark (20% mark) using a Pasteur pipette.

2. Blood was drawn upto 20 µl mark in the Hb-pipette. Blood column was adjusted carefully without bubbles. Excess of blood on the sides of the pipette was wiped out using a dry piece of cotton.

3. Blood was transferred to the acid in the graduated tube and rinsed well. The reaction mixture was mixed properly and allowed to stand for at least 10 minutes.

4. The solution was diluted with distilled water by using a few drops at a time and mixed until the colour of the reaction mixture matches with the glass plate on the comparator against natural light.

5. The level of the fluid was noted at its lower meniscus and the reading corresponding to this level on the scale was recorded in gm/dl. This is the value of haemoglobin in grams per 100 ml of blood.

(ii) Total Leucocyte Count (TLC)

The total leucocyte count of blood was done using improved Neubauer chamber by the method described by Boddie and Goe (1962).

Principle – Acid diluting fluid doesn't destroy WBCs. Stain added to diluting fluids makes nuclei visible in a counting chamber under the microscope.

Procedure – Blood was drawn in Throma WBC pipette having white bead upto 0.5 mark. If the blood has risen above the desired mark, pipette tip was touched with gauze and adjusted. Then, diluting fluid was drawn upto the mark 11. While drawing diluting fluid, the pipette was gently rotated. This kept the bead in motion and prevented air bubbles sticking to the bulb. The pipette was shaken for a few seconds while keeping finger or thumb on the tip of pipette. Rubber tubing was removed and pipette was held horizontally between thumb and finger. The contents of pipette were mixed first and a few drops were discarded. The tip of the pipette was touched to haemocytometer. The fluid was allowed to flow by capillary action slowly and the counting chamber was filled without any air bubbles. Both the counting areas were charged and average of final report was taken. The cells were allowed to settle for 1-2 minutes. The counting chamber was placed under microscope and the cells in four large squares were counted with ruled area under low magnification (10 X).

Calculation –

Number of leucocytes/mm^3 = leucocytes in 4 WBC squares × 50.

(iii) Differential Leucocyte Count (DLC)

The differential leucocyte count of blood was done by the method as advocated by Boddie and Goe (1962).

Preparation of blood film – For differential leucocyte count, first a thin blood smear was prepared on a glass slide. For preparing blood film, a small drop of fresh blood was placed near one end of slide. The blood was spread evenly with a spreader slide at 30^0 angles. A good slide film should have smooth appearance, free from holes, straight border and rainbow like appearance when seen against light. Then the blood film was air dried.

Staining – For staining, few drops of undiluted Leishman's stain was added and allowed to act for 1 minute, then the stain was diluted with double amount of buffered distilled water and allowed the diluted stain to act for 5 minutes. The slide was washed gently with distilled water and air dried. A good stained slide had a pinkish tinge.

Examination of blood smear

The inspection of blood film was first done under low power magnification (10X). A portion of smear near the thin end referred as counting area was selected and switched to oil immersion lens (100X). Examination of blood smear was done thoroughly and at least 100 leucocytes were counted by battlement or zig-zag method. Counted cells were neutrophils, lymphocytes, monocyctes, eosinophils and basophils. Results were expressed in percentage.

(C) Estimation of Biochemical Parameters

Estimations of biochemical parameters were done using commercially available kits from Nice chemicals Pvt., Ltd. and Span Diagnostics.

(i) ***Total plasma protein***

The estimation of blood proteins *i.e.* total protein and albumin were done by Biuret and Bromocresol method as advocated by Reinhold (1953).

Principle – The peptide linkages of amino acids in protein react with biuret reagent to form a violet coloured complex. This is measured colorimetrically at 530 nm. Albumin reacts with bromocresol green solution at pH 4.1 to from a green coloured derivative. This is measured colorimetrically at 620 nm.

Procedure

Three test tubes marked blank (B), standard (S) and test (T) were taken and 5 ml of biuret reagent was added to each of these. 0.1 ml of distilled water was added to the blank whereas 0.1 ml of serum was added to the test and 0.1 ml of protein standard was added to the standard. The content of tubes were mixed well and kept at room temperature for 30 minutes. Optical density was measured at 545 nm (green filter) against blank.

Calculation

$$\text{Serum protein in gm \%} = \frac{\text{O.D. of T}}{\text{O.D. of S}} \times 5$$

(ii) ***Blood Sugar***

The estimation of blood sugar was done by Folin and Wu method as described by Frankel *et al.* (1970).

Principle – The method is based on three stages –

(i) Precipitation of blood proteins with copper tungstate.

(ii) Reduction of cupric sulphate to cuprous oxide .

(iii) Colorimetric measurement of the subsequent green colour produced on the addition of molybdate reagent to the cuprous oxide.

Procedure

Test - 0.1 ml of whole blood was taken in a test tube containing 3.5 ml of distilled water, 0.2ml of sodium tungstate solution (10%) was added and mixed. Then, 0.2 ml of 2/3 N H_2SO_4 was added and mixed and allowed to react for 5 minutes and then centrifuged at 3000 rpm for 10 minutes. 2 ml of supernatant fluid was taken in a Folin and Wu tube marked T.

Blank – 2 ml of distilled water was taken in a Folin and Wu tube, marked B.

Standard – 1 ml of glucose working standard (0.1 mg / ml) and 1 ml distilled water was taken in a Folin and Wu tube marked S.

To each of the above tubes, 2 ml of alkaline copper tartarate reagent was added and mixed and placed in a boiling water bath exactly for 8 minutes, then cooled without shaking and added 2 ml of phosphomolybdic acid reagent and mixed. All the three tubes were allowed to stand for 5 minutes and diluted upto 12.5 ml mark with distilled water and mixed well. Readings were taken in colorimeter at 440 nm or by dark blue filter against blank set at zero.

Calculation – mg glucose in 100 ml blood = $\frac{\text{O.D. of T}}{\text{O.D. of S}} \times 200$

(iii) Serum Cholesterol

The estimation of blood cholesterol was done by Ferric Chloride method as described by Wootton (1964)

Principle – Cholesterol reacts with Ferric chloride in the presence of acetic acid and sulphuric acid. The red colour thus produced is measured colorimetrically.

Procedure – First, a working reagent was prepared by diluting 0.5 ml of ferric chloride to 50 ml with aldehyde free acetic acid. 9.9 ml of this working reagent was pipetted into a centrifuge tube and 0.1 ml of serum was added, mixed and allowed to stand for 15 minutes and then centrifuged. Thus, a protein free solution was obtained. Three test tubes marked as blank (B), standard (S) and test (T) were taken. 5 ml and 4.9 ml of working reagent was added to B and S, respectively. 5 ml of protein free solution was added to the test. 0.1 ml of cholesterol standard was added to S. Thereafter, 3 ml of concentrated sulphuric acid (36 N) was added to each test tube, mixed and allowed to stand for 30 minutes in the dark. Optical density was measured at 560 nm (yellow green filter) against distilled water set at zero.

Calculation

$$\text{Blood cholesterol in mg per 100 ml of blood} = \frac{T-B}{S-B} \times 200$$

(iv) Blood Urea Nitrogen (BUN)

The blood urea nitrogen was estimated by Diacetyl Monoxime method as described by Wootton (1964).

Principle – Urea reacts with diacetylmonoxime in the presence of an activator to form a pink coloured derivative. This is measured colorimetrically.

Procedure – First, dilution of serum and urea standard (1 : 20) was done with distilled water. Three test tubes marked as blank (B), standard (S) and test (T) were taken and then 2.0 ml of acid reagent was added to all the test tubes. 0.2 ml of distilled water was added in the blank. 0.2 ml of diluted standard and diluted serum were added to standard and test, respectively. Then, 2 ml of colour reagent (diacetylmonoxime 2% solution in 2% acetic acid) was added to each of the tubes and mixed properly.

After mixing, the tubes were placed in boiling water both exactly for 10 minutes and then cooled. Optical density was recorded at 540 nm (green filter) against blank.

Calculation

$$\text{mg of BUN per 100 ml of blood} = \frac{\text{O.D. of T}}{\text{O.D. of S}} \times 40 \times 0.467$$

(v) *Serum Glutamate Pyruvate Transaminase (SGPT) or Alanine Transaminase (ALT)*

The estimation of SGPT was done by the 2,4- DNPH method as described by Reitman and Frankel (1957).

Principle

Alanine + α - ketoglutaric acid $\xrightleftharpoons{SGPT}$ Pyruvic acid + gludamic acid

Pyruvic acid so formed is treated with 2,4 – DNPH and the brown colour produced is measured colorimetrically at 505 nm.

Procedure – Preparation of standard graph for SGPT -

Pipetted in the tubes labelled as below :-

	1	*2*	*3*	*4*	*5*
Buffered Alanine (ml)	0.5	0.45	0.4	0.35	0.3
Pyruvate standard (ml)	-	0.05	0.1	0.15	0.2
Distilled water (ml)	0.1	0.1	0.1	0.1	0.1
DNPH reagent (ml)	0.5	0.5	0.5	0.5	0.5
Mixed well and allowed to stand at room temperature for 20 min.					
0.4 N NaOH (ml)	5.0	5.0	5.0	5.0	5.0
Enzyme activity IU/L	0.0	13.4	27.4	46.6	72.0

The contents of the tubes were mixed and kept at room temperature for 10 minutes. Optical densities were measured at 505 nm (green filter) against purified water. A graph was plotted by drawing O.D. on y-axis and IU/L on x-axis.

Test - 0.25 ml of buffered alanine was taken in a test tube marked T and incubated at 37^{0}C for 5 minutes. Then, 0.05 ml of serum was placed in the tube, mixed and again incubated at 37^{0}C for 30 minutes. Thereafter, 0.25 ml of DNPH colour reagent was added to the tube, mixed and allowed to stand for 20 minutes at room temperature. Finally, 2.5 ml of 0.4 N NaOH was added, mixed and allowed to stand

for 10 minutes. Optical density was measured at 505 nm against purified water.

Calculation – O.D. of T was marked on y - axis of the standard curve and it was extrapolated to the corresponding enzyme activity on x - axis.

(vi) Serum Glutamate Oxaloacetate Transaminase (SGOT) or Aspartate Transaminase (AST)

The estimation of SGOT was done by 2, 4-DNPH method as described by Reitman and Frankel (1957).

Principle

Aspartic acid + α ketoglutaric acid $\xrightleftharpoons[\text{SGOT}]{}$ Oxaloacetic acid + glutamic acid.

Oxaloacetic acid so formed is coupled with 2,4- dinitro phenyl hydrazine (2, 4 - DNPH) to give the corresponding hydrazone, which gives brown colour in alkaline medium and this is measured colorimetrically.

Procedure – Preparation of standard graph for SGOT :

Pipetted in the tubes labelled as below : -

	1	2	3	4	5
Buffered Alanine (ml)	0.5	0.45	0.4	0.35	0.3
Pyruvate standard (ml)	-	0.05	0.1	0.15	0.2
Distilled water (ml)	0.1	0.1	0.1	0.1	0.1
DNPH reagent (ml)	0.5	0.5	0.5	0.5	0.5
Mixed well and allowed to stand at room temperature for 20 min.					
0.4 N NaOH (ml)	5	5	5	5	5
Enzyme activity (IU/L)	0.0	11.5	29.3	54.7	91.2

The content of the tubes were mixed well and allowed to stand at room temperature for 10 minutes. Optical densities of all the five tubes were measured against distilled water at 505 nm (green filter). A graph was plotted by drawing O.D. on y - axis and IU/L on x-axis.

Test - 0.25 ml of buffered aspartate was taken in a test tube marked T and incubated at 37^0C for 5 minutes. Then, 0.05 ml of serum was added to the tube, mixed and again incubated at 37^0C for an hour. 0.25 ml of DNPH colour reagent was added to the tube, mixed and allowed to stand for 20 minutes at room temperature. Finally, 2.5 ml of 0.4 N NaOH was added to T and mixed and allowed to stand for 10 minutes. Optical density of T was recorded against distilled water at 505 nm (green filter) on a colorimeter.

Calculation

O.D. of T was marked on the y - axis of the standard graph and it was extrapolated to the corresponding enzyme activity on x - axis.

(D) Parameters for Estimating Muscle Damage

(i) *Creatine phosphokinase (CPK)*

For creatine phosphokinase (CPK) estimation serum samples were collected from all the five healthy goats and they were sent to collecting centre of Thyrocare India Pvt. Ltd., Ashok Rajpath, Patna–4 and from there they were then sent to Bombay by flight for investigation.

(E) Statistical Analysis

Statistical analysis of data were done by using paired 't' test (Snedecor and Cochran, 1967).

Chapter - 4

Results

RESULTS

TOXICITY STUDIES ON ENROFLOXACIN

Acute toxicity study of enrofloxacin was conducted in each of five healthy goats after single i.m. administration of the drug at higher dose rate of 20 mg/kg body weight. The blood samples were collected at 0 h (before administration of the drug) and at 4, 8, 12 and 24 hours post drug administration. The following parameters were studied and the results are noted below.

ACUTE

A. CLINICAL PARAMETERS

(i) Respiration Rate

Effect of single i.m. injection of 20 mg/kg body weight of enrofloxacin on respiration rate at different hours are presented in Table 1. The values of respiration rate/min at 0 hour ranged from 15 to 20 with a mean of 16.8 ± 0.96. Analysis of data shows non-significant difference between the values noted at 0 h (pre treatment) and 4, 8, 12 and h hours port drug administration. This clearly shows that the drug has no effect on respiration rate.

Table – 1

Effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on respiratory rate/min.

Animal No.	Time in Hours				
	0	4	8	12	24
1	15	18	16	17	16
2	18	17	18	19	17
3	16	18	17	18	15
4	20	18	19	17	18
5	15	17	18	19	17
Mean ± S.E.M.	16.8 ± 0.96	17.6 ± 0.24	17.6 ± 0.50	18.0 ± 0.44	16.6 ± 0.50

Table – 1 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	7.04	1.76	1.023^{NS}
Error	20	34.40	1.72	

NS = Non-significant

(ii) Pulse Rate

Table 2 presents the effect of single i.m. injection of enrofloxacin (20 mg/kg) on pulse rate. Mean ± S. E. M. values of 76.6 ± 1.88/min was noted at 0 hour which slightly increased to 77.2 ± 0.58 and 78.2 ± 1.35/min at 4 and 8 h, respectively. Thereafter, the values slightly decreased to 76.0 ± 0.83 at 12 h and further to 74.2 ± 1.01 at 24 h. Analysis of data by single factor ANOVA (Table 2 A) does not show any significant difference. Thus, it seems that enrofloxacin may not have any effect on pulse rate.

(iii) Body temperature

Effect of single i.m injection of enrofloxacin (20 mg/kg) on body temperature is presented in Table 3. Mean ± S.E.M. value of 103.44 ± 0.13°F was noted at oh, which increased non-significantly to 104.16 ± 0.13, 104.56 ± 0.31, 104.20 ± 0.46 and 104.00 ± 0.22°F at 4, 8, 12 and 24 hour post administration of the drug.

(iv) Ruminal Movement

Table 4 presents the effect of single i.m. injection of enrofloxacin (20mg/kg) on ruminal movement. At 0 h, the ruminal movement/5 min ranged from 3 to 8 with a mean of 5.2 ± 0.86. Mean ± S.E.M. values of 5.2 ± 0.58, 6.0 ± 70, 5.0 ± 0.70 and 5.6 ± 0.50 /5 min were obtained at 4, 8, 12 and 24 h, respectively. These values do not differ significantly between hours (Table 4 A), which denote that enrofloxacin does not have any significant effect on ruminal movement.

Table – 2

Effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on pulse rate/min.

Animal No.	Time in Hours				
	0	4	8	12	24
1	72	77	80	75	72
2	75	78	82	78	74
3	80	76	78	74	72
4	82	79	77	75	76
5	74	76	74	78	77
Mean ± S.E.M.	76.6 ± 1.88	77.2 ± 0.58	78.2 ± 1.35	76.0 ± 0.83	74.2 ± 1.01

Table – 2 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	44.56	11.14	1.48^{NS}
Error	20	149.60	7.48	

NS = Non-significant

Table – 3

Effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on body temperature (^0F)

Animal No.	Time in Hours				
	0	4	8	12	24
1	103.4	104.0	105.2	103.8	104.0
2	103.0	103.8	104.4	103.4	103.8
3	103.6	104.2	105.0	103.2	103.4
4	103.4	104.6	104.8	105.0	104.0
5	103.8	104.2	103.4	105.6	104.8
Mean ± S.E.M.	103.44 ±0.13	104.16 ± 0.13	104.56 ±0.31	104.20±0.46	104.00±0.22

Table – 3 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	3.31	0.82	2.00^{NS}
Error	20	8.20	0.41	

NS = Non-significant

Table – 4

Effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on ruminal movement (rate/5 min)

Animal No.	Time in Hours				
	0	4	8	12	24
1	3	4	7	6	4
2	4	5	4	3	6
3	6	7	5	4	5
4	8	4	6	5	7
5	5	6	8	7	6
Mean ± S.E.M.	5.2 ± 0.86	5.2 ± 0.58	6.0 ± 0.70	5.0 ± 0.70	5.6 ± 0.50

Table – 4 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	3.20	0.80	0.34^{NS}
Error	20	46.80	2.34	

NS = Non-significant

B. HAEMATOLOGICAL PARAMETERS –

(i) Hemoglobin (Hb in g/dl)

Different values of hemoglobin noted at different times (0, 4, 8, 12 and 24 h) after single i.m. injection of enrofloxacin are presented in Table 5 and Fig.1. The values of hemoglobin before drug administration (0 h) varied from 8.2 to 8.9 g/dl with a mean of 8.50 ± 0.12 g/dl. Mean ± S.E.M. values of 8.66 ± 0.08, 8.66 ± 0.12, 8.48 ± 0.10 and 8.48 ± 0.11 g/dl were noted at 4, 8, 12 and 24 h respectively, after i.m. administration of enrofloxacin. Analysis of data shows non-significant difference between hours. (Table 5A) This indicates that enrofloxacin has no significant effect on hemoglobin.

(ii) Total Leucocyte count (TLC)

Effect of enrofloxacin on total leucocytes count (TLC) after single i.m. administration (20 mg/kg) is shown in Table 6 and Fig. 2. At 0 h, the mean ± S.E.M. of TLC was noted to be 7045. 6 ± 1.63/mm^3 of blood, which non-significantly increased to 7046.6 ± 1.63, 7048.8 ± 1.71 and 7051.0 ± 1.51/mm^3 at 4, 8 and 12 h post i.m. injection. At 24 h, a non-significantly decreased value of 7047.4 ± 1.72/ mm^3 was noted as compared to 0 h. Analysis of data by single factor ANOVA reveals non-significant difference between hours (Tables 6 A). It seems that enrofloxacin has no significant acute effect on TLC.

Table – 5

Effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on haemoglobin (Hb in g/dl)

Animal No.	Time in Hours				
	0	4	8	12	24
1	8.5	8.8	8.4	8.3	8.6
2	8.3	8.5	8.9	8.7	8.4
3	8.6	8.9	8.8	8.2	8.5
4	8.9	8.6	8.3	8.5	8.8
5	8.2	8.5	8.9	8.7	8.1
Mean ± S.E.M.	8.50 ± 0.12	8.66 ± 0.08	8.66 ± 0.12	8.48 ± 0.10	8.48 ± 0.11

Table – 5 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	0.17	0.04	0.80^{NS}
Error	20	1.05	0.05	

NS = Non-significant

Table – 6

Effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on Total Leucocyte Count (TLC)

Animal No.	Time in Hours				
	0	4	8	12	24
1	7041	7042	7044	7047	7045
2	7043	7044	7046	7049	7043
3	7046	7047	7049	7050	7047
4	7048	7049	7052	7054	7049
5	7050	7051	7053	7055	7053
Mean ± S.E.M.	7045.6 ±1.63	7046.6 ±1.63	7048.8 ±1.71	7051.0 ±1.51	7047.4 ±1.72

Table – 6 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	100	25	1.66^{NS}
Error	20	300	15	

NS = Non-significant

Fig. 1 : *Showing effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on haemoglobin (Hb in gm/dl)*

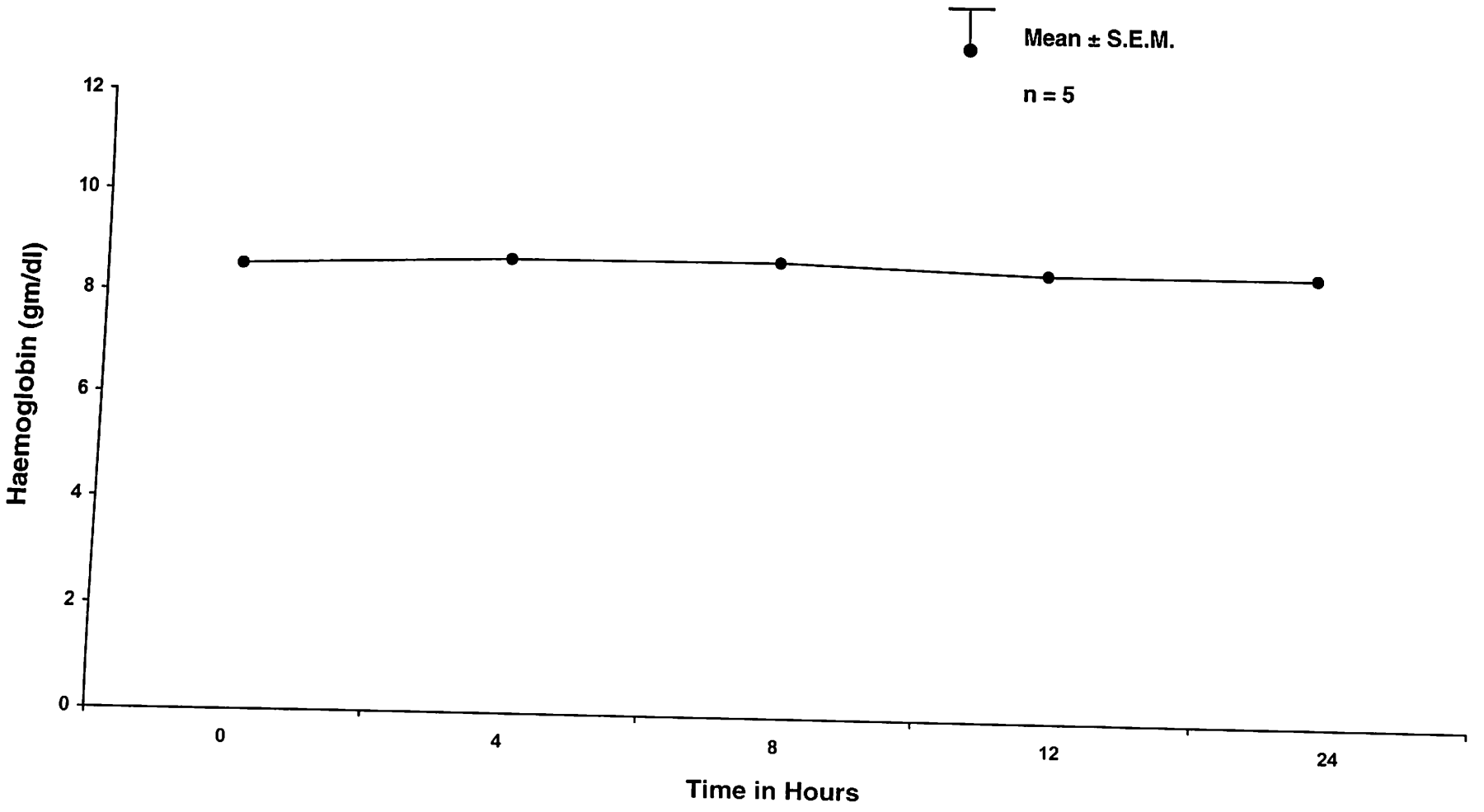
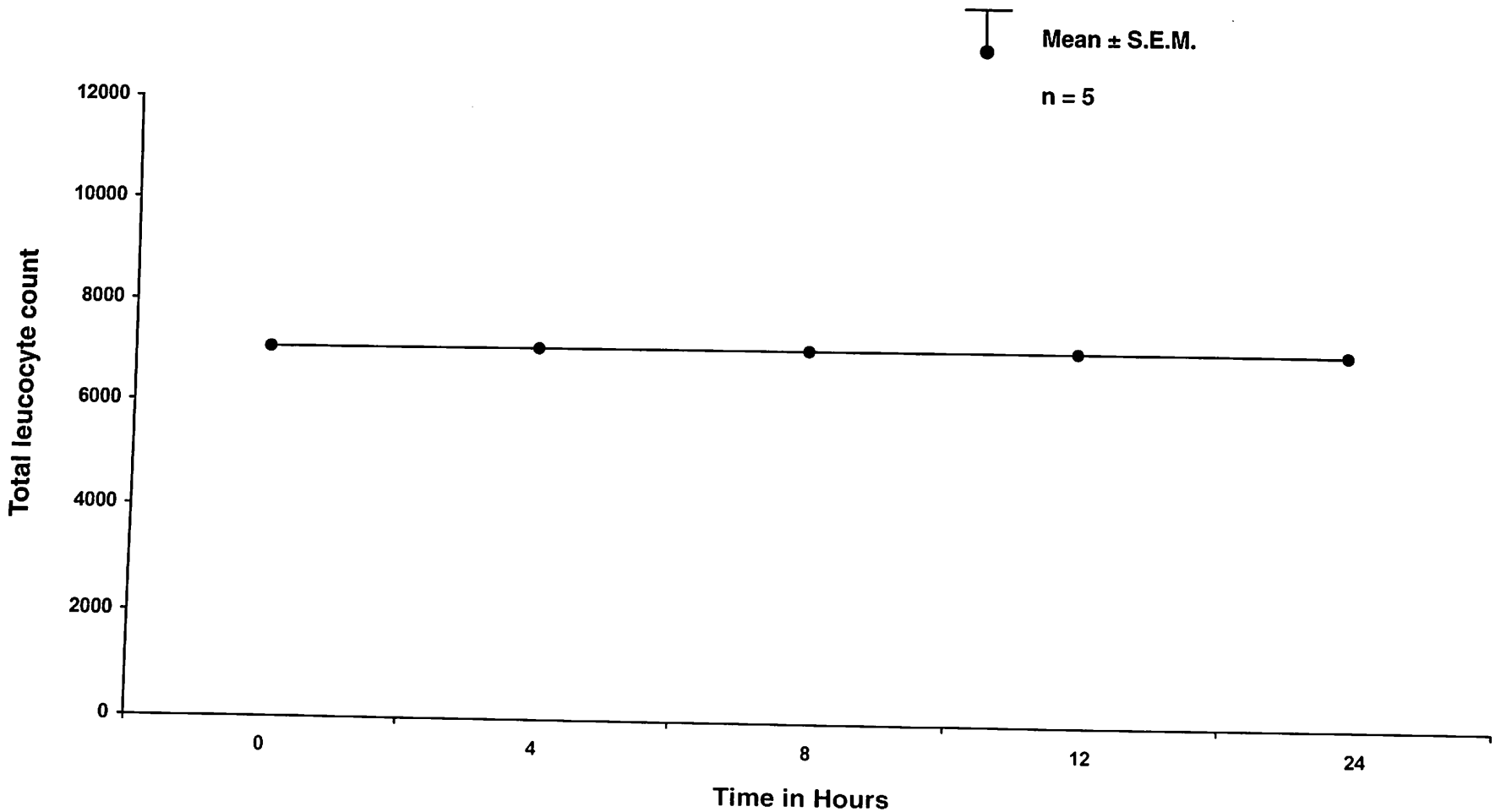


Fig. 2 : *Showing effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on Total Leucocyte Count (TLC)*



(iii) Differential leucocyte count (DLC)

Differential leucocytes count noted at different hours (0, 4, 8, 12, 24 h) after single i.m. injection of enrofloxacin (20 mg/kg) are presented in Table 7 and Fig.3. In parenthesis the original values converted to arc sin values are presented for proper statistical analysis. Single factor ANOVA test reveals that there is no significant effect of enrofloxacin on different leucocytes *viz.*, neutrophil, lymphocyte, monocyte, eosinophil and basophil.

C. BIOCHEMICAL PARAMETERS

(i) Total plasma protein

Table 8 presents the values of total plasma protein before (0 h) and 4, 8, 12 and 24 h post single i.m. injection of enrofloxacin (20 mg/kg) in each of five female goats. At 0 h, a mean ± S.E.M. value of 7.30 ± 0.11 g/dl was estimated as total plasma protein value in goats, which does not show any significant difference from the values obtained at 4, 8, 12 and 24 h (Table 8 A and Fig. 4). Mean ± S.E.M. values of 7.36 ± 0.05, 7.50 ± 0.0 7, 7.60 ± 0.0 7 and 7.34 ± 0.08 g/dl were obtained at 4, 8, 12 and 24 h post i.m. injection of enrofloxacin.

Table – 7

Effect of single i.m. injection of enrofloxacin (20mg/kg)at various time intervals in goats on Differential leucocyte count (DLC)

ıimal ımber	Time in Hours																								
	0					4					8					12					24				
	N	L	M	E	B	N	L	M	E	B	N	L	M	E	B	N	L	M	E	B	N	L	M	E	B
1	36% (36.87)	54% (47.29)	3% (9.98)	2% (8.13)	1% (5.74)	35% (36.27)	55% (47.87)	4% (11.54)	3% (9.98)	0% (0)	37% (37.47)	55% (47.87)	4% (11.54)	2% (8.13)	1% (5.74)	36% (36.87)	55% (47.87)	4% (11.54)	3% (9.98)	1% (5.74)	39% (38.65)	50% (45.00)	4% (11.54)	2% (8.13)	0% (0)
2	39% (38.65)	50% (45.00)	3% (9.98)	3% (9.98)	1% (5.74)	40% (39.23)	50% (45.00)	3% (9.98)	4% (11.54)	1% (5.74)	38% (38.06)	52% (46.15)	3% (9.98)	3% (9.98)	1% (5.74)	41% (39.82)	51% (45.57)	3% (9.98)	3% (9.98)	0% (0)	37% (37.47)	52% (46.15)	3% (9.98)	4% (11.54)	1% (5.74)
3	35% (36.27)	55% (47.87)	3% (9.98)	3% (9.98)	0% (0)	36% (36.87)	54% (47.29)	3% (9.98)	2% (8.13)	1% (5.74)	38% (38.06)	52% (46.15)	3% (9.98)	4% (11.54)	0% (0)	40% (39.23)	52% (46.15)	2% (8.13)	2% (8.13)	1% (5.74)	38% (38.06)	53% (46.72)	4% (11.54)	3% (9.98)	1% (5.74)
4	40% (39.23)	50% (45.00)	4% (11.54)	3% (9.98)	1% (5.74)	39% (38.65)	51% 3 (45.57)	3% (9.98)	4% (11.54)	1% (5.74)	41% (39.82)	49% (44.43)	4% (11.54)	3% (9.98)	0% (0)	37% (37.47)	52% (46.15)	3% (9.98)	4% (11.54)	1% (5.74)	40% (39.23)	49% (44.43)	3% (9.98)	2% (8.13)	0% (0)
5	38% (38.06)	52% (46.15)	3% (9.98)	3% (9.98)	0% (0)	39% (38.65)	53% (46.72)	4% (11.54)	4% (11.54)	1% (5.74)	40% (39.23)	52% (46.15)	3% (9.98)	4% (11.54)	1% (5.74)	36% (36.87)	54% (47.29)	3% (9.98)	3% (9.98)	1% (5.74)	36% (36.87)	55% (47.87)	2% (8.13)	4% (11.54)	1% (5.74)
Mean ± S.E.M.	37.81 ± 0.54	46.26 ± 0.58	10.29 ± 0.30	9.61 ± 0.37	3.44 ± 1.40	37.93 ± 0.57	46.49 ± 0.53	10.60 ± 0.38	10.54 ± 0.67	4.59 ± 1.14	38.52 ± 0.43	46.15 ± 0.54	10.60 ± 0.38	10.23 ± 0.63	3.44 ± 1.40	38.05 ± 0.61	46.60 ± 0.42	9.92 ± 0.54	9.92 ± 0.54	4.59 ± 1.14	38.05 ± 0.41	46.03 ± 0.61	10.23 ± 0.63	9.86 ± 0.76	3.44 ± 1.40
Geom. mean	37.55%	52.16%	3.17%	2.76%	2.58% (n=3)	37.74 %	52.56 %	3.36%	3.28%	2.18% (n=4)	38.77 %	51.96 %	3.36%	3.10%	2.58% (n=3)	37.94 %	52.77 %	2.93%	2.93%	2.18% (n=4)	37.97 %	51.75 %	3.10%	2.86%	2.58% (n=3)

N = Neutrophil, L = lymphocyte, M = Monocyte, E = Eosinophil, B = Basophil.

The data in parenthesis indicate Arc sin value

Table – 7 A

Analysis of data by single factor ANOVA showing effect of single i. m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on differential leucocyte count (DLC)

Leucocytes	Sources of variation	D.F.	C.S.S.	M.S.	F
Neutrophil	Between days	4	1·45	0·36	$0·26^{NS}$
	Error	20	27·49	1·37	
Lymphocyte	Between days	4	1·11	0·27	$0·18^{NS}$
	Error	20	29·47	1·47	
Monocyte	Between days	4	1·62	0·40	$0·37^{NS}$
	Error	20	21·59	1·07	
Eosinophil	Between days	4	2·61	0·65	$0·34^{NS}$
	Error	20	37·29	1·86	
Basophil	Between days	4	7·89	1·97	$0·23^{NS}$
	Error	20	171·34	8·56	

NS = Non-significant

Table – 8

Effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on Total plasma protein (g/dl)

Animal No.	Time in Hours				
	0	4	8	12	24
1	7.5	7.4	7.6	7.7	7.5
2	7.1	7.3	7.5	7.6	7.2
3	7.3	7.5	7.7	7.8	7.4
4	7.6	7.4	7.3	7.4	7.5
5	7.0	7.2	7.4	7.5	7.1
Mean ± S.E.M.	7.30 ± 0.11	7.36 ± 0.05	7.50 ± 0.07	7.60 ± 0.07	7.34 ± 0.08

Table – 8 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	0.30	0.07	1.75^{NS}
Error	20	0.86	0.04	

NS = Non-significant

(ii) Blood Glucose

Table 9 presents the values of blood glucose before and after i.m. injection of enrofloxacin (20 mg/kg). Mean ± S.E.M. value of 87. 60 ± 1.02 mg/dl was obtained at 0 h. Mean values of 87.67 ± 0.84, 89 .05 ± 0.87, 89.41 ± 0.77 and 87.41 ± 1.03 mg/dl were obtained at 4, 8, 12 and 24 h after injection of enrofloxacin. Analysis of data by single factor ANOVA reveals non-significant difference (Table 9 A and Fig.5), which denotes that single injection of enrofloxacin does not have any significant effect on blood glucose.

(iii) Serum Cholesterol

Effect of single i.m. injection of enrofloxacin 20 mg/kg at various time intervals in goat on serum cholesterol is shown in Table 10 and Fig. 6. Mean ± S.E.M. value of 54.44 ± 0.67 mg/dl was obtained at 0 h which did not differ significantly from that of 4 h (55.76 ± 0.74 mg/dl), 8 h (56.30 ± 0.74 mg/dl), 12 h (57.39 ± 0.78 mg/dl) and 24 h (55.00 ± 0.62 mg/dl). The result shows that single i.m. injection of enrofloxacin does not have any significant effect on serum cholesterol.

Table – 9

Effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on blood glucose level (mg/dl)

Animal No.	Time in Hours				
	0	4	8	12	24
1	85	85.50	86.20	87.05	85.70
2	86	86.20	88.05	88.35	84.90
3	88	88.15	90.54	90.80	87.72
4	91	90.30	91.05	91.25	90.86
5	88	88.20	89.43	89.61	87.87
Mean ± S.E.M.	87.60 ± 1.02	87.67 ± 0.84	89.05 ± 0.87	89.41 ± 0.77	87.41 ± 1.03

Table – 9 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	17.28	4.32	1.02^{NS}
Error	20	84.48	4.22	

NS = Non-significant

Table – 9

Effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on blood glucose level (mg/dl)

Animal No.	Time in Hours				
	0	4	8	12	24
1	85	85.50	86.20	87.05	85.70
2	86	86.20	88.05	88.35	84.90
3	88	88.15	90.54	90.80	87.72
4	91	90.30	91.05	91.25	90.86
5	88	88.20	89.43	89.61	87.87
Mean ± S.E.M.	87.60 ± 1.02	87.67 ± 0.84	89.05 ± 0.87	89.41 ± 0.77	87.41 ± 1.03

Table – 9 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	17.28	4.32	1.02^{NS}
Error	20	84.48	4.22	

NS = Non-significant

Table – 10

Effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on serum cholesterol (mg/dl)

Animal No.	Time in Hours				
	0	4	8	12	24
1	53.50	54.80	55.10	56.20	55.60
2	52.40	53.60	54.30	55.00	52.80
3	54.80	55.70	56.20	57.80	55.00
4	56.30	57.80	58.20	59.09	56.60
5	55.20	56.90	57.70	58.90	55.00
Mean ± S.E.M.	54.44 ± 0.67	55.76 ± 0.74	56.30 ± 0.74	57.39 ± 0.78	55.00 ± 0.62

Table – 10 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	26.45	6.61	2.57^{NS}
Error	20	51.52	2.57	

NS = Non-significant

(iv) Blood Urea Nitrogen (BUN)

Table 11 presents the values of BUN at 0 h (before administration) and at 4, 8, 12 and 24 h post single i.m. injection of enrofloxacin (20 mg/kg). Mean ± S.E.M. value of 13.46 ± 0.26 mg/dl was obtained before administration of the drug (0 h), which did not differ significantly from that of 4, 8,12 and 24 h post i.m. injection of enrofloxacin (Table 11 A and Fig. 7).

(v) Serum Glutamate Pyruvate Transaminase (SGPT)

Effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on SGPT is presented in Table 12 and Fig. 8. A mean value of 5.59 ± 0.87 IU/L was obtained before administration of the drug that steadily increased to 19.38 ± 1.19 at 4 h, which further increased to 24.28 ± 1.28, 41. 55 ± 1.85 and 49.74 ± 1.39 IU/L at 8, 12 and 24 h, respectively, post i.m. injection of enrofloxacin. Analysis of data by single factor ANOVA denotes that there is a significant increase between hours. The data shows increasing trend with time and maximum value is obtained at 24 h (49.74 ± 1.39 IU/L).

Table – 11

Effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on blood urea nitrogen (BUN) (mg/dl)

Animal No.	Time in Hours				
	0	4	8	12	24
1	13.61	12.23	13.77	12.42	13.68
2	12.93	13.40	12.42	12.80	12.23
3	13.77	11.53	11.90	12.84	13.20
4	14.22	11.67	13.20	13.23	14.08
5	12.80	13.61	12.23	13.77	13.77
Mean ± S.E.M.	13.46 ± 0.26	12.48 ± 0.43	12.70 ± 0.34	13.01 ± 0.22	13.39 ± 0.32

Table – 11 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	3.60	0.90	1.69^{NS}
Error	20	10.60	0.53	

NS = Non-significant

Table – 12

Effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on serum glutamate pyruvate transaminase (SGPT) (IU/L)

Animal No.	Time in Hours				
	0	4	8	12	24
1	7.68	23.04	26.88	46.08	53.76
2	3.84	17.28	23.04	36.48	46.08
3	5.96	19.20	26.84	38.02	47.25
4	7.19	16.51	20.05	42.70	50.20
5	3.29	20.90	24.59	44.50	51.45
Mean ± S.E.M.	$5.59^{a} \pm 0.87$	$19.38^{b} \pm 1.19$	$24.28^{c} \pm 1.28$	$41.55^{d} \pm 1.85$	$49.74^{e} \pm 1.39$

Different superscripts differ significantly at $p < 0.05$

Table – 12 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	6234.16	1558.54	169.22*
Error	20	184.24	9.21	

* $p < 0.05$

Fig. 3 : *Showing effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on Differential Leucocyte Count (DLC)*

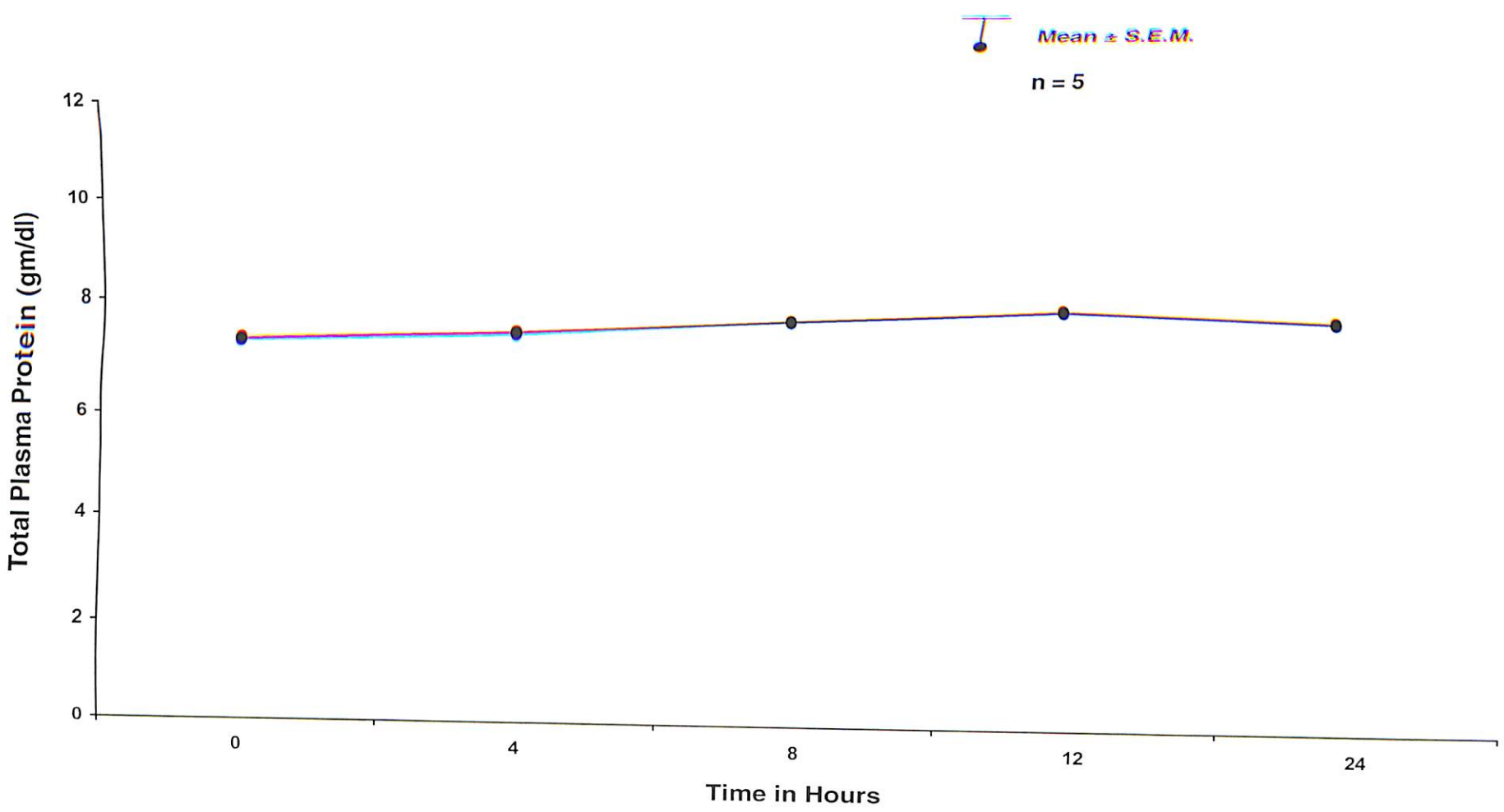

Mean ± S.E.M.
n = 5
Total Plasma Protein (gm/dl)
12
10
8
6
4
2
0
0
4
8
12
24
Time in Hours

Fig. 4 : *Showing effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on Total Plasma Protein (gm/dl)*

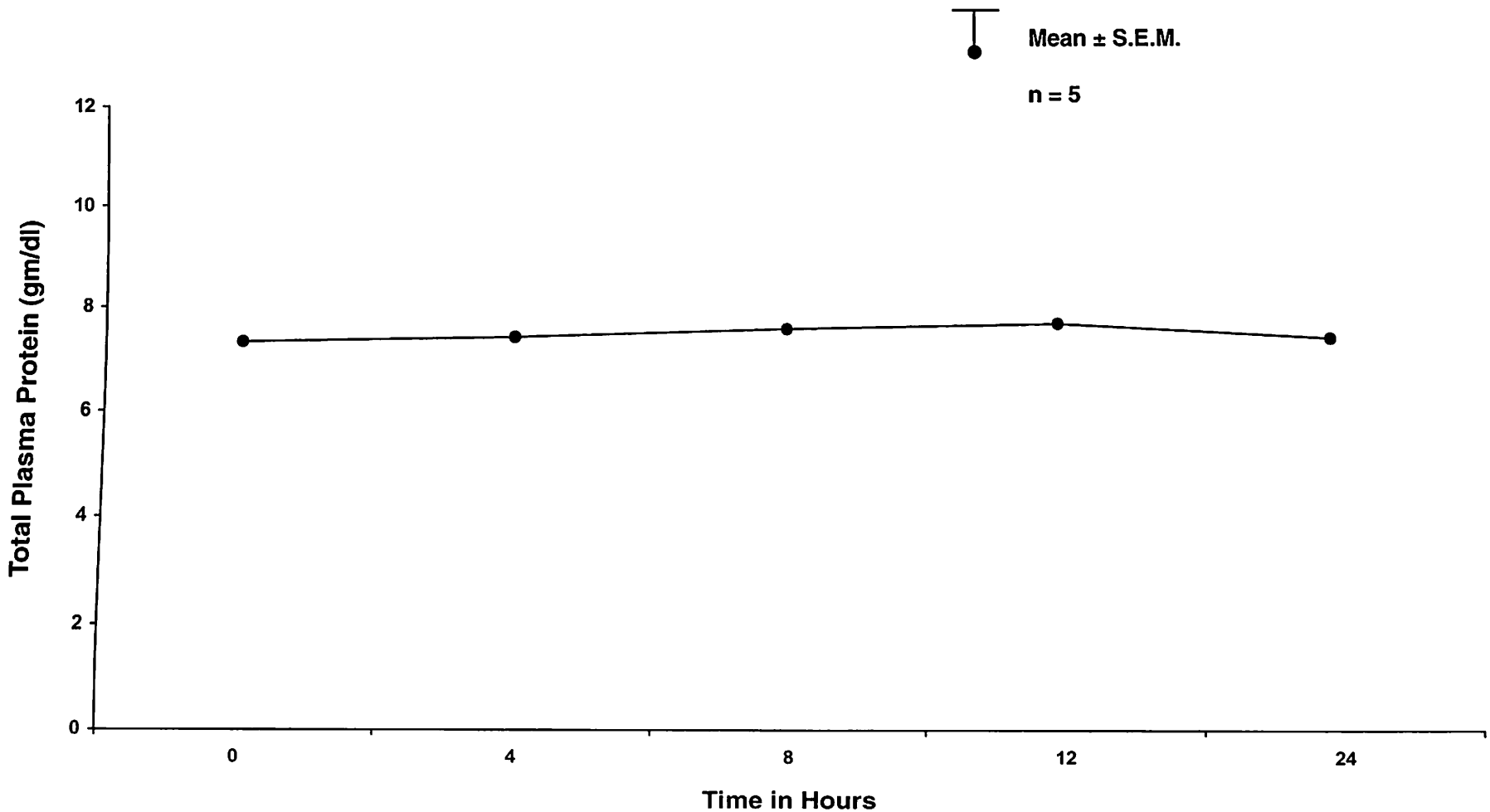


Fig. 5 : *Showing effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on Blood Glucose Level (mg/dl)*

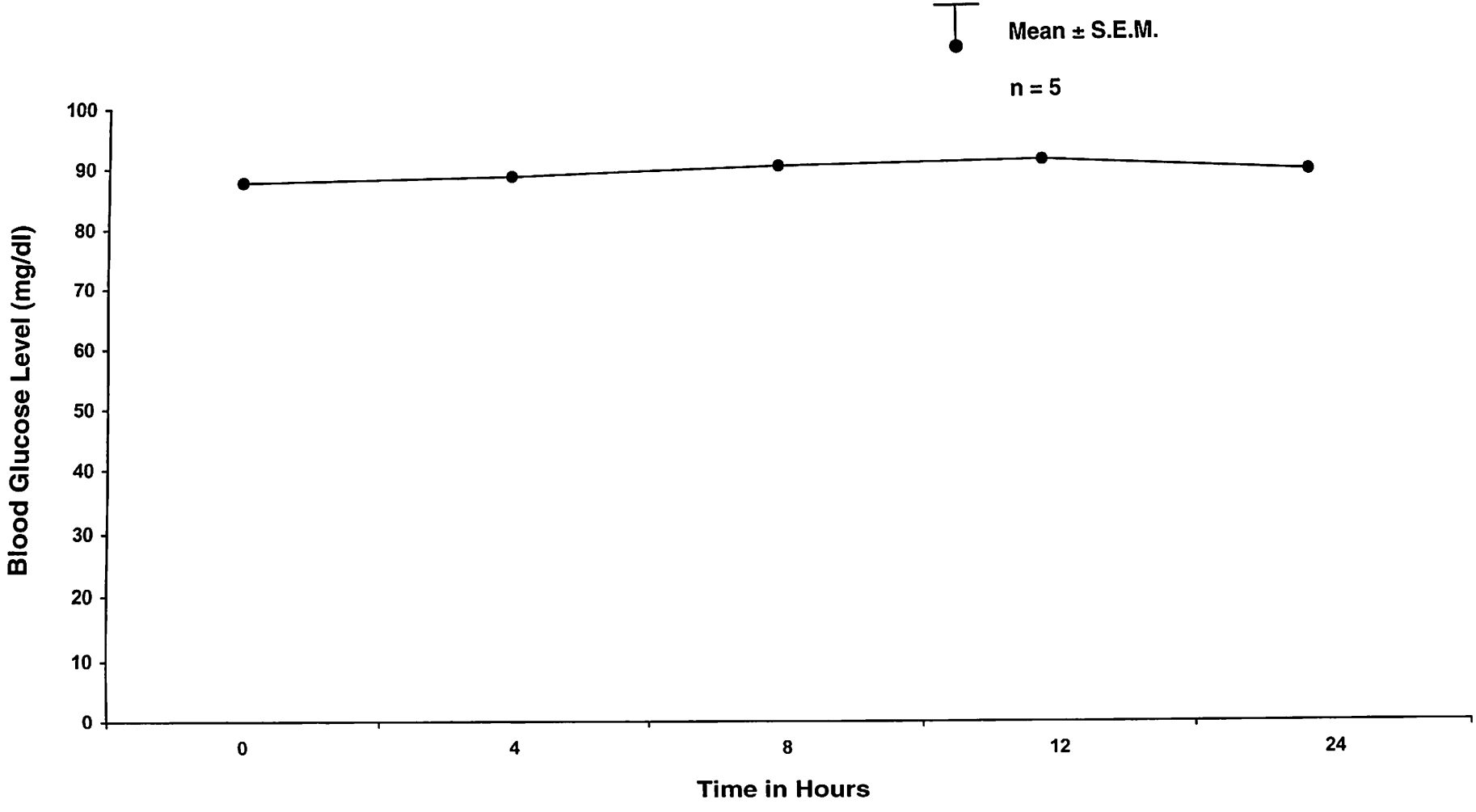


Fig. 6 : *Showing effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on Serum Cholesterol (mg/dl)*

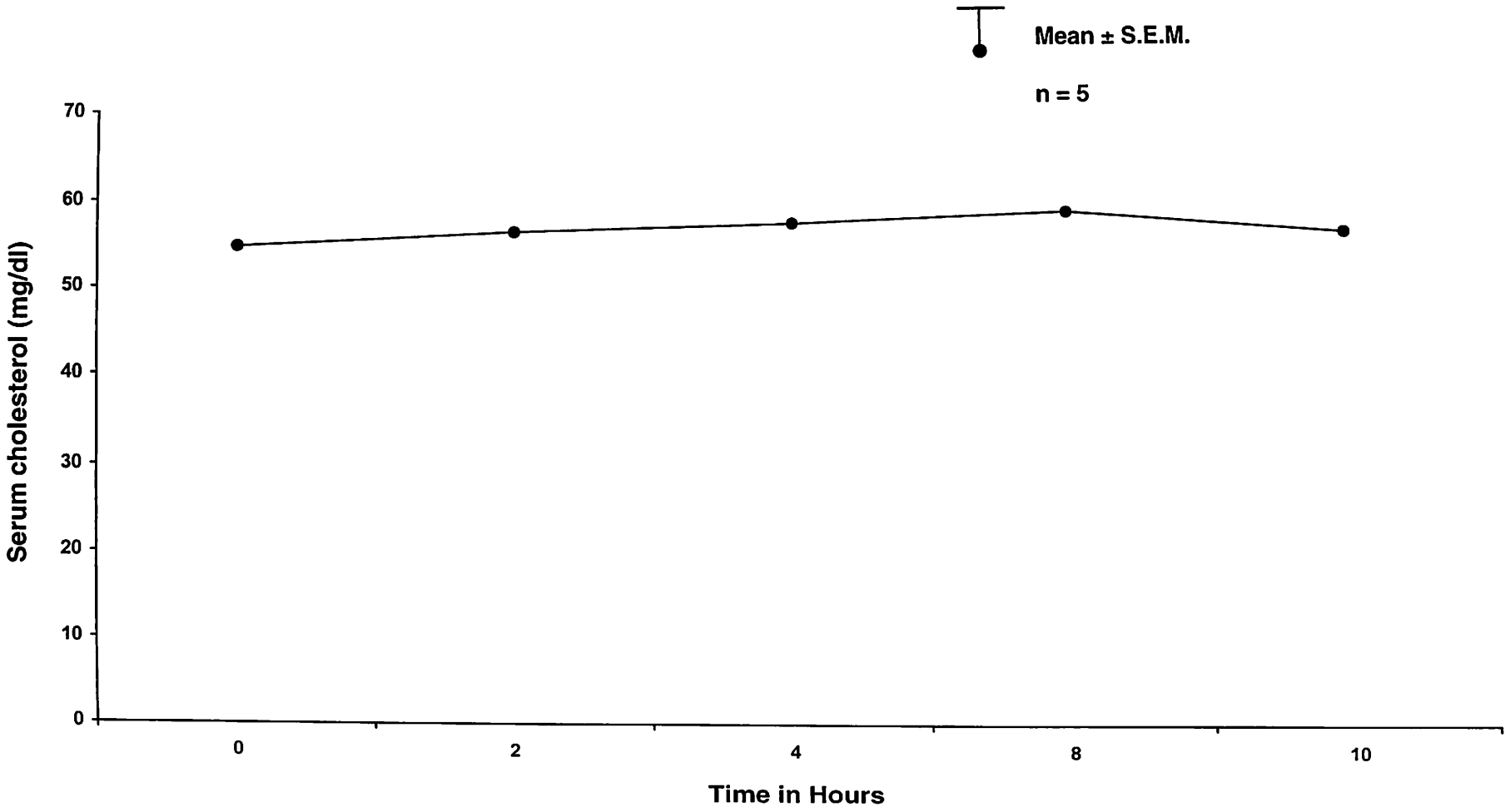


Fig. 7 : *Showing effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on Blood Urea Nitrogen (mg/dl)*

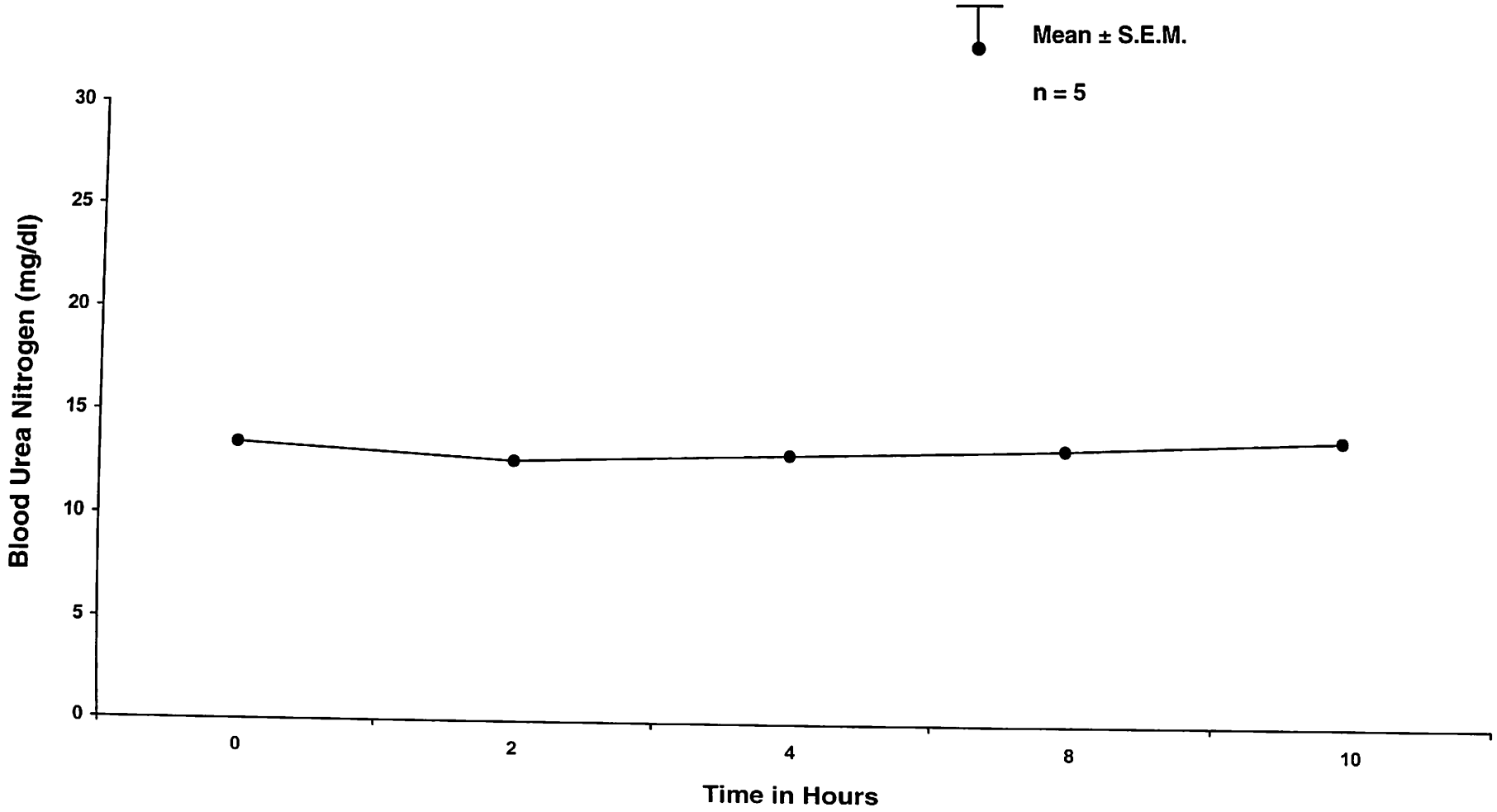


Fig. 8 : *Showing effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on Serum Glutamate Pyruvate Transaminase (SGPT) (IU/L)*

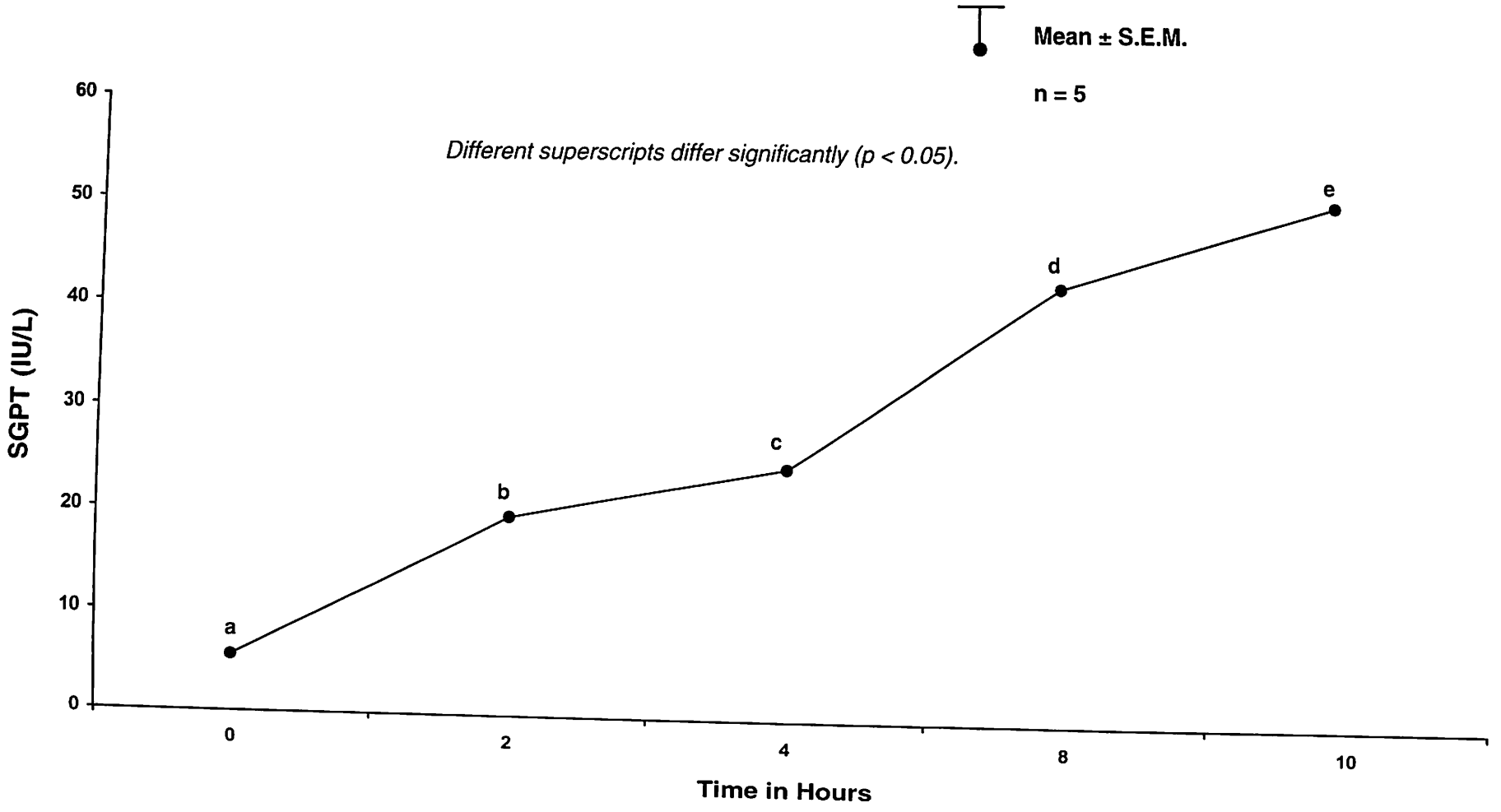


Fig. 9 : *Showing effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on Serum Glutamate Oxaloacetate Transaminase (SGOT) (IU/L)*

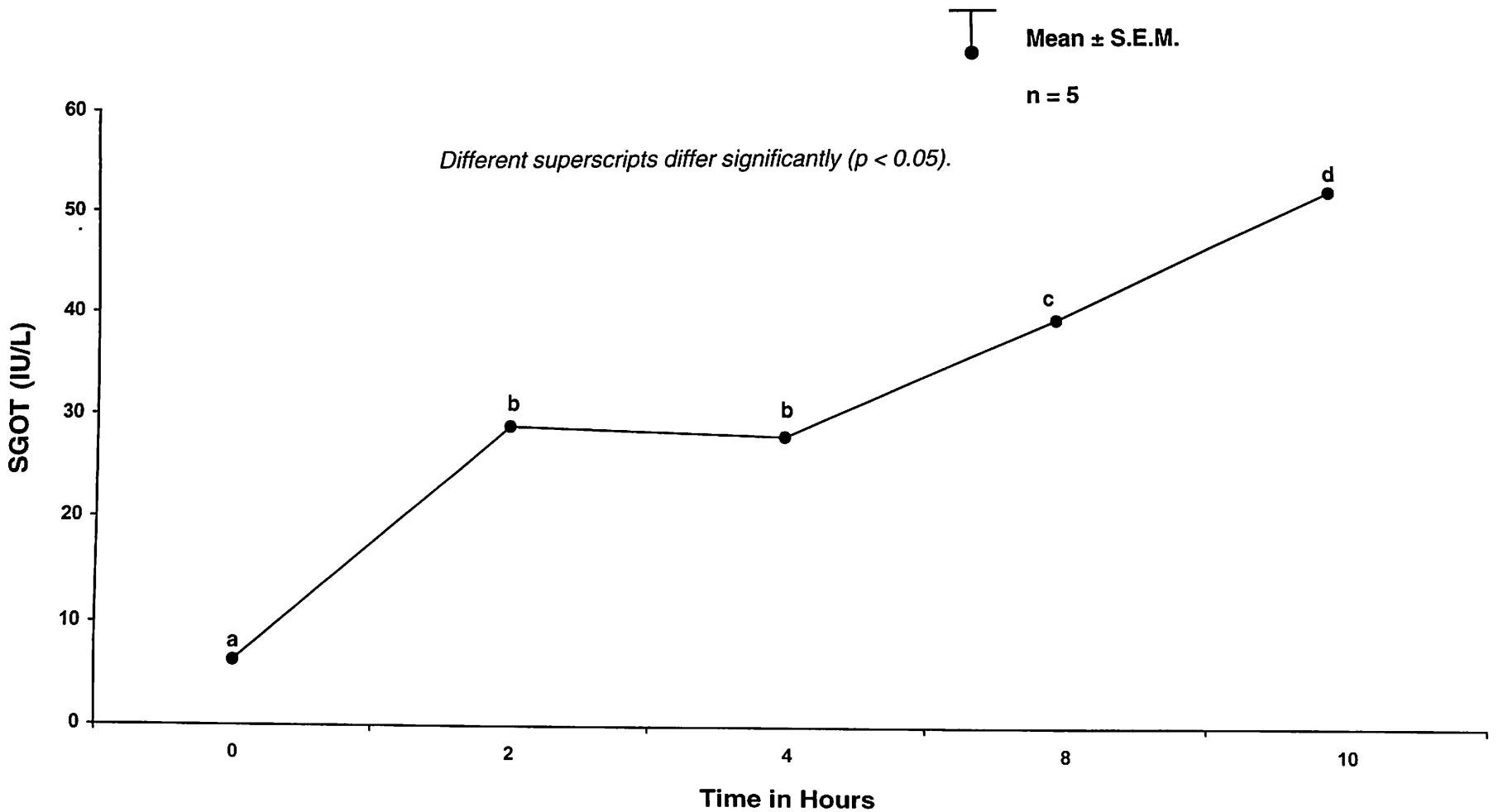
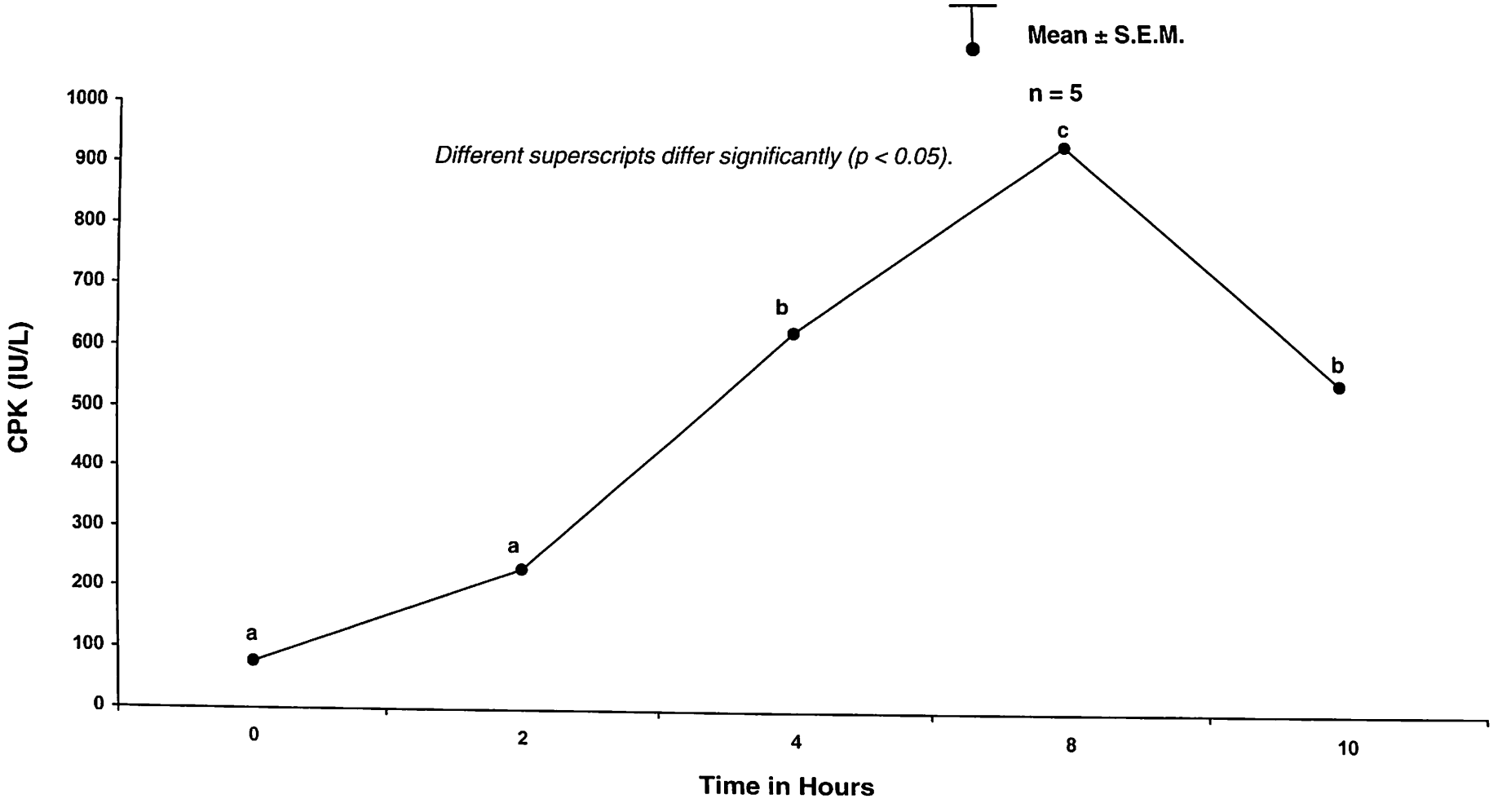


Fig. 10 : *Showing effect of single i.m. injection of enrofloxacin (20 mg/kg) at various time intervals in goats on Creatine Phosphokinase (CPK) (IU/L)*



SUB ACUTE

Sub acute toxicity study of enrofloxacin was conducted post i.m. administration of 5 mg/kg, i.m. once daily for 7 days in each of five healthy goat. The blood samples were collected on day 0 (before administration of the drug) and on 1, 2, 4, and 7 days post drug administration. The following parameters were studied and the results are noted below.

A. CLINICAL PARAMETERS

(i) Respiration Rate

Effect of single i.m. injection of 5 mg/kg body weight of enrofloxacin on respiration rate at different days are presented in Table 15. The value of respiration rate/minute on day 0 ranged from 16 to 20 with a mean of 16.8 ± 0.73/min. Analysis of data shows non-significant difference between the values noted on day 0 (pretreatment) and on 1, 2, 4 and 7 days post drug administration (Table 15 A). This clearly shows that the drug has no effect on respiration rate.

(ii) Pulse Rate

Table 16 presents the effect of i.m. injection of enrofloxacin (5mg/kg body weight once daily for 7 days) on pulse rate. Mean ± S.E.M. values of 76 .6 ± 2.42 /min was noted on day 0 which slightly increased to 78.2 ± 1.56 and 76.8 ± 1.24/min on day 2 and 4, respectively. The value slightly decreased to 74.2 ± 1.35/min on day 7. Analysis of data by single factor ANOVA (Table 16 A) does not show any significant difference. Thus, it seems that enrofloxacin may not have any effect on pulse rate.

Table – 15

Effect of enrofloxacin (5 mg/kg once daily for 7 days) at various day intervals in goats on respiration rate/min

Animal No.	Time in Days				
	0	1	2	4	7
1	16	18	17	18	15
2	15	18	16	17	16
3	18	17	18	19	17
4	19	18	19	17	18
5	16	17	18	19	17
Mean ± S.E.M.	16.8 ± 0.73	17.6 ± 0.24	17.6 ± 0.50	18.0 ± 0.44	16.6 ± 0.50

Table - 15 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	7.07	1.76	1.33^{NS}
Error	20	26.40	1.32	

NS = Non-significant

Table – 16

Effect of enrofloxacin (5 mg/kg once daily for 7 days) at various day intervals in goats on pulse rate/min

Animal No.	Time in Days				
	0	1	2	4	7
1	74	76	78	78	74
2	73	79	83	75	73
3	79	75	80	78	70
4	85	80	76	80	78
5	72	76	74	73	76
Mean ± S.E.M.	76.6 ± 2.42	77.2 ± 0.96	78.2 ± 1.56	76.8 ± 1.24	74.2 ± 1.35

Table – 16 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	43.60	10.90	0.86^{NS}
Error	20	252.40	12.62	

NS = Non-significant

(iii) Body Temperature

Effect of i.m injection of enrofloxacin (5 mg/kg daily for 7 day) on body temperature is presented in Table 17. Mean ± S.E.M. value of 102.64 ± 0.11°F was noted on day 0 which increased slightly to 102.84 ± 0.11, 102.80 ± 0.20, 103.12 ± 0.10 and 102.88 ± 0.20°F on day 1, 2, 4 and 7 post administration of the drug. Non-significant difference in body temperature is noted between days (Table 17 A), indicating that the drug may not have any effect on body temperature.

(iv) Ruminal Movement

Table 18 presents the effect of daily i.m. injection of enrofloxacin (5 mg/kg for 7 days) on ruminal movement. On day 0, the ruminal movement/ 5 min ranged from 3 to 6 with a mean of 4.2 ± 0.58. Mean ± S.E.M. values of 4.8 ± 0.58, 4.8 ± 0.58, 4.2 ± 0.37 and 4.2 ± 0.58/5 min were obtained on day 1, 2, 4 and 7, respectively. These values do not differ significantly between days (Table 18 A), which, denotes that enrofloxacin does not have any significant effect on ruminal movement.

Table – 17

Effect of enrofloxacin (5 mg/kg once daily for 7 days) at various day intervals in goats on body temperature (^0F)

Animal No.	Time in Days				
	0	1	2	4	7
1	102.4	102.6	102.4	102.8	102.4
2	102.6	102.8	102.6	103.2	103.0
3	102.4	103.0	103.2	103.4	102.4
4	103.0	103.2	103.4	103.2	103.2
5	102.8	102.6	102.4	103.0	103.4
Mean ± S.E.M.	102.64±0.11	102.84±0.11	102.80±0.20	103.12±0.10	102.88±0.20

Table – 17 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	0.59	0.14	1.16^{NS}
Error	20	2.49	0.12	

NS = Non-significant

Table – 18

Effect of enrofloxacin (5 mg/kg once daily for 7 days) at various day intervals in goats on ruminal movement/5 minutes

Animal No.	Time in Days				
	0	1	2	4	7
1	3	5	6	4	3
2	4	6	5	3	5
3	6	4	3	5	6
4	5	3	6	4	3
5	3	6	4	5	4
Mean ± S.E.M.	4.2 ± 0.58	4.8 ± 0.58	4.8 ± 0.58	4.2 ± 0.37	4.2 ± 0.58

Table – 18 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	2.16	0.54	0.07^{NS}
Error	20	152	7.6	

NS = Non-significant

B. HAEMOTOLOGICAL PARAMETERS

(i) Hemoglobin (Hb in g /dl)

Different values of hemoglobin noted on different days (0, 1, 2, 4 and 7) after daily i.m. injection of enroflocacin are presented in Table 19 and Fig.11. The values of hemoglobin before drug administration on day 0 varied from 8.0 to 8.5 g/dl with a mean of 8.28 ± 0.10 g/dl. Mean ± S.E.M. values of 8.36 ± 0.06, 8.20 ± 0.10, 8.30 ± 0.10 and 10 ± 0.05 g/dl were noted on day, 1, 2, 4 and 7, respectively, after i.m. administration of enrofloxacin. Analysis of data shows non-significant difference between days (Table 19 A). This indicates that enrofloxacin has no significant effect on hemoglobin.

(ii) Total Lecucucyte Count (TLC)

Effect of enrofloxacin on total leucocyte count (TLC) after daily i.m administration (5 mg /kg) of enrofloxacin is shown in Table 20 and Fig. 12. On day 0, the mean ± S.E.M. of TLC was noted to be 7066.0 ± 1.70/mm^3 of blood, which slightly increased to 7066.8 ± 1.71, 7069.2 ± 1.49, 7070.8 ± 1.42 and 7067 .8 ± 1.52 / mm^3 on day 1,2, 4 and 7 post i.m. injection. However, analysis of data by single factor ANOVA reveals non-significant difference between days (Table 20 A). It seems that enrofloxacin has no significant effect on TLC.

Table – 19

Effect of enrofloxacin (5 mg/kg once daily for 7 days) at various days intervals in goats on haemoglobin (Hb in g/dl)

Animal No.	Time in Days				
	0	1	2	4	7
1	8.1	8.3	8.2	8.5	8.0
2	8.3	8.5	8.0	8.5	8.1
3	8.0	8.2	8.6	8.1	8.3
4	8.6	8.3	8.0	8.4	8.1
5	8.4	8.5	8.2	8.0	8.0
Mean ± S.E.M.	8.28 ± 0.10	8.36 ± 0.06	8.20 ± 0.10	8.30 ± 0.10	8.10 ± 0.05

Table – 19 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	0.19	0.04	1.00^{NS}
Error	20	0.80	0.04	

NS = Non-significant

Table – 20

Effect of enrofloxacin (5 mg/kg once daily for 7 days) at various days intervals in goats on total leucocyte count (TLC)

Animal No.	Time in Days				
	0	1	2	4	7
1	7061	7062	7065	7067	7066
2	7064	7065	7067	7069	7064
3	7066	7066	7069	7070	7067
4	7068	7069	7072	7073	7069
5	7071	7072	7073	7075	7073
Mean ± S.E.M.	7066.0±1.70	7066.8±1.71	7069.2±1.49	7070.8±1.42	7067.8±1.52

Table – 20 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	100	25	2.50^{NS}
Error	20	200	10	

NS = Non-significant

(iii) Differential Leucocyte Count (DLC)

Differential leucocyte counts noted on different days (0, 1, 2, 4 and 7) after daily i.m. injection of enrofloxacin (5 mg/kg) are presented in Table 21 and Fig 13. In parenthesis the original values converted to arc sin values are presented for proper statistical analysis. Single factor ANOVA test reveals that there is no significant effect of ernrofloxacin on different leucocytes *viz.* neutrophils, lymphocytes, monocytes, eosinophils and basophils. Statistical analysis reveals non-significant effect of enrofloxacin on different cells of leucocytes after daily i.m. administration of enrofloxacin (Table 21 and Fig.13).

C. BIOCHEMICAL PARAMETERS

(i) Total plasma protein

Table 22 presents the values of total plasma protein before (day 0) and after day 1, 2, 4 and 7 on daily i.m. injection of enrofloxacin (5 mg/kg for 7 days) in each of five female goats. On day 0 mean ± S.E.M. value of 7.30 ± 0.11 g/dl was noted as the value of total plasma protein in goats, which did not show any significant difference from the values obtained on day 1, 2, 4 and 7 (Table 22 A and Fig 14). Mean ± S.E.M. values of 7.36 ± 0.05, 7.46 ± 0.07, 7.22 ± 0.05 and 7.34 ± 0.08 were obtained on day 1, 2, 4 and 7 post i.m injection of enrofloxacin.

Table - 21

Effect of enrofloxacin (5 mg/kg once daily for 7 days) at various days intervals in goats on differential leucocyte count (DLC)

nimal umber	Time in Days																									
	0					1					2					4					7					
	N	L	M	E	B	N	L	M	E	B	N	L	M	E	B	N	L	M	E	B	N	L	M	E	B
1	35% (36.27)	53% (46.72)	4% (11.54)	2% (8.13)	0% (0)	34% (35.67)	55% (47.87)	3% (9.98)	4% (11.54)	1% (5.74)	36% (36.87)	54% (47.29)	3% (9.98)	2% (8.13)	0% (0)	35% (36.27)	54% (47.29)	4% (11.54)	2% (8.13)	1% (5.74)	38% (38.06)	49% (44.43)	3% (9.98)	2% (8.13)	1% (5.74)
2	38% (38.06)	49% (44.43)	4% (11.54)	4% (11.54)	0% (0)	39% (38.65)	49% (44.43)	4% (11.54)	3% (9.98)	0% (0)	37% (37.47)	51% (45.57)	4% (11.54)	3% (9.98)	0% (0)	40% (39.23)	50% (45.00)	4% (11.54)	2% (8.13)	1% (5.74)	36% (36.87)	51% (45.57)	4% (11.54)	3% (9.98)	0% (0)
3	34% (35.67)	54% (47.29)	4% (11.54)	4% (11.54)	1% (5.74)	35% (36.27)	53% (46.72)	4% (11.54)	3% (9.98)	0% (0)	37% (37.47)	51% (45.57)	4% (11.54)	3% (9.98)	1% (5.74)	39% (38.65)	51% (45.57)	2% (8.13)	2% (8.13)	1% (5.74)	37% (37.47)	52% (46.15)	3% (9.98)	4% (11.54)	0% (0)
4	39% (38.65)	49% (44.43)	3% (9.98)	4% (11.54)	0% (0)	38% (36.06)	50% (45.00)	4% (11.54)	3% (9.98)	0% (0)	40% (39.23)	48% (4385)	4% (11.54)	4% (11.54)	1% (5.74)	36% (36.87)	51% (45.57)	4% (11.54)	4% (11.54)	0% (0)	39% (38.65)	48% (43.85)	4% (11.54)	2% (8.13)	1% (5.74)
5	37% (37.47)	51% (45.57)	4% (11.54)	4% (11.54)	1% (5.74)	38% (36.06)	52% (46.15)	3% (9.98)	3% (9.98)	1% (5.74)	39% (38.65)	51% (45.57)	3% (9.98)	3% (9.98)	1% (5.74)	35% (36.27)	53% (46.72)	4% (11.54)	4% (11.54)	0% (0)	35% (36.27)	54% (47.29)	2% (8.13)	3% (9.98)	0% (0)
Mean ± S.E.M.	37.22 ± 0.55	45.68 ± 0.58	11.22 ± 0.31	10.68 ± 0.76	2.29 ± 1.40	36.54 ± 0.53	46.03 ± 0.61	10.91 ± 0.38	10.29 ± 0.31	2.29 ± 1.40	37.53 ± 0.45	45.57 ± 0.54	10.91 ± 0.38	9.92 ± 0.54	3.44 ± 1.40	37.45 ± 0.62	46.03 ± 0.42	10.85 ± 0.68	9.49 ± 0.83	3.44 ± 1.40	37.58 ± 0.50	45.45 ± 0.61	10.23 ± 0.63	9.55 ± 0.64	2.29 ± 1.40
Geom. mean	36.55%	51.15%	3.77%	3.48%	3.38 % (n=2)	36.74 %	51.75 %	3.56%	3.17%	3.38% (n=2)	37.77 %	50.96 %	3.56%	2.93%	2.58% (n=3)	36.94 %	51.77 %	3.48%	2.63%	2.58 % (n=3)	36.97 %	50.75 %	3.10%	2.70%	3.38% (n=2)

N = Neutrophil, L = lymphocyte, M = Monocyte, E = Eosinophil, B = Basophil.

The data in parenthesis indicate Arc sin value

Table – 21 A

Analysis of data by single factor ANOVA showing effect of enrofloxacin (5 mg/kg once daily for seven days) at various days intervals in goats on differential leucocyte count (DLC)

Leucocytes	Sources of variation	D.F.	C.S.S.	M.S.	F
Neutrophil	Between days	4	3·65	0·91	$0·63^{NS}$
	Error	20	28·88	1·44	
Lymphocyte	Between days	4	1·38	0·34	0.21^{NS}
	Error	20	31·27	1·56	
Monocyte	Between days	4	2·63	0·65	$0·52^{NS}$
	Error	20	25·06	1·25	
Eosinophil	Between days	4	6·40	1·60	$0·81^{NS}$
	Error	20	39·39	1·96	
Basophil	Between days	4	7·88	1·97	$0·19^{NS}$
	Error	20	197·70	9·88	

NS = Non-significant

(ii) ***Blood Glucose***

Table 23 presents the values of blood glucose before (day 0) and after i.m. injection of enrofloxacin (5 mg/kg) on day 1, 2, 4 and 7. Mean ± S.E.M. value of 85.60 ± 1.02 mg/dl was obtained on day 0. Mean values of 85.50 ± 0.84, 87.18 ± 0.70, 87.43 ± 0.65 and 85.40 ± 1.03 mg/dl were obtained on day 1, 2, 4 and 7 after injection of enrofloxacin. Analysis of data by single factor ANOVA reveals non-significant difference (Table 23 A and Fig. 15) between days, which denotes that injection of enrofloxacin does not have any significant effect on blood glucose.

(Iii) Serum Cholesterol

Effect of daily i.m. injection of enrofloxacin mg/kg on different days in goat on serum cholesterol is shown in Table 24 and Fig. 16. Mean ± S.E.M. value of 56.74 ±0.74 mg/dl was obtained on day 0, which did not differ significantly from that of day 1 (54.44 ± 0.67) mg/dl), day 2 (58.40 ± 2.30 mg/dl), day 4 (60.30 ± 1.42mg/dl) and day 7 (58.38±2.30 mg/dl). The result shows that single i.m. injection of enrofloxacin does not have any significant effect on serum cholesterol (Table 24 A).

Table – 22

Effect of enrofloxacin (5 mg/kg once daily for 7 days) at various days intervals in goats on total plasma protein (g/dl)

Animal No.	Time in Days				
	0	1	2	4	7
1	7.3	7.5	7.6	7.1	7.4
2	7.0	7.2	7.4	7.2	7.1
3	7.5	7.4	7.6	7.4	7.5
4	7.1	7.3	7.5	7.3	7.2
5	7.6	7.4	7.2	7.1	7.5
Mean ± S.E.M.	7.30 ± 0.11	7.36 ± 0.05	7.46 ± 0.07	7.22 ± 0.05	7.34 ± 0.08

Table – 22 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	0.20	0.05	2.50^{NS}
Error	20	0.52	0.02	

NS = Non-significant

Fig. 11 : *Showing effect of enrofloxacin (5 mg/kg daily for 7 days) at various days intervals in goats on haemoglobin (Hb in gm/dl)*

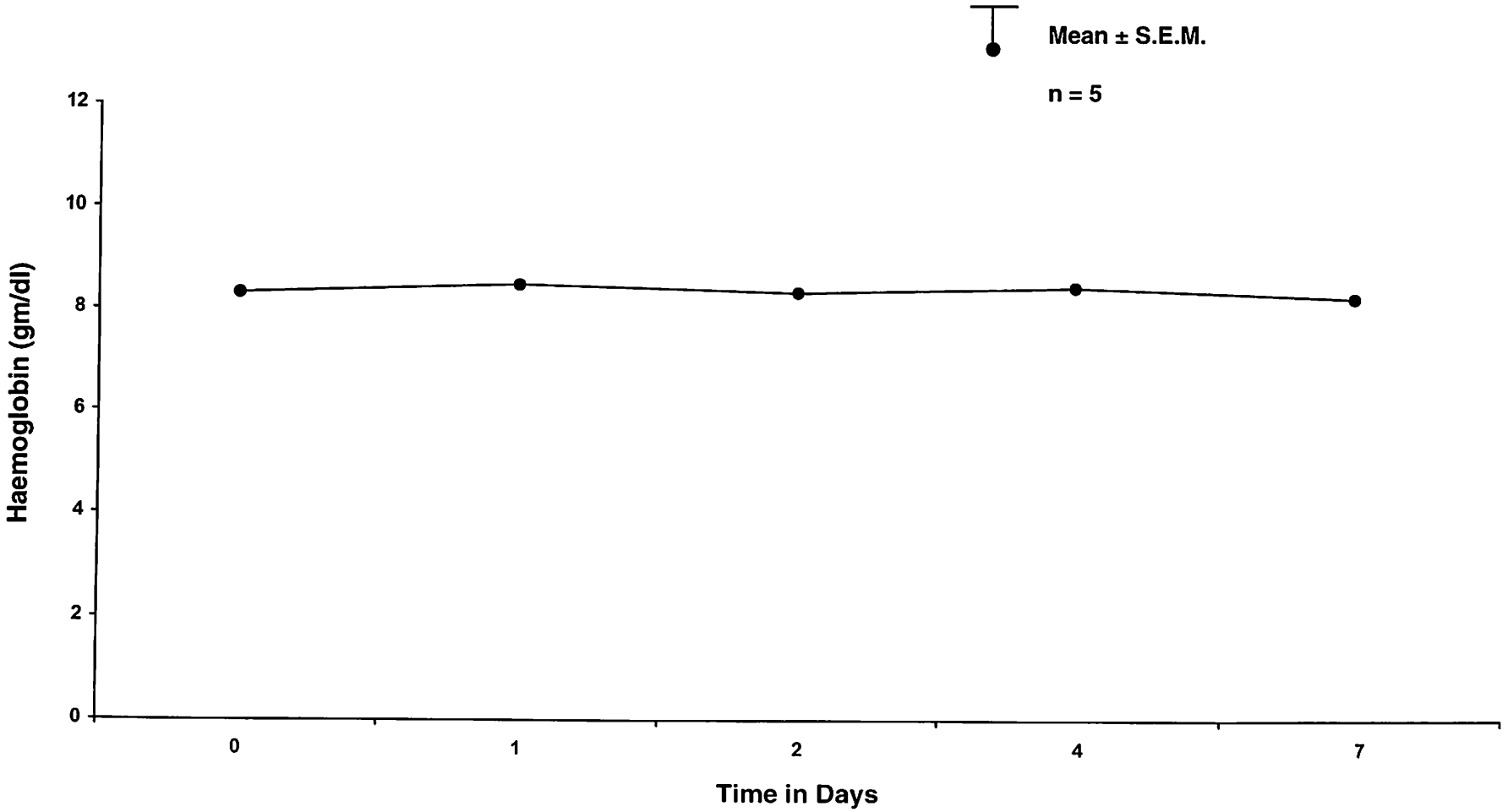


Fig. 11 : *Showing effect of enrofloxacin (5 mg/kg daily for 7 days) at various days intervals in goats on haemoglobin (Hb in gm/dl)*

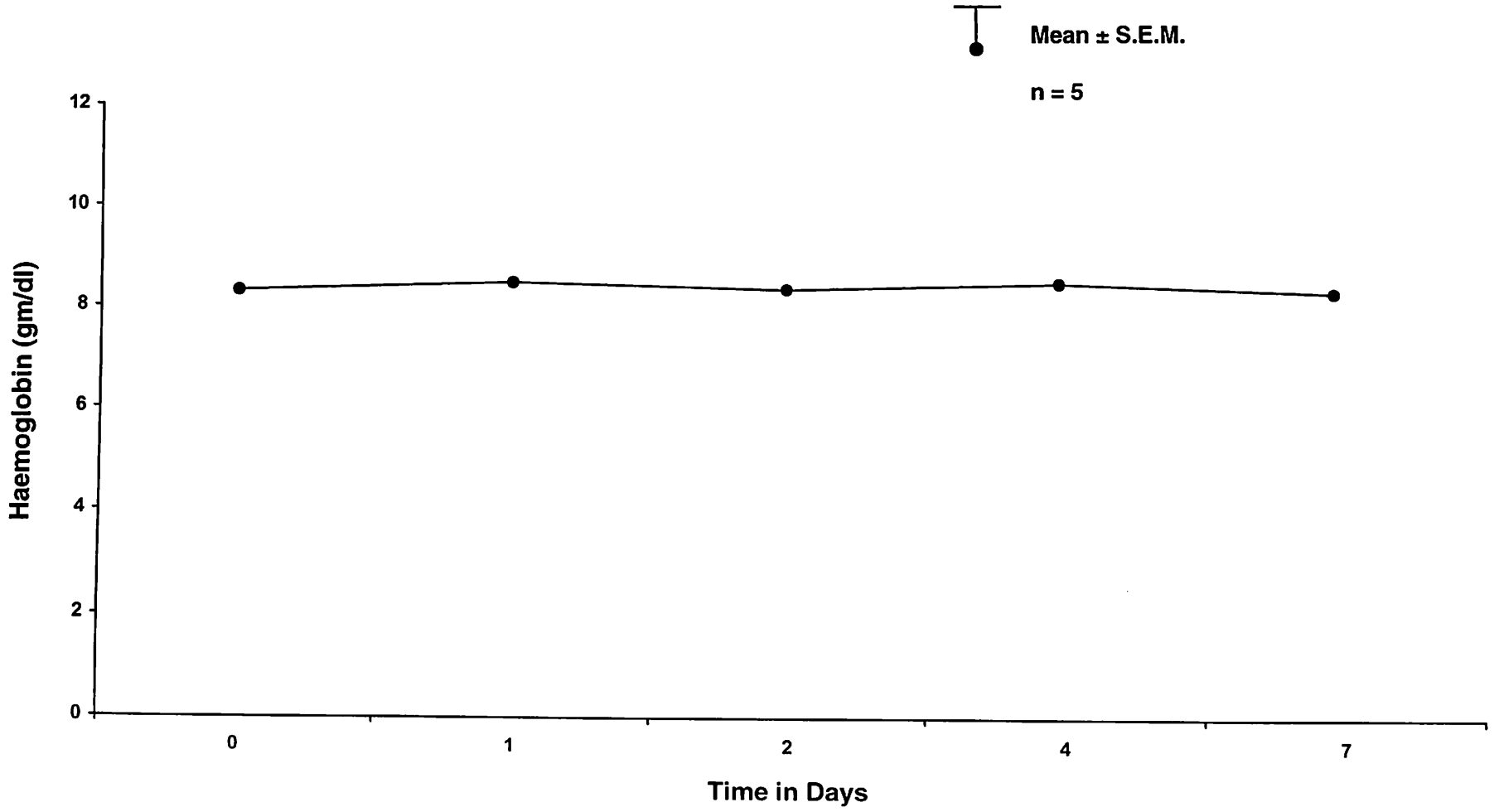


Fig. 12 : *Showing effect of enrofloxacin (5 mg/kg once daily for 7 days) at various days intervals in goats on Total Leucocyte Count (TLC)*

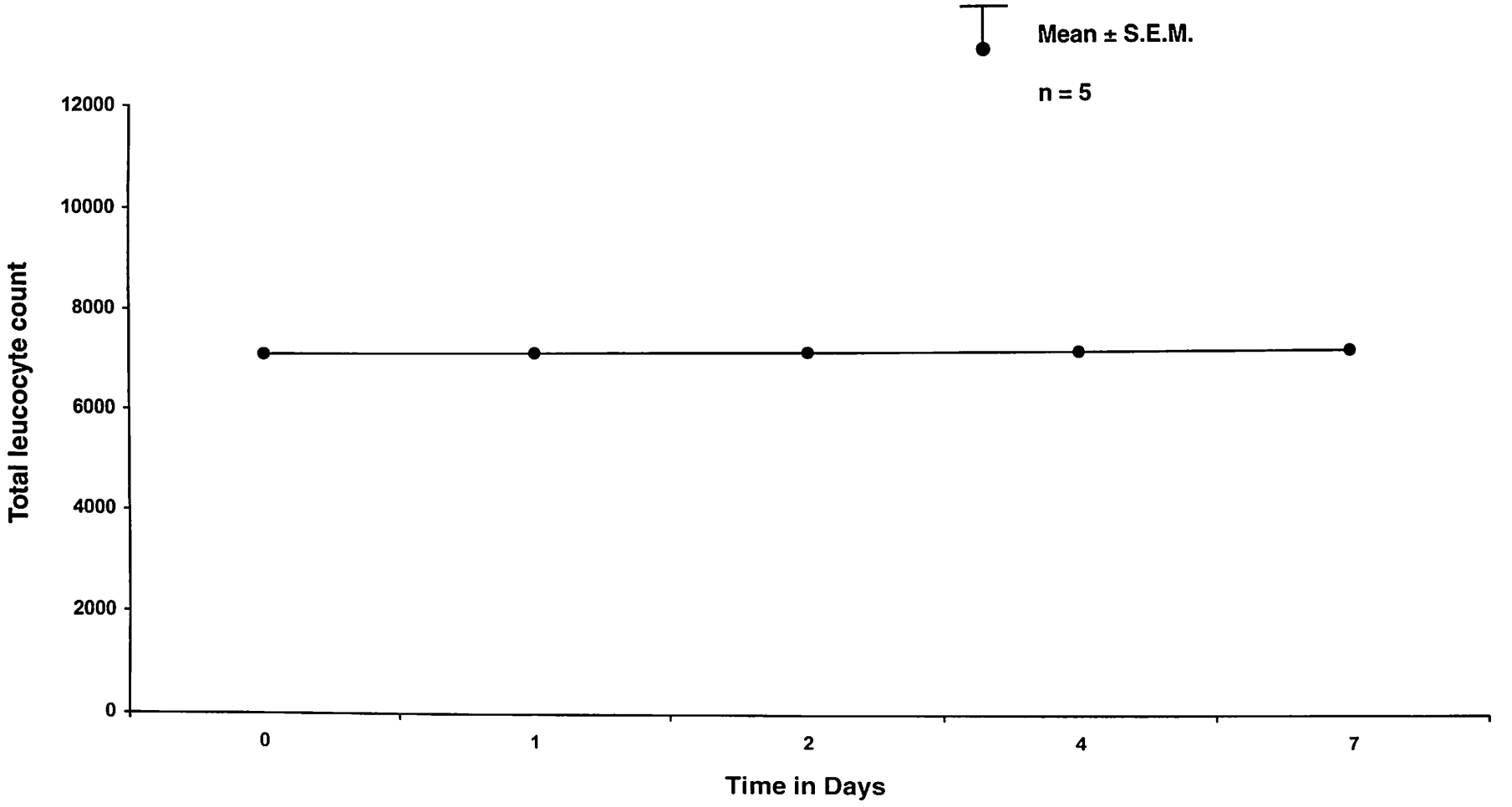


Fig. 13 : *Showing effect of enrofloxacin (5 mg/kg once daily for 7 days) at various days intervals in goats on Differential Leucocyte Count (DLC)*

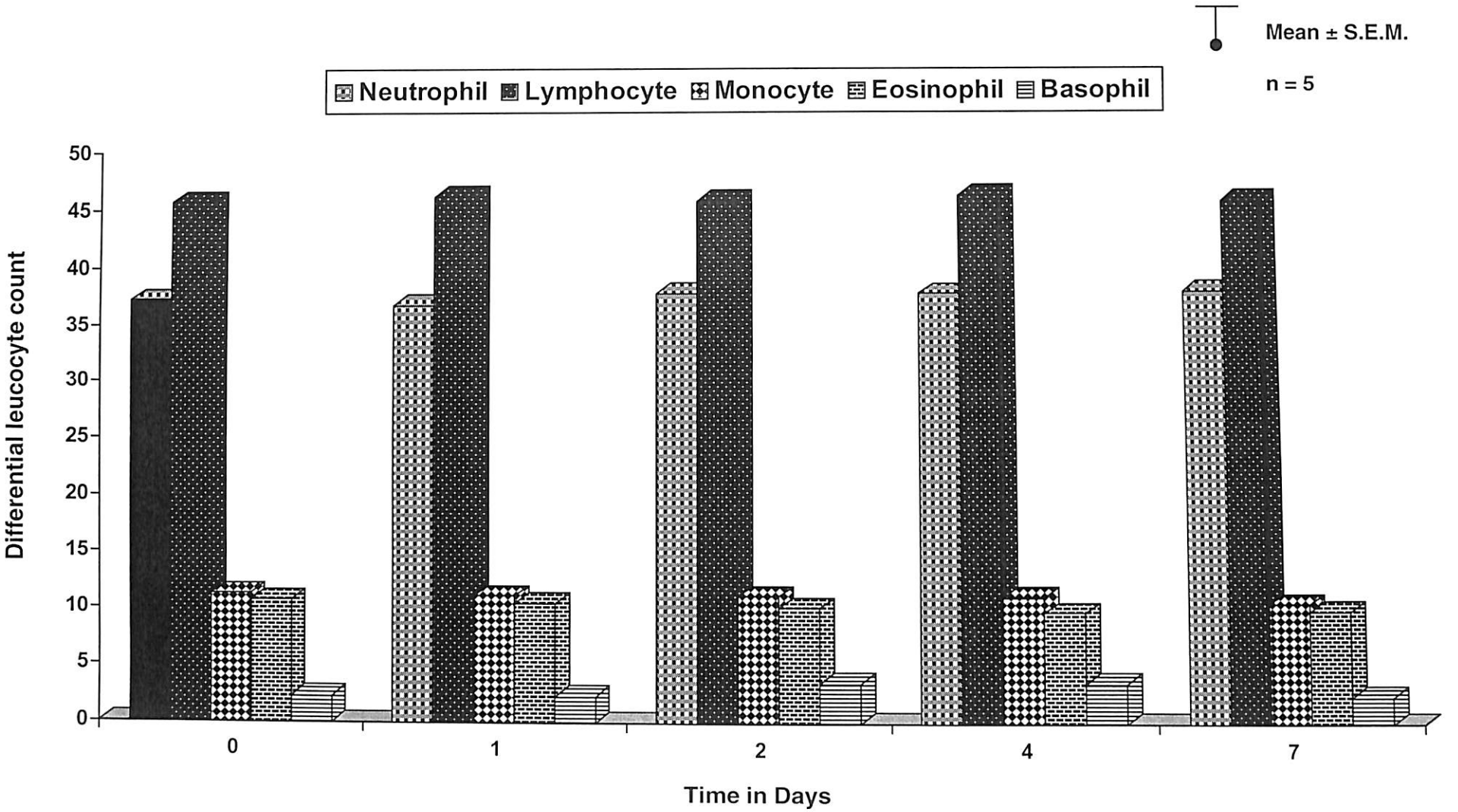
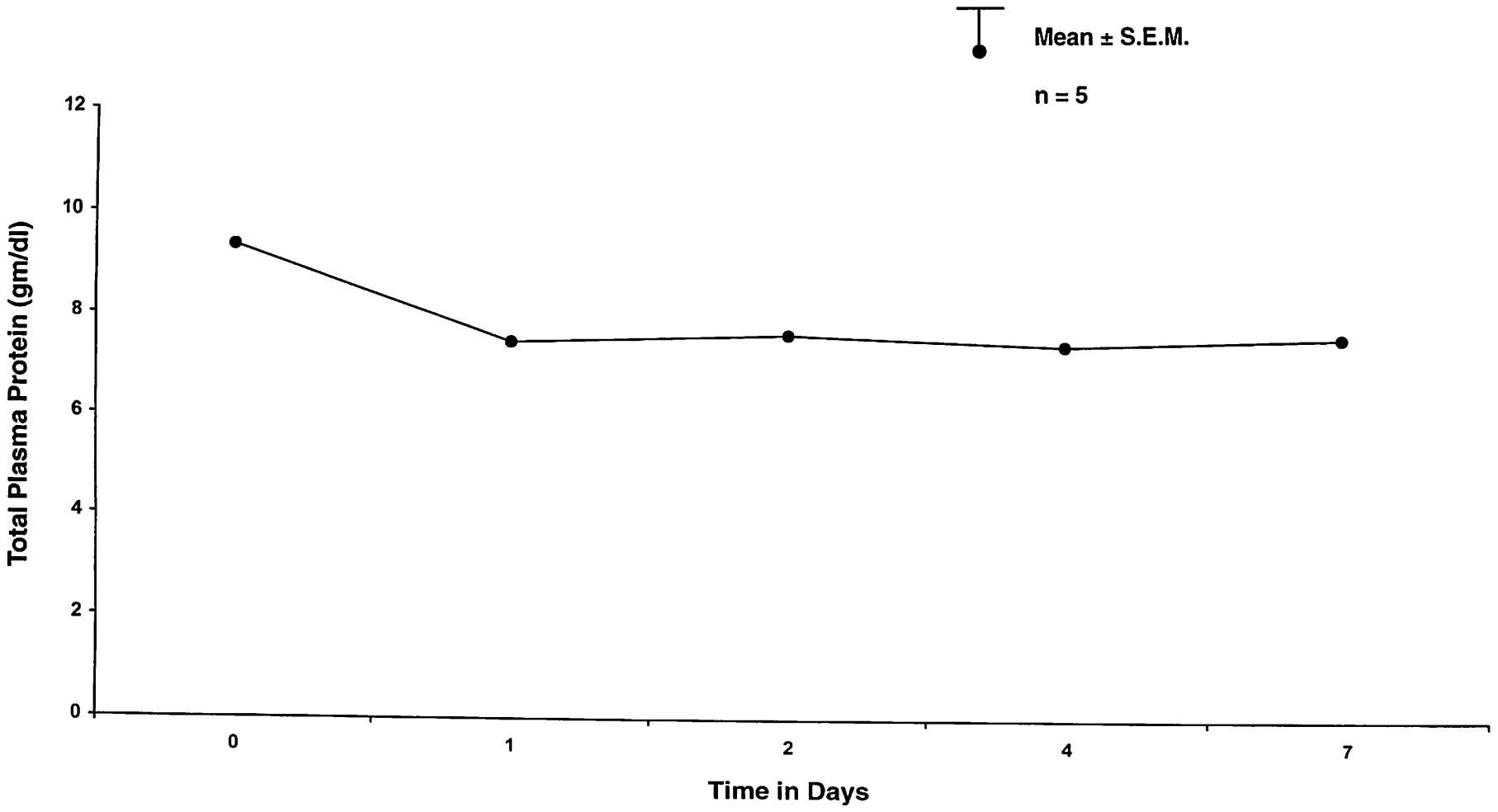


Fig. 14: *Showing effect of enrofloxacin (5 mg/kg once daily for 7 days) at various days intervals in goats on Total Plasma Protein (gm/dl)*



(ii) *Blood Glucose*

Table 23 presents the values of blood glucose before (day 0) and after i.m. injection of enrofloxacin (5 mg/kg) on day 1, 2, 4 and 7. Mean ± S.E.M. value of 85.60 ± 1.02 mg/dl was obtained on day 0. Mean values of 85.50 ± 0.84, 87.18 ± 0.70, 87.43 ± 0.65 and 85.40 ± 1.03 mg/dl were obtained on day 1, 2, 4 and 7 after injection of enrofloxacin. Analysis of data by single factor ANOVA reveals non-significant difference (Table 23 A and Fig. 15) between days, which denotes that injection of enrofloxacin does not have any significant effect on blood glucose.

(Iii) Serum Cholesterol

Effect of daily i.m. injection of enrofloxacin mg/kg on different days in goat on serum cholesterol is shown in Table 24 and Fig. 16. Mean ± S.E.M. value of 56.74 ±0.74 mg/dl was obtained on day 0, which did not differ significantly from that of day 1 (54.44 ± 0.67) mg/dl), day 2 (58.40 ± 2.30 mg/dl), day 4 (60.30 ± 1.42mg/dl) and day 7 (58.38±2.30 mg/dl). The result shows that single i.m. injection of enrofloxacin does not have any significant effect on serum cholesterol (Table 24 A).

Table – 23

Effect of enrofloxacin (5 mg/kg once daily for 7 days) at various days intervals in goats on blood glucose level (mg/dl)

Animal No.	Time in Days				
	0	1	2	4	7
1	83.00	83.50	85.22	85.50	83.75
2	84.00	84.10	86.17	86.70	82.95
3	86.00	86.41	88.30	88.50	85.63
4	89.00	88.20	89.10	89.20	88.96
5	86.00	85.30	87.13	87.25	85.75
Mean ± S.E.M.	85.60 ± 1.02	85.50 ± 0.84	87.18 ± 0.70	87.43 ± 0.65	85.40 ± 1.03

Table – 23 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	19.75	4.93	1.31^{NS}
Error	20	75.26	3.76	

NS = Non-significant

(iv) Blood Urea Nitrogen (Bun)

Table 25 presents the values of BUN on day 0 (before administration) and on day 1, 2, 4 and 7 post daily i.m. injection of enrofloxacin, (5 mg/kg for 7 days). Mean ± S.E.M. value of 12.92 ± 0.46 mg/dl was obtained before administration of the drug (day 0), which did not differ significantly from that of day 1, 2, 4 and 7 post i.m. injection of enrofloxacin (Table 25 A and Fig 17).

(v) *Serum Glutamate Pyruvate Transaminase (SGPT)*

Effect of daily i.m. injection of enrofloxacin (5mg/kg) at various time intervals in goats on SGPT is presented in Table 26 and Fig. 18. A mean value of 7.68 ± 1.49 IU/L was obtained before administration of the drug, which steadily increased to 16.72 ± 1.97 on day 1, which further increased to 23.28 ± 3.87, 34.03 ± 3.29 and 42. 10 ± 2.56 IU/L on day 2, 4 and 7, respectively, post i.m. injection of enrofloxacin. Analysis of data by single factor ANOVA denotes that there is a significant increase between days. The data showed increasing trend with time and maximum value was obtained on day 7 (42.10 ± 2.56 IU/L).

Table – 25

Effect of enrofloxacin (5 mg/kg once daily for 7 days) at various days intervals in goats on blood urea nitrogen (BUN) (mg/dl)

Animal No.	Time in Days				
	0	1	2	4	7
1	11.67	14.08	14.22	12.84	12.14
2	12.14	12.80	13.20	13.77	11.90
3	13.77	13.61	14.36	14.39	13.40
4	14.10	11.53	13.23	13.68	13.65
5	12.93	11.76	12.42	12.80	12.23
Mean ± S.E.M.	12.92 ± 0.46	12.75 ± 0.49	13.48 ± 0.35	13.49 ± 0.30	12.66 ± 0.35

Table – 25 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	3.19	0.79	0.98^{NS}
Error	20	16.22	0.81	

NS = Non-significant

Table – 26

Effect of enrofloxacin (5 mg/kg once daily for 7 days) at various days intervals in goats on serum glutamate pyruvate transaminase (SGPT) (IU/L)

Animal No.	Time in Days				
	0	1	2	4	7
1	11.52	23.04	36.48	46.08	51.84
2	3.84	13.44	19.20	32.64	42.24
3	5.32	12.50	14.90	28.40	40.00
4	7.01	15.25	18.53	27.90	37.27
5	10.71	19.40	27.31	35.14	39.15
Mean ± S.E.M.	7.68^{a} ± 1.49	16.72^{b} ±1.97	23.28^{b}±3.87	34.03^{c}±3.29	42.10^{c}±2.56

Different superscripts differ significantly at $p < 0.05$

Table – 26 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	3725.47	931.36	24.14*
Error	20	771.43	38.57	

* $p < 0.05$

Table – 27

Effect of enrofloxacin (5 mg/kg once daily for 7 days) at various days intervals in goats on serum glutamate oxaloacetate transaminase (SGOT) (IU/L)

Animal No.	Time in Days				
	0	1	2	4	7
1	11.52	17.28	26.88	38.40	46.08
2	3.84	11.52	21.12	30.72	42.24
3	5.34	12.26	14.25	29.10	39.50
4	7.05	16.20	18.27	28.34	38.57
5	10.42	20.00	27.34	36.10	39.50
Mean ± S.E.M.	$7.63^{a} \pm 1.46$	$15.45^{b} \pm 1.58$	$21.57^{c} \pm 2.51$	$32.53^{d} \pm 1.99$	$41.17^{e} \pm 1.37$

Different superscripts differ significantly at $p < 0.05$

Table – 27 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	4	3570.75	892.68	53.04*
Error	20	336.65	16.83	

* $p < 0.05$

Table – 28

Effect of enrofloxacin (5 mg/kg once daily for 7 days) at various days intervals in goats on creatine phosphokinase level (CPK) (IU/L)

Animal No.	Time in Days					
	0	1	2	4	7	12
1	68	989	561	444	316	57
2	109	236	378	385	95	78
3	78	440	692	410	214	62
4	84	325	409	320	115	64
5	98	286	247	405	202	60
Mean ± S.E.M.	$87.4^{ab} \pm 7.26$	$455.2^{cd} \pm 137.62$	$457.4^{ce} \pm 77.03$	$392.8^{de} \pm 20.52$	$188.4^{ab} \pm 39.51$	$64.2^{b} \pm 3.63$

Different superscripts differ significantly at $p < 0.05$

Table – 28 A

Showing analysis of data by single factor ANOVA

Sources of variation	d.f.	C.S.S.	M.S.	F
Between hours	5	833725.40	166745.08	7.43*
Error	24	538468.00	22436.16	

* $p < 0.05$

(vi) Serum Glutamate Oxaloacetate Transminase (SGOT)

Effect of daily i.m. injection of enrofloxacin (5 mg/kg) on different days (0, 1, 2, 4 and 7) is presented in Table 27 and Fig. 19. A mean value of 7.63 ± 1.46 IU/L was obtained before administration of the drug which steadily increased to 15.45 ± 1.58 on day 1, which further increased to 21.57 ± 2.51, 32.53 ± 1.99 and 41.17 ± 1.37 IU/L on day 2, 4 and 7, respectively, post i.m. injection of enrofloxacin. Analysis of data by single factor ANOVA denotes that there is a significant increase between days. The data showed increasing trend with time and maximum value was obtained on day 7 (41.17 ± 1.37 IU/L)

D. PARAMETERS FOR ESTIMATING MUSLE DAMAGE

(i) Creatine phosphokinase (CPK)

Table 28 presents the values of creatine phosphokinase level before (day 0) and after daily i.m. injection of enrofloxacin (5 mg/kg) in each of five female goats. On day 0, mean ± S.E.M. value of CPK in healthy goats was observed to be 87.4± 7.26 IU/L, which increased significantly to 455 .2 ± 137.62, 457.4 ± 77.03, 392.80 ± 20.52 and 188.4 ± 39.51 IU/L on day 1, 2, 4 and 7, respectively. After drug withdrawal it reaches to 64.2 ± 3.63 IU/l on day 12. Statistical analysis of the data showed significant differences between days. Thus, enrofloxacin has significant effect on creatine phosphokinase level, which indicates the muscle damage property of enrofloxacin (Table 28 and Fig. 20).

Fig. 15 : *Showing effect of enrofloxacin (5 mg/kg once daily for 7 days) at various days intervals in goats on Blood Glucose Level (mg/dl)*

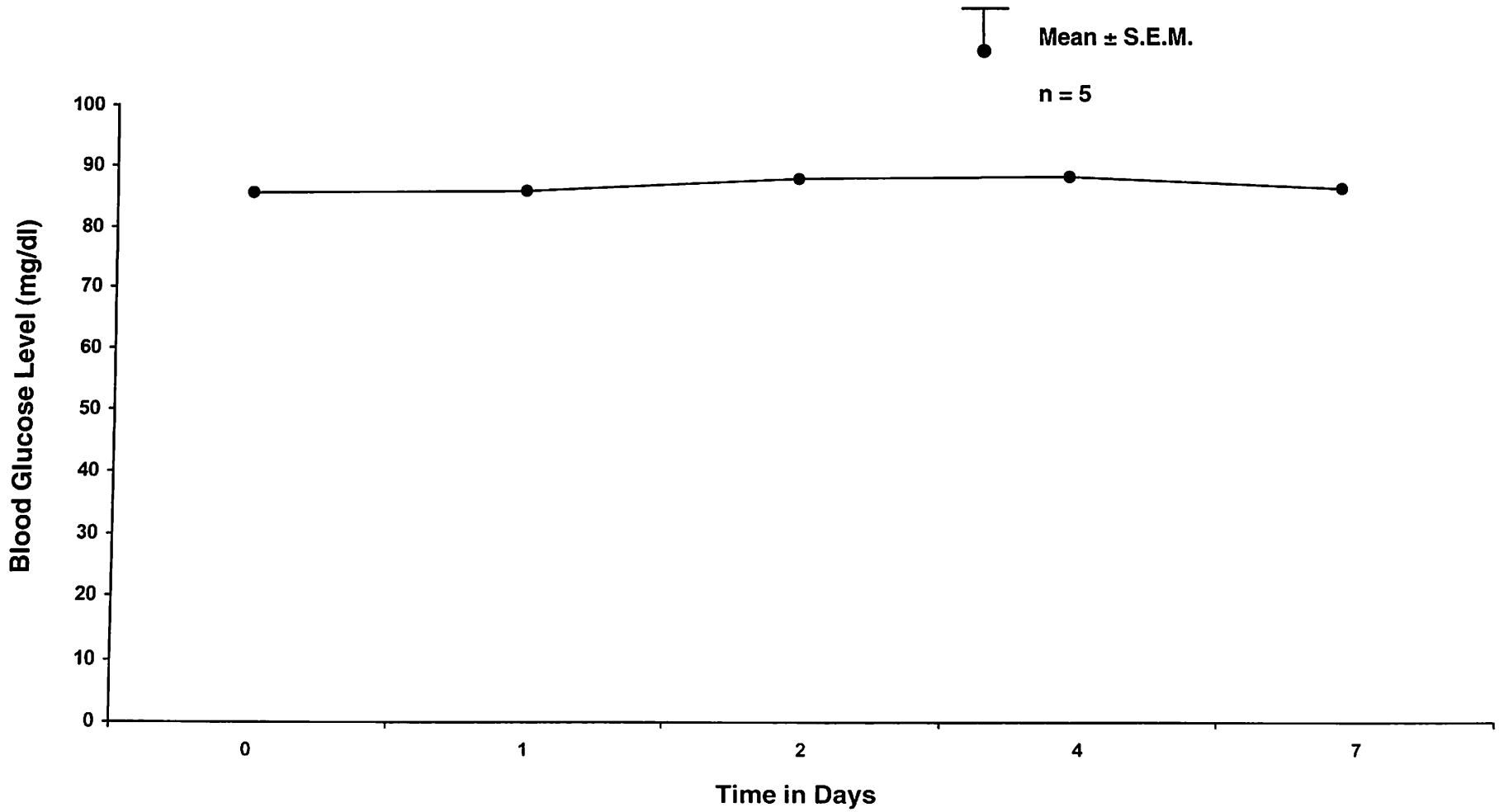


Fig. 16 : *Showing effect of enrofloxacin (5 mg/kg once daily for 7 days) at various days intervals in goats on Serum Cholesterol (mg/dl)*

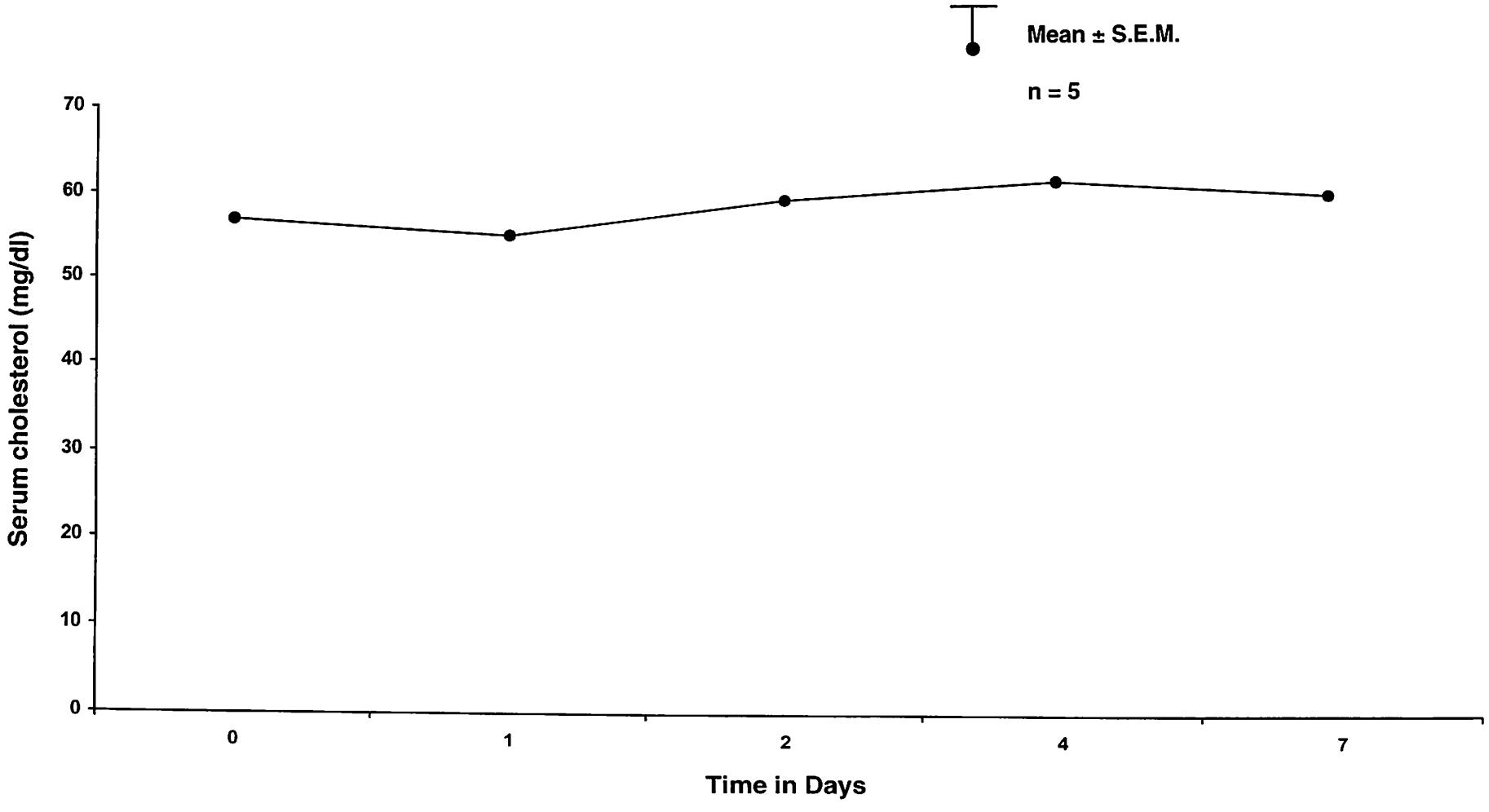


Fig. 17 : *Showing effect of enrofloxacin (5 mg/kg once daily for 7 days) at various days intervals in goats on Blood Urea Nitrogen (mg/dl)*

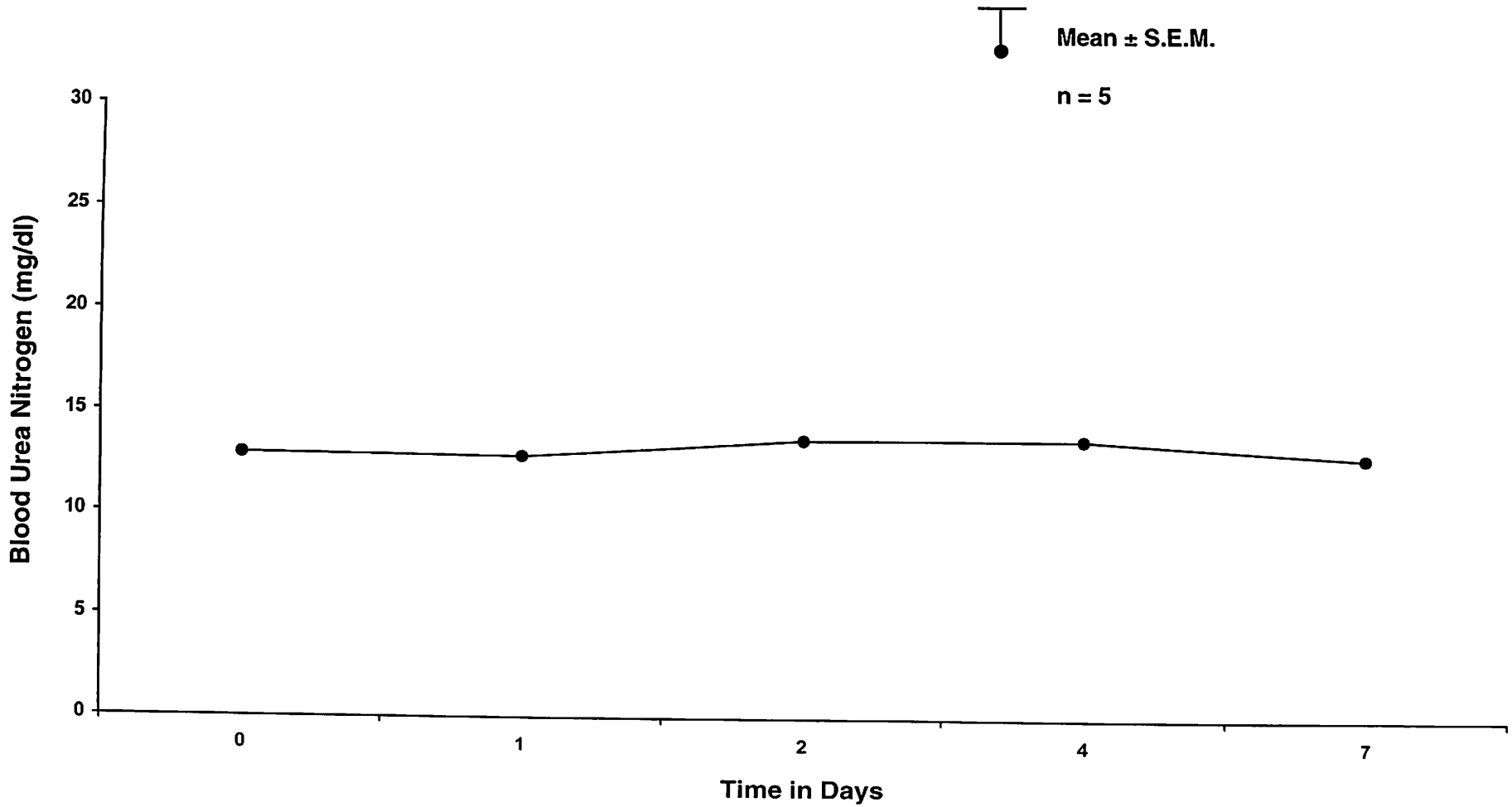


Fig. 18 : *Showing effect of enrofloxacin (5 mg/kg once daily for 7 days) at various days intervals in goats on Serum Glutamate Pyruvate Transaminase (SGPT) (IU/L)*

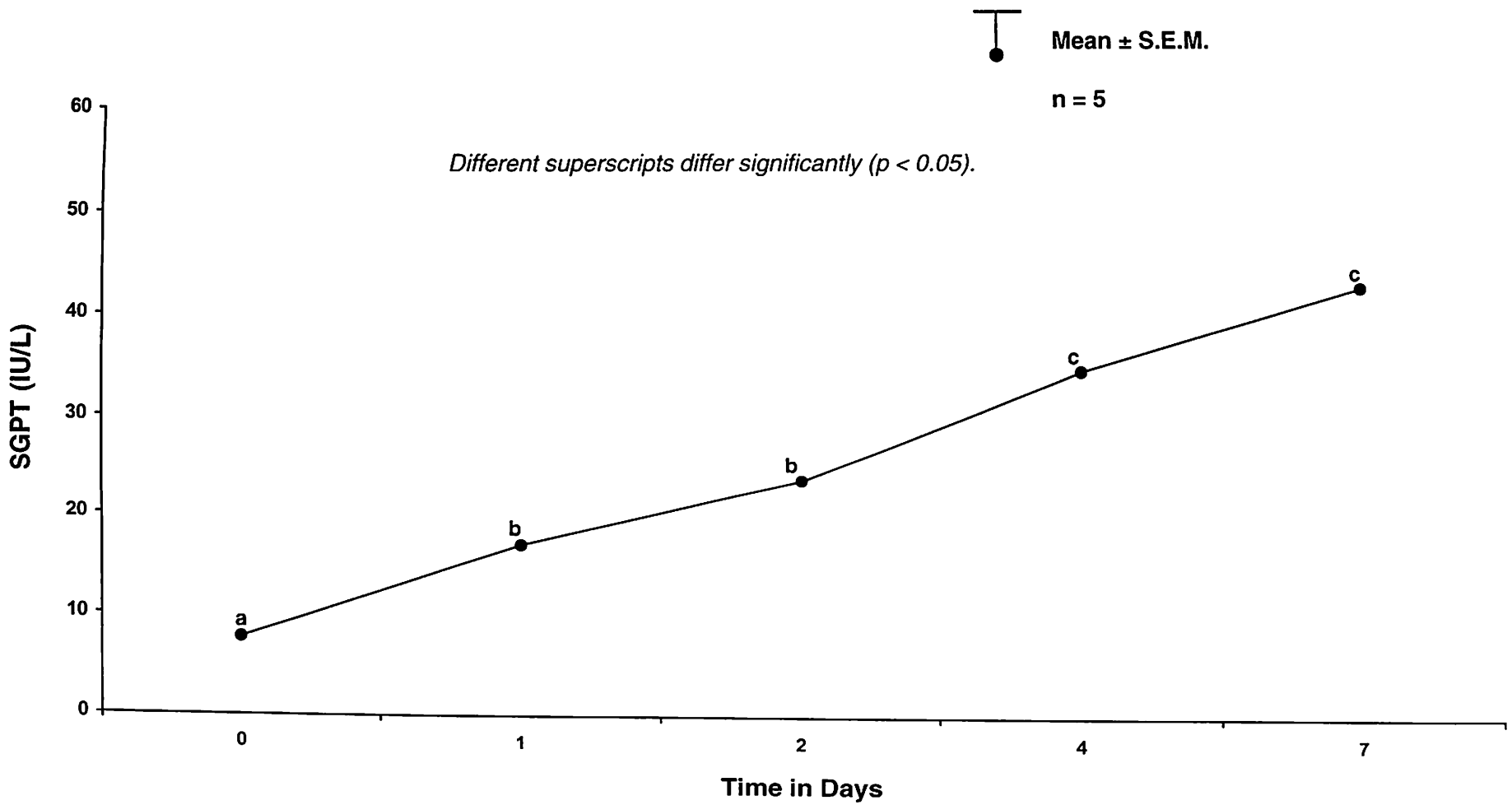


Fig. 19 : *Showing effect of enrofloxacin (5 mg/kg once daily for 7 days) at various days intervals in goats on Serum Glutamate Oxaloacetate Transaminase (SGOT) (IU/L)*

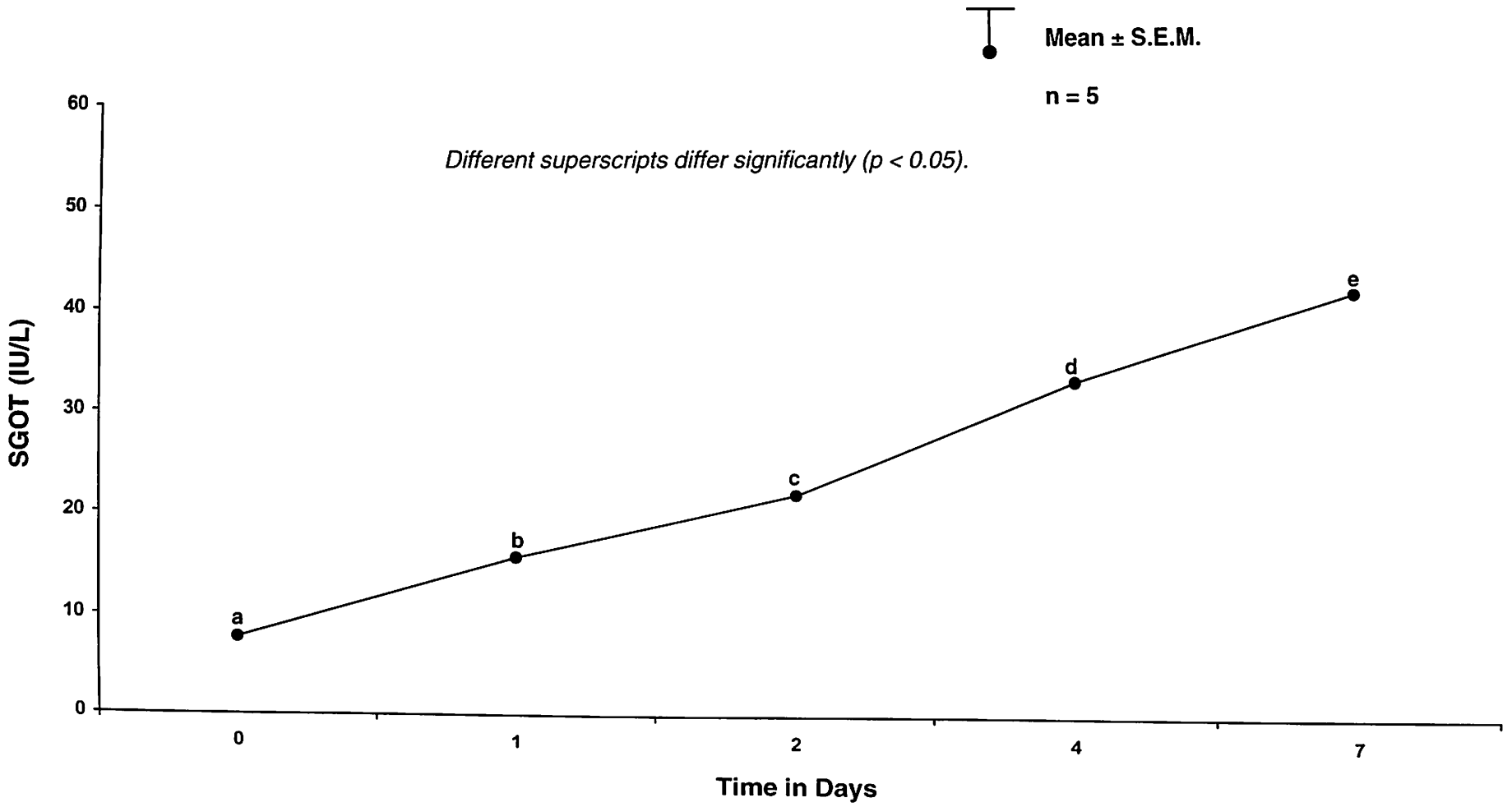
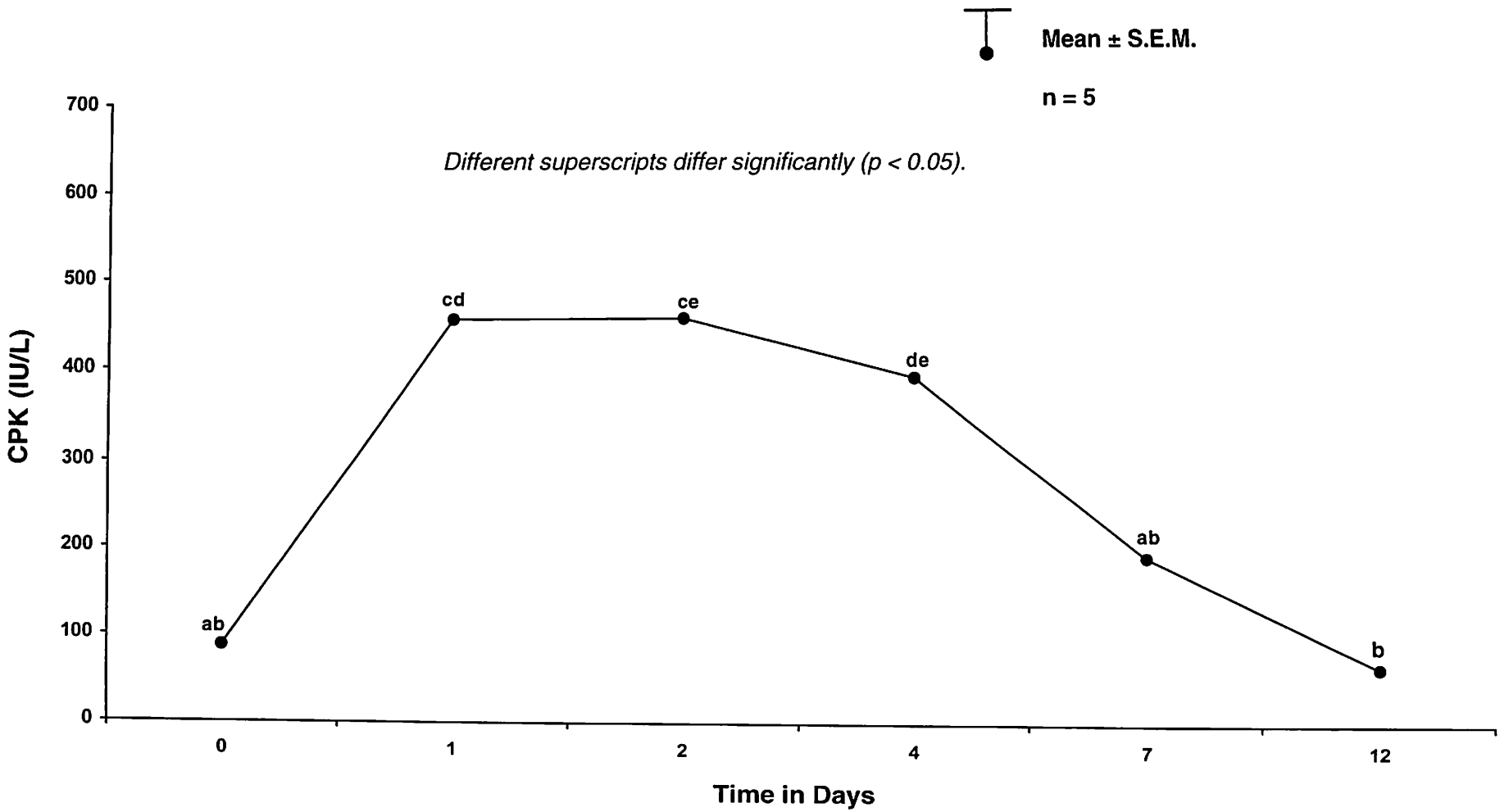


Fig. 20 : *Showing effect of enrofloxacin (5 mg/kg once daily for 12 days) at various days intervals in goats on Creatine Phosphokinase level (CPK) (IU/L)*



Chapter - 5

Discussion

DISCUSSION

Enrofloxacin, a recent member of fluoroquinolones, possesses certain advantages such as bactericidal and broad spectrum activity, no cross resistance with other group of chemotherapeutic agents and effective against micro-organisms that are resistant to β-lactum antibiotics, aminoglycosides etc. The drug is reported to have better distribution in different organs and tissues in various species of animals. The drug has been exclusively developed for veterinary use. Pharmacokinetic studies were carried out in all species of animals including goats and toxicity studies of enrofloxacin were also carried out in many species but very little work was done in goats. Therefore, the present study was undertaken to know the toxicity of enrofloxacin in goats.

TOXICITY STUDY

A. *Clinical Parameters* :

In the present toxicity study, acute untoward effects of enrofloxacin on different clinical parameters namely respiration rate, pulse rate, body temperature and ruminal movement were studied after its single i.m. injection of 20 mg/kg at different time intervals *viz.*, 0, 4, 8, 12 and 24 hour. Similarly, the sub acute effects of

enrofloxacin on different clinical parameters as noted above were studied after daily i.m. injection of 5 mg/kg for 7 days at different days intervals *viz.*, 0, 1, 2, 4 and 7 days.

The acute study revealed that there was no significant change in respiration rate/min which varied from 16.8 ± 0.96 to 18.0 ± 0.44 from 0 to 24 hours. The other parameters i.e., pulse rate, body temperature and ruminal movement also showed insignificant change.

Similar to acute toxicity study, the sub acute toxicity study revealed that there was no significant change in respiration rate and the value varied from 16.8 ± 0.73 to 18.0 ± 0.44 from 0 to 7 days. It was also observed that sub acute toxicity study showed the similar pattern on pulse rate, body temperature and ruminal movement which altered non-significantly.

B. Haematological Parameters :

In the present toxicity study, acute untoward effects of enrofloxacin on different haematogical parameters namely haemoglobin, total leukocyte count (TLC) and differential leukocyte count (DLC) were studied after its single i.m. injection of 20 mg/kg at different time intervals *viz.*, 0, 4, 8, 12 and 24 hours. Likewise, the sub acute effects of enrofloxacin on different haemotological parameters as mentioned under acute toxicity study were studied

after daily i.m. injection of 5 mg/kg for 7 days at different days intervals viz., 0, 1, 2, 4 and 7 days.

The acute toxicity study revealed that there was no significant change in haemoglobin (g/dl) and the value varied from 8.48 ± 0.10 g/dl to 8.66 ± 0.12 g/dl from 0 to 24 hours. Likewise, there was no significant effect on differential leucocyte count (DLC) and total lecucocyte count (TLC) and they differed only non-significantly from 0 to 24 hours.

The sub acute toxicity study showed similar pattern of change on haemoglobin (g/dl) and the value varied from 8.10 ± 0.05 g/dl to 8.36 ± 0.06 g/dl from 0 to 7 days. It was also observed that there was no significant effect on differential leucocyte count (DLC) and total leucocyte count (TLC) and they also differed only non-significantly from 0 to 7 days.

C. Biochemical Parameters :

The present acute toxicity study revealed that the value of blood glucose noted before the injection of enrofloxacin (0 h) was found to be 87.60 ± 1.02 mg/dl, which differs non-significantly from that of 4 h (87.67 ± 0.84 mg/.dl), 8 h (89.05 ± 0.87 mg/dl), 12 h (89.41 ± 0.77 mg/dl) and 24 h (87.41 ± 1.03 mg/dl). This clearly indicates that enrofloxacin has no effect on glucose metabolism. Similarly, enrofloxacin was found to have no significant effect on other

biochemical parameters *viz.*, total plasma protein, serum cholesterol and blood urea nitrogen (BUN) and the values at 4, 8, 12 and 24 h did not differ significantly from the values noted before injection of enrofloxacin.

The sub acute effect of enrofloxacin on blood glucose has been presented in Table 23 and 23 A. The values noted on day 0 (before injection of enrofloxacin) was found to be 85.60 ± 1.02 mg/dl, which differed non-significantly from that of day 1 (85.50 ± 0.84 mg/dl), day 2 (87.18 ± 0.70 mg/dl), day 4 (87.43 ± 0.65 mg/dl) and day 7 (85.40 ± 1.03 mg/dl). This clearly indicates that enrofloxacin has no effect on blood glucose metabolism. Similarly, enrofloxacin was found to have no significant effect on other biochemical parameters *viz.*, total plasma protein, serum cholesterol, and blood urea nitrogen.

A marked significant effect of enrofloxacin on serum glutamate pyruvate transaminase (SGPT) was observed in both acute and sub acute studies. During single dose administration (Table 12 and 12 A) the value noted at 0 h (5.59 ± 0.87 IU/L) showed a steady significant, gradual increase with time at 4 h (19.38 ± 1.19 IU/L), 8 h (24.28 ± 1.28 IU/L), 12 h (41.55 ± 1.85 IU/L) and 24 h (49. 74 ± 1.39 IU/L). All the above values noted at different time differed significantly with each other as well. This indicates that there is progressive significant increase in the values of SGPT with time indicating the significant untoward effect of enrofloxacin on liver

tissues. The normal value in goat for SGPT varies from 7 to 24 IU/L (Kaneko *et al.*, 1997). In the present study, SGPT levels increased higher than the normal value of 24 IU/L at 12 and 24 h after higher dose of enrofloxacin (20mg/kg). This denotes that definitely some liver damage may occur at higher doses.

The values of SGPT noted after daily i.m. injection of enrofloxacin (5mg/kg) for 7 days have been presented in Table 26 and Table 26 A. The value noted on day 0 (7.68 ± 1.49 IU/L), which increased subsequently on day 1 (16.72 ± 1.97 IU/L), day 2 (23.28 ± 3.87 IU/L), day 4 (34.03 ± 3.29 IU/L) and day 7 (42.10 ± 2.56 IU/L). Though, there was an increase of SGPT on day 2 from that of day 1 but it was not statistically significant whereas, the other values noted on day 4 and day 7 were found to be significantly higher than that of day 0, 1 and 2. The value noted on day 7 was not significantly higher than that of day 4, which may be due to development of liver tissues tolerance.

In case of serum glutamate oxaloacetate transaminase (SGOT), the values noted at 4 h (28.41 ± 5.27 IU/L) and 8 h (27.30 ± 3.61 IU/L) were noted to be significantly different from 0 h (6.33 ± 0.82 IU/L). Further increase in SGOT values were noted at 12 h (38.30 ± 2.09IU/L) and very high value was noted at 24 h (50.85 ± 3.07). These values significantly higher than that of 0, 4 and 8 h. This

indicates that enrofloxacin at higher than the therapeutic dose (20 mg/kg) has significant effect with increase in time on different tissues as well. The normal value for SGOT in goat varies from 43-132 IU/L (Kaneko *et al..*, 1997). Though there is a significant effect of enrofloxacin on SGOT values obtained at different time intervals but the values are within the physiological limits indicating lesser cardiac muscle damage.

The sub acute effect of enrofloxacin on SGOT has been presented in (Table 27 and 27 A). The value noted on day 0 (7.63 ± 1.46 IU/L), day 1 (15.45 ± 1.58 IU/L), day 2 (21.57 ± 2.51 IU/L), day 4 (32.53 ± 1.99 IU/L) and day 7 (41.17 ± 1.37 IU/L). All the above values noted on different days differed significantly with each other. This indicates that there is progressive significant increase in the values of SGOT with time. This indicates the significant effect of enrofloxacin on SGOT.

It has been reported that SGOT increases 10,000 times in myocardial cells and 500 times in liver cells than the serum values in myocardial infarction and liver diseases whereas, SGPT increases 3000 times more in liver and 400 times more in heart in diseased condition (Vasudevan and Kumari, 1998). In the present acute toxicity study the results reveal that there may be some liver damage if enrofloxacin administered at higher than the therapeutic dose (20mg/kg) but it may not cause much damage to cardiac muscle.

D. Parameters for estimating muscle damage :

In the present acute toxicity study, creatine phosphokinase (CPK) activity before injection of enrofloxacin (0 h) was noted to be 75.8 ± 2.57 IU/L). This value increased subsequently at 4 h (226.6 ± 48.52 IU/L), 8 h (613.6 ± 78.81 IU/L), 12 h (921.4 ± 141. 34 IU/L) and 24 h (526.4 ± 126.59 IU/L). But the increase of CPK at 4 h was not statistically significant from 0 h whereas other values noted at 8, 12 and 24 h significantly higher than that of 0 and 4 h. The value noted at 12 h was significantly higher than that of 8 h but the value at 24 h returned to the level similar to that of 8 h.

In the sub acute toxicity study, the value of creatine phosphokinase (CPK) was noted to be 87.4 ± 7.26 IU/L) on day 0 (before injection of enrofloxacin) which increased to 455.2 ± 137.62 IU/L on day 1, 457.4 ± 77.03 IU/L on day 2 , 392.8 ± 20.52 on day 4 and 188.4 ± 39.51 IU/L on day 7. After withdrawal of the drug it decreases to 64.2 ± 3.63 IU/L on day 12 which have been undertaken in the present study. Thus, the acute and sub acute toxicity studies indicate the skeletal muscle damaging activity of enrofloxacin. The sub acute toxicity study further indicates that the muscle damaging activity may be of temporary nature since after withdrawal of drug on day 7, the CPK value returned to normal value on day 12 (i.e., after 5 days of drug withdrawal).

A report of local tissue damage in cows after 2 deep intramuscular injections of eight antimicrobial agents showed highest elevation of serum creatine phosphokinase (CPK) activity with enrofloxacin followed by tylosin and trimethoprin-sulfadoxine (Pyorala, 1994).

Thus the present acute and sub acute toxicity studies on enrofloxacin reveals that the drug has no toxic effect on various clinical parameters (respiration, pulse, body temperature and ruminal movement), haematological parameters [haemoglobin %, total leucocyte count (TLC), differential leucocyte count (DLC)] and biochemical parameters (total plasma protein, blood glucose, serum cholesterol and blood urea nitrogen). However, a slight increase in SGOT was noted which is within physiological limit, but the increase in SGPT was found to be beyond physiological limit during acute as well as sub acute toxicity studies. Hence, the drug should be preferably be avoided in animals suffering from hepatic diseases. Muscle toxicity was noted in acute as well as sub acute toxicity studies on enrofloxacin on i.m. administration but on withdrawal of the drug, CPK value returns to normal within a short span (5 days) indicating the temporary nature of muscle damage caused by intramuscular administration. Hence, the drug should be used preferably by subcutaneous or intravenous route for treatment of systemic infections.

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Chapter - 6

Summary

SUMMARY

Toxicological studies of enrofloxacin were conducted in five healthy female goats of non-descript breed weighing between 16-22 kg. For acute toxicity study, enrofloxacin was administered separately in each of five healthy goats by i.m. route at higher dose rate of 20 mg/kg once only. For sub acute toxicity study, enrofloxacin was administered by i.m. route at therapeutic doses of 5 mg/kg once daily for 7 days.

Blood and serum samples were collected for estimating various haematological parameters (haemoglobin %, total leucocyte count and differential leucocyte count), biochemical parameters (total plasma protein, blood glucose, serum cholesterol, blood urea nitrogen, serum glutamate pyrurate transaminase and serum glutamate oxaloscetate transaminase) and muscle damage parameter (creatine phosphokinase level) in healthy goats. Various clinical parameters (respiration rate, pulse rate, body temperature and ruminal movement) were also taken into consideration.

TOXICITY STUDY :

A. Clinical Parameters :

Values of respiration rate/min, pulse rate/min, body temperature (°F) and ruminal movement/5 min in acute and sub

acute toxicity study after i.m. administration of enrofloxacin did not show any significant change from that of pretreatment (before administration of drug) values.

B. Haematological parameters :

(i) In acute toxicity study haemoglobin values between pretreatment (0 hour) and 4, 8, 12 and 24 h post treatment of enrofloxacin did not show any significant change. Similarly in subacute toxicity study, haemoglobin values between pretreatment (0 day) and 1, 2, 4 and 7 days after administration of enrofloxacin (5 mg/kg daily for 7 days) did not show any significant change.

(ii) Acute and sub acute toxicity study of total leucocyte count and differential leucocyte count values obtained between pretreatment and post-treatment did not show any significant change.

C. Biochemical Parameters :

(i) In acute study, total plasma protein value of 7·30 ± 0·11 mg/dl was noted as pretreatment value which did not differ significantly from the values noted at 4, 8, 12 and 24 h post treatment. The sub acute toxicity study also showed similar pattern of values which were altered non-significantly.

(ii) In case blood glucose, the values noted on pretreatment (0 hour) did not differ significantly from those noted at 4, 8, 12 and 24 h post treatment. The sub acute toxicity study also showed insignificant change on various days of post treatment as compared to pretreatment value.

(iii) In acute toxicity study, a value of 54·44 ± 0·67 mg/dl was noted for serum cholesterol as pretreatment value which did not differ significantly from 4, 8, 12 and 24 h post treatment values. The sub acute toxicity study also showed similar pattern of change which were altered non-significantly on day 1, 2, 4 and 7 days of post treatment as compared to pretreatment value (day 0).

(iv) In case of blood urea nitrogen (BUN), the values noted on pretreatment (0 h) did not differ significantly from those of 4, 8, 12 and 24 h post treatment. The sub acute toxicity study on blood urea nitrogen (BUN) revealed similar pattern of change which differed non-significantly.

(v) The study of the effect of enrofloxacin on serum glutamate pyruvate transaminase (SGPT) revealed that the SGPT value noted at 0 h (5·59 ± 0·87 IU/L) increased significantly at 4 h (19·38 ± 1·19 IU/L), which further increased significantly at 8 h (24·28 ± 1·28 IU/L), 12 h (41·55 ± 1·85 IU/L) and 24 h

(49·74 ± 1·39 IU/L) respectively. Hence, a high dose of enrofloxacin (20 mg/kg) may cause some liver damage. Sub acute toxicity study of enrofloxacin (5 mg/kg i.m. once daily for 7 days) revealed that the values of SGPT increased significantly with increasing days of post treatment as compared to pretreatment (day 0) value (7.68 ± 1·49 IU/L). The mean ± S.E.M. values of 16·72 ± 1·97 IU/L, 23·28 ± 3·87 IU/L, 34·03 ± 3·29 IU/L, and 42·10 ± 2·56 IU/L were noted on day 1, 2, 4 and 7, respectively. The sub acute toxicity study indicates possibility of liver damage.

(vi) In the acute toxicity study, the values of serum glutamate oxaloacetate transaminase (SGOT) increased significantly with increasing hours of post treatment as compared to pretreatment (0 h) value. The mean values of 6·33 ± 0·82 28·41 ± 5·27, 27·30 ± 3·61, 38·30 ± 2·09 and 50·85 ± 3·07 IU/L were noted on 0, 4, 8, 12 and 24 h, respectively. The sub acute toxicity study of enrofloxacin on SGOT revealed that the SGOT value noted on day 0 (7·63±1·46 IU/L) increased significantly on day 4 (32·53 ± 1·99 IU/L), which further increased to 41·17 ± 1·37 IU/L on day 7. The above data indicates the possibility of muscle damaging property of enrofloxacin, particularly on cardiac muscle.

D. Parameters for estimating muscle damage :

Creatine phosphokinase (CPK) test was done for estimating muscle damage. The value of creatine phosphokinase (CPK) noted on 0 h (75·8 ± 2·57 IU/L) increased significantly on subsequent hours of post treatment of enrofloxacin upto 12 h and the values were noted to be 226·6 ± 48· 52 IU/L, 613·6 ± 78·81 IU/L and 921·4 ± 141·34 IU/L at 4, 8 and 12 h. After 12 h the value was noted to be decreased and mean ± S.E.M. value of 526·4 ± 126·59 IU/L was noted at 24 h. In sub acute toxicity study, similar to the value noted for SGPT and SGOT which differed significantly, creatine phosphokinase (CPK) values was also found to increase on subsequent days of post treatment of enrofloxacin as compared to pretreatment (0 day) value. The value of CPK noted on day 0 (87·4±7·26 IU/L) increased significantly on subsequent days of post treatment of enrofloxacin and the value were noted to be (457·4 ± 77·03 IU/L), (392·8 ± 20·52 IU/L) and (188·4 ± 39·51 IU/L)on day 2,4 and 7 respectively. After termination of the drug administration on 7^{th} day, the value decreased subsequently and noted to be near pretreatment value on day 12 (64·2 ± 3·63 IU/L).

There is significant rise in level of SGOT and SGPT values post i.m. injection of enrofloxacin as compared to pretreatment value. It is noted that SGOT increases 10,000 times in myocardial cells and 500 times in liver cells than the serum values in myocardial infarction and liver disease whereas, SGPT increases 3000 times more

in liver and 400 times more in heart in diseased condition (Vasudevan *et al.*,1998). The significant increases in SGOT level noted in the present study after injection of enrofloxacin is within the physiological limit.

The normal value in goat for SGOT and SGPT varies 43-132 IU/L and 7-24 IU/L, respectively (Kaneko, 1997). Hence, the drug should be used carefully in case of liver and muscular disorders, particularly in cardiac dysfunction.

The acute and sub acute toxicity studies indicate the skeletal muscle damaging activity of enrofloxacin since there is a significant rise in CPK level. The sub acute toxicity study further indicates that the muscle damaging activity may be of temporary nature since after withdrawal of drug on day 7, the CPK value returned to normal value on day 12 (i.e., after 5 days of drug withdrawal).

By taking into account of various advantages and its least toxicity as noted above, the drug can be used safely in animals, particularly on goat. Precaution may be taken before prescribing the drug particularly through i.m. route in animals, which have the history of liver and heart diseases. Further, the drug should be used preferably by subcutaneous or intravenous route for treatment of systemic infections.

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