Pharmacokinetics and Toxicity of Pefloxacin 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

Nirbhay Kumar

Reg No.- M/V. Phar/40/2002-2003

DEPARTMENT OF VETERINARY PHARMACOLOGY AND TOXICOLOGY

BIHAR VETERINARY COLLEGE

P A T N A - 800 014

2004

Pharmacokinetics and Toxicity of Pefloxacin 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

Nirbhay Kumar

Reg No.- M/V. Phar/40/2002-2003

DEPARTMENT OF VETERINARY PHARMACOLOGY AND TOXICOLOGY

BIHAR VETERINARY COLLEGE

P A T N A - 800 014

2004

Dedicated to

my benevolent

and

adorable

PARENTS

&

TEACHERS

PHARMACOKINETICS AND TOXICITY OF PEFLOXACIN 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

NIRBHAY KUMAR

Reg. No. M/V.Phar/40/2002-2003

DEPARTMENT OF VETERINARY PHARMACOLOGY AND TOXICOLOGY
BIHAR VETERINARY COLLEGE
PATNA – 800014
2004

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

Dr. S.D. Singh
Ph.D.
Associate Professor & Head
Deptt. of Pharmacology & Toxicology
Bihar Veterinary College, Patna-800014

CERTIFICATE - I

This is to certify that the thesis entitled **"PHARMACOKINETICS AND TOXICITY OF PEFLOXACIN IN GOAT"** 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. NIRBHAY KUMAR**, 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.

23/05/04

(S.D. Singh)
Major Advisor

Endorsed :

23/05/04.

(Chairman / Head of the Department)

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

Dr. S.D. Singh
Ph.D.
Associate Professor & Head
Deptt. of Pharmacology & Toxicology
Bihar Veterinary College, Patna-800014

CERTIFICATE - I

This is to certify that the thesis entitled **"PHARMACOKINETICS AND TOXICITY OF PEFLOXACIN IN GOAT"** 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. NIRBHAY KUMAR**, 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.

30/05/04

(S.D. Singh)
Major Advisor

Endorsed :

30/05/04.

(Chairman / Head of the Department)

DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY

Bihar Veterinary College, Patna-800014
Rajendra Agricultural University, Pusa, Bihar

CERTIFICATE - II

We, the undersigned, members of the Advisory Committee of **DR. NIRBHAY KUMAR**, 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 **"PHARMACOKINETICS AND TOXICITY OF PEFLOXACIN IN GOAT"** may be submitted by **DR. NIRBHAY KUMAR** in partial fulfillment of the requirements for the degree.

23/05/04.

[Dr. S.D. Singh]
Chairman
Advisory Committee

Members of the Advisory Committee :

(i) **Dr. C. Jayachandran**
Associate Professor
Department of Veterinary Pharmacology & Toxicology,
Bihar Veterinary College, Patna-14.

Jayachandran 23/5/04

(ii) **Dr. S. P. Verma**
Associate Professor and Head,
Department of Veterinary Medicine
Bihar Veterinary College, Patna-14

S.P. Verma 22-05-04

(iii) **Dr. C. Singh**
Associate Professor and Head,
Department of Veterinary Physiology
Bihar Veterinary College, Patna-14
[Nominee of DRI-cum-Dean, P.G. Studies]

23/05/04

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

CERTIFICATE - III

This is to certify that the thesis entitled **"PHARMACOKINETICS AND TOXICITY OF PEFLOXACIN IN GOAT"** submitted by **DR. NIRBHAY KUMAR** , 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 .....24-11-2004..... .

24/11/04

[Dr. S.D. Singh]
Chairman
Advisory / Examination
Committee

Members of the Advisory / Examination Committee :

(i) **Dr. C. Jayachandran** 24/11/04

(ii) **Dr. S. P. Verma** Verma. 24.11.04

(iii) **Dr. C. Singh** 24/11/2004

[DRI-cum-Dean, P.G. Studies]

CONTENTS

LIST OF TABLES

LIST OF FIGURE

10.	Mean total protein, albumin and globulin (gm/dl) following repeated i.v. administration of pefloxacin (10 mg/kg) for 7 days in goat.
11.	Mean serum glutamate pyruvate transaminase (SGPT) following repeated i.v. administration of pefloxacin (10 mg/kg) for 7 days in goat.
12.	Mean serum glutamate oxaloacetate pyruvate transaminase (SGOT) following repeated i.v. administration of pefloxacin (10 mg/kg) for 7 days in goat.

ACKNOWLEDGEMENT

It is great privilege and pleasure to express my deep sense of gratitude and indebtedness to my Major Advisor, Dr. S.D. Singh, M.V.Sc., Ph.D., Associate Professor and Head, Department of Pharmacology and Toxicology and Associate Dean-cum-Principal, Bihar Veterinary College, Patna for his precious guidance, keen interest, critical comments and constructive suggestions throughout the course of study and in preparation of this manuscript. I am highly thankful to him as a Principal of the college for providing necessary facilities timely to conduct the present study.

I feel pleasure to express my whole hearted appreciation, heartily and warm thanks to Dr. C. Jayachandran, Associate Professor, Department of Pharmacology and Toxicology, Bihar Veterinary College, Patna for his immense help and excellent guidance during the course of investigation and in preparation of this manuscript.

I gratefully acknowledge the painstaking advice and useful suggestions offered from time to time by other members of my advisory committee, Dr. S.P. Verma, Associate Professor and Head, Department of Veterinary Medicine and Dr. C. Singh, Associate Professor and Head, Department of Veterinary Physiology.

The author takes pleasure in expressing his sincere gratitude to Dr. S.B. Verma, Associate Professor, Animal Breeding and Genetics and Dr. K.G. Mandal, Assistant Professor, Animal Breeding and Genetics for making critical suggestions during data analysis.

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

It is a pleasure to express my thanks to my dear friends, Dr. Deepak Kumar Prasad, Dr. Thakur Balwant Singh, Dr. Archana, Dr. Anuradha Kumari, Dr. Rajeev Kumar, Dr. Ram Pravesh, Dr. Bipin, Dr. S.M. Kushwaha, Dr. R.K. Sinha, Dr. R.K. Nirala and my juniors Dr. Praveen, Dr. Smita, Dr. Mritunjay, Dr. Prem, Dr. Shailendra, Dr. Raj Kumar and Dr. Nilay along with other research scholars, the company of whom helped me to overcome the stressful moments of my research work.

Thanks and appreciation are also due to the non-teaching staff members, particularly Sri Nathun Pandit, Md. Naim, Sri Ramashish Paswan and Sri Vijay Kumar Singh of the Department of Pharmacology and Toxicology.

Finally, I shall fail in my duty if I do not extend special gratitude to my father Sri Yogendra Prasad Singh and my mother Smt. Kamla Devi who had to toil to bring me up to this stage and who were a constant source of inspiration throughout the period of study. I am also thankful to my Bhaiya Mr. Abhay Kumar, Bhabhi Smt. Sabita Kumari and my younger sisters Shobha and Neha. My sincere affection and love are also extended to my nephew Monu and niece Priyadarshini.

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

Nirbhay Kumar. 22.05.04.

Date : 22.05.2004 **(Nirbhay Kumar)**

Place : Patna

Chapter - 1

Introduction

INTRODUCTION

The advent of antimicrobials had been a landmark against the infectious diseases. The antimicrobial therapy now constitutes a major component of modern medical and veterinary practices. Keeping in view the increasing problem of microbial resistance against various antimicrobial agents, there is always a need to have a drug that would be highly effective in combating different infections. The past decades have witnessed the advent of new series of antibacterials like fluoroquinolones in the armamentarium of modern clinicians especially against infectious diseases.

The fluoroquinolones represent a major breakthrough in the chemotherapy against the microbial infections and they are playing an important role in medical and veterinary practices. Unfortunately, these compounds also have great potential for overuse and misuse, which in turn, may result in the emergence of resistant strains which are currently susceptible to quinolones.

The history of the newer quinolones began with the discovery of nalidixic acid in 1962 as an accidental by-product during the synthesis of the antimalarial compound, chloroquine (Lesher *et al.*, 1962). Nalidixic acid is very commonly used to treat urinary tract

infections caused by gram-negative aerobic organisms. However, its use for systemic infections has been limited due to various factors like modest and variable serum and tissue concentrations; reports of toxicities (Cahal *et al.*, 1965); clinical failures and development of resistance (Barlow, 1963). In 1970s, another group of quinolones (eg. oxolinic acid, pipemidic acid and cinoxacin) was launched, but these compounds were only marginally better than nalidixic acid. However, a breakthrough was achieved in early 1980s with the development of fluorinated 4 - quinolones (Wolfson *et al.*, 1985) such as norfloxacin and ciprofloxacin, since these agents have broad antimicrobial activity and are effective orally against wide varieties of infectious diseases with fewer side effects and non- development of microbial resistance.

Pefloxacin, a member of third generation fluoroquinolone is a broad spectrum, antibacterial agent having potent bactericidal activity against a wide range of gram- negative and gram-positive organisms. It is effective against various diseases such as gastro-intestinal, respiratory, urinary, genital, skin, soft tissues, bone and joint infections etc. It has excellent bioavailability with superior pharmacokinetic profiles (better absorption and distribution in body fluids). It also possesses excellent penetration in tissue macrophages and body fluids. The drug acts against bacteria even in the presence of purulent material.

Pefloxacin is transformed into several metabolites in the body (Montay *et al.*, 1983). The main metabolites are pefloxacin N-oxide, desmethyl pefloxacin or norfloxacin and oxonorfloxacin. Norfloxacin is the main active metabolite. The structure of pefloxacin differs from that of norfloxacin only in addition of a methyl group to position 4 of the piperazinyl substituent at position 7.

Pefloxacin like other quinolones has toxic potentials in the muscle, tendon and synovial membrane (Kashida and Kato, 1997). One of the most common problems is the gastrointestinal disturbance (like nausea, vomiting, diarrhoea etc.) but these are produced at higher doses and are not serious. Pefloxacin also induces arthropathy in juvenile animals (Machida *et al.*, 1990). Thrombocytopenia at very high doses on prolonged administration in human has also been reported (Chichmanian *et al.*, 1992). Photosensitivity and photoallergenicity of norfloxacin in some cases of guinea pigs has also been described (Horio *et al.*, 1994).

Though many reports of pharmacokinetic study of pefloxacin are available in animals, but the pharmacokinetic studies of pefloxacin and its active metabolite norfloxacin in goats are scarcely reported. Further, it seems that there is lack of toxicological reports in animals including goats.

Goat is a versatile animal and is mainly reared in tropical countries like India. Goat farming is an important tool to overcome poverty and unemployment and for improving socio-economic conditions particularly for weaker sections of society. Hence, it is essential that proper health coverage should be given to goat husbandry for optimal economic gain. To use a drug in therapy, it is essential to study its detailed pharmacokinetic and toxicological parameters in this species.

Keeping in view the above noted facts, the detailed pharmacokinetic and toxicological studies of pefloxacin were undertaken with the following aims and objectives.

1. Estimation of concentrations of pefloxacin at different time intervals in body fluids following its intravenous administration in goats.
2. Determination of kinetic parameters of pefloxacin in goats.
3. Calculation of dosage regimen of pefloxacin in goats.
4. Toxicological studies of pefloxacin following its repeated administration in goats.

Chapter - 2

Review of Literature

REVIEW OF LITERATURE

Quinolone carboxylic acid derivatives are synthetic antimicrobial agents that are becoming more popular in medical and veterinary practices. Initially, nalidixic acid was used mainly for treating urinary tract infections, but the use of this drug in clinical practice greatly reduced due to emergence of resistant organisms. It led to the introduction of fluoroquinolones in clinical practice. The use of fluoroquinolone antibacterial agents in veterinary medicine has increased tremendously in the last ten years. It is due to the fact that they are rapidly bactericidal against a wide variety of clinically important bacterial organisms. Further, they are potent, well tolerated by animals and have been administered *via* a wide variety of routes (orally *via* tablets and drinking water, subcutaneously, intramuscularly, and intravenously).

Pefloxacin is one of the derivatives having mega spectrum bactericidal activity with several advantages over older quinolones.

History

The history of newer quinolone agents began with the discovery of nalidixic acid (Lesher *et al.*, 1962). Nalidixic acid was discovered as an accidental by-product during the synthesis of an

antimalarial compound chloroquine. The initial popularity of nalidixic acid was rapidly diminished because of some reports of its failure in complicated urinary tract infections and development of resistant bacteria (Barlow *et al.,* 1963). A breakthrough was achieved in early 1980s with the development of fluorinated 4-quinolones such as norfloxacin and ciprofloxacin (Wolfson *et al.,* 1985). Since then, a number of other newer quinolones have been synthesized *viz.,* enoxacin, ofloxacin, enrofloxacin, pefloxacin, ciprofloxacin, amifloxacin and difloxacin (Harold, 1987). These agents have broad spectrum of antibacterial activity, fewer side effects and lesser development of microbial resistance (Andriole, 1988).

Chemistry

The fluoroquinolones have a basic structure of quinolone carboxylic acid (Figure I). The modifications of basic molecule at N_1, C_6, C_7 and C_8 positions result in major changes in antimicrobial activity, pharmacokinetic and metabolic properties.

- Addition of 'F' atom at C_6 enhances DNA gyrase inhibitory activity and extends activity against *Staphylococcus* spp. also.
- Addition of second 'F' atom at C_8 provides better absorption and longer half life.

- Addition of piperazine group at C_7 extends antibacterial activity against *Staphylococcus* spp. and *Pseudomonas* spp.
- Substitution of methyl group for piperazine group also results in better absorption and longer half-life, and
- Addition of cyclopropyl group at N_1, amino group at C_5 and 'F" at C_8 extends spectrum against *Mycoplasma* spp. and *Chlamydia* spp. (Neu, 1992).

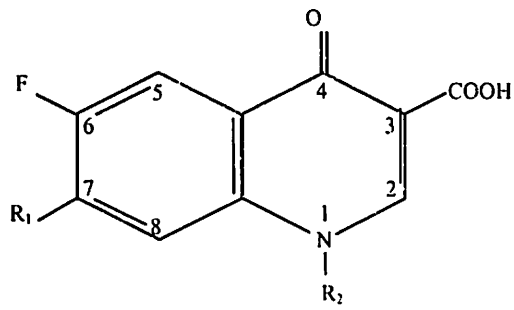


Fig. I : *Structure of Quinolone carboxylic acid*

Physico-Chemical Properties of Pefloxacin

Pefloxacin is a yellowish white powder, very slightly soluble in water, alcohol and chloroform. With methane sulphonic acid it gives a dihydrated monosalt, pefloxacin methyl sulphonate dihydrate. This salt is a white crystalline powder, readily soluble in water, slightly soluble in 95% alcohol and very slightly soluble in chloroform. The pH of 1% w/v aqueous solution ranges between 3 to 4.5. At physiological pH, it exists as zwitterion.

When concentrated form of pefloxacin dihydrate is exposed to light for long periods, it turns slightly pink but its other physico-chemical properties are similar to those of fresh solution (Goueffon *et al.*, 1981).

The chemical structure of pefloxacin is shown in figure II.

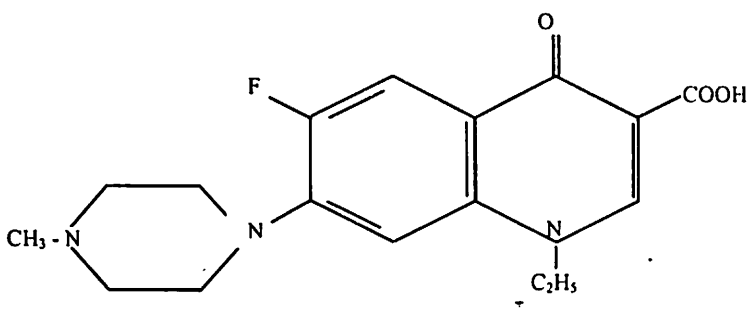


Fig. II

1-ethyl - 6 - fluoro - 7- (4 - methyl - 1 - piperazinyl) - 4 - oxo - 1, 4 - dihydro - 3 - quinolone carboxylic acid.

Empirical Formula : $C_{17}\ H_{20}\ FN_3\ O_3$

Molecular weight of pefloxacin methyl sulfonate dihydrate = 465.5

Antimicrobial Activity :

Pefloxacin is a broad spectrum antimicrobial agent active against a wide range of gram-negative and gram-positive organisms such as *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella* spp., *Shigella* spp. (Aldridge *et al.*, 1986), *Proteus* spp. (Auckenthaler *et al.*,

1986), *Yersinia* spp. (Felmingham *et al.*, 1985), *vibrio* spp. (Wall *et al.*, 1985), *Neisseria gonorrhoeae* (Gonzalez and Henwood, 1989), *Neisseria meningitidis*, *Campylobacter coli* and *Campylobacter jejuni* (Gonzalez and Henwood, 1989) and *Haemophilus influenzae* (Quantin *et al.*, 1988).

Pefloxacin is also reported to act against gram- positive organisms such as *Staphylococcus aureus* (including penicillin and oxacillin resistant strains), *S. aureus* (including methicillin resistant strains) and *Staphylococcus* spp. (including saprophyticus and coagulase-negative strains) (Arpi *et al.*, 1987), *Streptococcus agalactiae*, *Streptococcus faecalis*, *Streptococcus pneumoniae* and *Streptococcus* spp. (including β–haemolytic viridans strains) (Aldrige *et al.*, 1986). Pefloxacin is moderately active against *Mycobacterium tuberculosis* (Davies *et al.*, 1994).

The therapeutic concentration of pefloxacin for antibacterial action ranges from 0.03 to 2.0 μg/ml (Gonzalez and Henwood, 1989; Lightvoet *et al.*, 1985). The minimum inhibitory concentrations (MIC) of pefloxacin against several susceptible organisms are shown below.

Organisms	*MIC (μg/ml)*
Acinetobacter spp.	2.00
Branhamello catarrh	0.10
Campylobacter jejuni	0.30
Citrobacter freundii	0.25
Enterobacter aerogenes	0.20
Enterobacter cloacae	0.50
Escherichia coli	0.06
Haemophilus ducreyi	0.30
Haemophilus influenzae	0.05
Klebsiella pneumoniae	0.25
Morganella morganii	0.25
Neisseria gonorrhoeae	0.30
Neisseria meningitidis	0.06
Proteus mirabilis	0.25
Proteus vulgaris	0.25
Providencia retteri	0.25
Providencia stuartii	0.50
Pseudomonas aeruginosa	2.00
Salmonella typhi	0.06
Serratia marcescens	0.25
Shigella spp.	0.06
Staphylococcus aureus	0.25
Staphylococcus epidermidis	0.03
Staphylococcus faecalis	0.50
Staphylococcus pneumoniae	0.06
Yersinia enterocolitica	0.06

Source : Gonzalez and Henwood (1989).

Mechanism of Action

Primarily, pefloxacin has retained the biochemical mechanism of quinolone action. It is a bactericidal antimicrobial agent. It acts by inhibiting the enzyme DNA gyrase, 4 subunits (two A subunits and two B subunits) of which have been identified (Higgins *et al.,* 1978; Pedrini *et al.,* 1979). DNA gyrase, an enzyme found in every organism is responsible for supercoiling of bacterial DNA strands in bacterial cell (Wang, 1974, 1985). The process of supercoiling involves firstly, the conversion of double stranded DNA into two single strand DNA by nicking which is brought about by subunit A of DNA gyrase. These single strands are then supercoiled under action of B subunits. The supercoiling facilitates the compaction of genome in a tiny cell. After supercoiling, the two single strands are again sealed together by A submit (Smith *et al.,* 1988). Pefloxacin inhibits bacterial growth by inhibiting both the subunits of enzyme. In addition, DNA gyrase inhibition leads to uncontrolled synthesis of mRNA and protein, extensive filamentation and vacuole formation, degeneration of chromosomal DNA by exonucleases and hence, a bactericidal action is exhibited. The structural differences between bacterial and mammalian DNA gyrase enzyme account for the selectivity of quinolones for bacterial enzyme.

All fluoroquinolones are bactericidal and this activity is maximally seen at specified therapeutic plasma concentration. The concentration lower or higher than this range demonstrates reduced bacterial activity. The paradoxical effect of decreased killing at greater concentration is most probably caused by a dose dependent inhibition of RNA and protein synthesis (Corret *et al.*, 1991).

KINETIC STUDIES OF PEFLOXACIN

Kinetic studies of pefloxacin were conducted in man and animals by different workers and are noted below.

Man

Frydman *et al.* (1986) studied multiple - dose kinetics of pefloxacin in young human volunteers by i.v. or oral administration of 400 mg every 12 hourly. The bioavailability of pefloxacin was complete and plasma concentrations after i.v. and oral administration were similar. Pefloxacin was rapidly absorbed from the gastrointestinal tract and reached maximum plasma concentration about 1 h after dosing. Pefloxacin elimination ($t_{1/2}$ β) increased from 11.00 ± 2.64 h after the first i.v. dose to 13.93 ± 3.58 h after the last i.v. dose . Apparent total body clearance decreased from 148.5 ± 47.6

to 106.9 ± 39.2 $ml.kg^{-1}.min^{-1}$ because of decreased non-renal clearance. Similar results were obtained after repeated oral dosing. The results showed that concentration of pefloxacin in excess of minimum inhibitory concentration for many pathogens could be achieved in plasma and urine with 4 mg bid regimen with both i.v. and oral routes.

Webberley *et al.* (1987) determined the pharmacokinetics of pefloxacin in human volunteers following a 400 mg oral dose. The mean peak serum level of 6.6 $\mu g.\ ml^{-1}$ was attained rapidly 0.8 h after administration. Mean elimination half life was 11.6 h. Urinary recovery of pefloxacin and metabolites was 33.1 % of the dose. The study suggested that a twice or possibly once daily dosing might be sufficient to treat systemic infections caused by susceptible pathogens. Once daily dosing should be sufficient for urinary tract infections.

Cow

Patil *et al.* (1996) investigated pharmacokinetics of pefloxacin in lactating cows following single intravenous administration of 5 mg/kg. The therapeutic plasma concentration (0.03 to 2 $\mu g.ml^{-1}$) of pefloxacin was maintained upto 4.5 h. Following

i.v. administration, distribution half life ($t_{1/2}$ α), elimination half-life ($t_{1/2}$ β), total body clearance (Cl_B) and volume of distribution (Vd) were 0.15 ± 0.03 h, 2.53 ± 0.09 h , 3.67 ± 0.04 ml. $min^{-1}.kg^{-1}$ and 0.68 ± 0.03 $L.kg^{-1}$, respectively. Pefloxacin was detected in milk after 30 min of i.v. administration. The mean peak milk concentration was 4.14 μg. ml^{-1}. The good diffusion of pefloxacin might be related to its low molecular weight and to its strong lipophilicity. This could prove beneficial in the treatment of mastitis.

Crossbred Calves

Srivastava *et al.* (2000) investigated the disposition kinetics and urinary excretion of pefloxacin after a single i.v. administration of 5 mg/kg in crossbred calves. At 1 min after injection, the concentration of pefloxacin in plasma was 18.95 ± 0.892 $\mu g.ml^{-1}$ which declined to 0.13 ± 0.02 $\mu g.ml^{-1}$ at 10 h. Pefloxacin was rapidly distributed from the blood to the tissue compartment as shown by the high values for the initial distribution coefficient, α (12.1 ± 1.21 h^{-1}) and the constant for the rate of transfer of drug from the central to the peripheral compartment, K_{12} (8.49 ± 0.99h^{-1}). The elimination half life and volume of distribution were 2.21 ± 0.111 h and 1.44 ± 0.084 $L.kg^{-1}$, respectively. The total body clearance (Cl_B)

and the ratio to the drug present in the peripheral to that in the central compartment (P/C ratio) were 0.454 ± 0.026 $L.kg^{-1}.h$ and 5.52 ± 0.519, respectively. Pefloxacin at the rate of 6.4 mg/kg repeated at 12 h intervals was recommended for most of the drug sensitive pathogens.

Buffalo calves

Prakash (2003) studied disposition kinetics of pefloxacin in prepubertal buffalo calves after single i.v. administration of 5 mg/kg . The apparent volume of distribution into periphery (Vd_{area}) was 4.62 ± 0.04 $L.kg^{-1}$ and the estimate of total body clearance (Cl_B) was 20.69 ± 2.21 $ml.kg^{-1}.min$. The plasma half life and rate constant for elimination were 155.93 ± 17.33 min and 0.038 min^{-1}, respectively. AUC was 243.22 ± 25.08 $\mu g.ml^{-1}.min$ and the volume of distribution at steady state (Vd_{SS}) was 4.07 ± 0.37 $L.kg^{-1}$. Mean residence time was 3.34 ± 0.35 h. The drug was recommended to be administered at the rate of 7.5 mg/kg i.v. repeated at 12 h interval in buffalo calves.

Goat

Ansari *et al.* (2000) reported pharmacokinetics of pefloxacin in healthy female goats after single i.v. administration of 4 mg/kg. The peak concentration of 9.06 ± 1.30, 4.29 0.32 and 46.45 ±

2.16 $\mu g.ml^{-1}$ were attained at 5, 45 and 45 minutes in plasma, milk and urine, respectively. The therapeutic concentration ($\geq$ 0.12 $\mu g.ml^{-1}$) was maintained upto 8, 8 and 30 h in plasma, milk and urine, respectively. The mean distribution half-life ($t_{1/2}\ \alpha$), elimination half life ($t_{1/2}\ \beta$) and total body clearance (Cl_B) of 0.27 ± 0.06 h, 3.53 ± 0.26 h and 5.07 ± 1.15 $ml.kg^{-1}.min^{-1}$, respectively were estimated. A high Vd_{area} of 1.59 ± 0.42 $L.Kg^{-1}$ and tissue to plasma concentration ratio (T≈P) of 1.46 ± 0.26 indicate that the drug is well distributed.

Malik *et al.* (2002) investigated pharmacokinetics of pefloxacin in healthy female goats after administration of single i.v. (10mg/kg) or oral (20 mg/kg) dose. Concentrations of the drug more or equal to 0.25 $\mu g.ml^{-1}$ were maintained in plasma for upto 6 and 10h after i.v. or oral administration of pefloxacin, respectively. Plasma pefloxacin concentrations decreased rapidly during the initial phase after i.v. injection with a distribution half-life ($t_{1/2}\ \alpha$) of 0.10 ± 0.01 h. The terminal phase had a half-life ($t_{1/2}\ \beta$) of 1.12 ± 0.21 h. The volume of distribution at steady state (Vd_{ss}),mean residence time (MRT) and total systemic clearance (Cl_B) of pefloxacin were 1.08 ± 0.09 $L.kg^{-1}$, 1.39 ± 0.23 h and 821 ± 88 $ml.kg^{-1}.h^{-1}$, respectively. Following oral administration of pefloxacin, the maximum concentration in plasma (C_{max}) was 2.22 ± 0.48 $\mu g.ml^{-1}$ and the interval from administration

Sheep

Six healthy sheep weighing 53 to 65 kg were administered intravenously or intramuscularly an aqueous solution of pefloxacin (PFL) at 10 mg/kg. After i.v. administration, the mean distribution and elimination half life were 1.07 ± 0.05 and 6.88 ± 0.62 h, respectively. Area under curve was 55.89 ± 0.44 $\mu g.ml^{-1}.h$ with total body clearance of 0.176 ± 0.002 $L.kg^{-1}.h^{-1}$ and mean residual time (MRT) of 4.63 ± 0.23 h. Following i.m. administration, the mean distribution half life was 0.32 ± 0.03 h. Peak serum PFL concentration occurred at 1.44 ± 0.09 h and peak value was 3.58 ± 0.10 $\mu g.ml^{-1}$. The apparent half life was 5.68 ±0.14 h with MRT of 8.70 ± 0.17 h. The bioavailability was 82.42 ± 6.25%. Using MIC_{90} concentration of 1.9 $\mu g.ml^{-1}$, PFL dose of 8 mg/kg body weight administered i.m. twice daily is recommended for treatment in sheep. (Moutafchieva and Djouvinov, 1997).

Dog

Montay *et al.* (1984) reported pharmacokinetics of pefloxacin mesylate in female beagle dogs after a single oral dose of 50mg/kg. Mean peak plasma level was 27.5 ± 1.0 $\mu g.\ ml^{-1}$. Area under curve (AUC) and elimination half life ($t_{1/2}\ \beta$) were 156.4±7.2 $\mu g.ml^{-1}.h$

until maximum concentration (T_{max}) was 2.3 ± 0.7h. The absorption half-life ($t_{1/2}$ Ka), mean absorption time (MAT) and elimination half life were 0.82 ± 0.40 h , 4.2 ± 1.0 h and 2.91 ± 0.50 h, respectively. The oral bioavailability of pefloxacin was 42 ± 5.8%. Pefloxacin at the rate of 20 mg/kg repeated after 8 h intervals or orally twice daily is suggested for treating infections caused by drug sensitive pathogens in goats.

Abd El-Aty and Goudah (2002) studied some pharmacokinetic parameters of pefloxacin in lactating goat following i.v. and i.m. injection of 10 mg/kg. The maximum serum concentration was 8.4 ± 0.48 $\mu g.ml^{-1}$, elimination half-life ($t_{1/2}$ β) was 1.6 ± 0.3 h; total body clearance was 3.6 ± 0.3 $L.kg^{-1}.h^{-1}$; steady state volume of distribution (Vdss) was 5.14 ± 0.21 $L.kg^{-1}$ and the area under curve (AUC) was 2.78 ± 0.22 $\mu g.ml^{-1}h$.Pefloxacin was absorbed rapidly after i.m. injection with an absorption half-life ($t_{1/2}$ Ka) of 0.32 ± 0.02 h. The peak serum concentration (C_{max}) of 0.86 ± 0.08 $\mu g.ml^{-1}$ was attained at 0.75 h (T_{max}). The absolute bioavailability after i.m. administration was 70.63 ± 1.13% and the serum protein-bound fraction ranged from 7.2 to 14.3%. The drug was detected in milk and urine for 10 and 72 h, respectively.

Sheep

Six healthy sheep weighing 53 to 65 kg were administered intravenously or intramuscularly an aqueous solution of pefloxacin (PFL) at 10 mg/kg. After i.v. administration, the mean distribution and elimination half life were 1.07 ± 0.05 and 6.88 ± 0.62 h, respectively. Area under curve was 55.89 ± 0.44 $\mu g.ml^{-1}.h$ with total body clearance of 0.176 ± 0.002 $L.kg^{-1}.h^{-1}$ and mean residual time (MRT) of 4.63 ± 0.23 h. Following i.m. administration, the mean distribution half life was 0.32 ± 0.03 h. Peak serum PFL concentration occurred at 1.44 ± 0.09 h and peak value was 3.58 ± 0.10 $\mu g.ml^{-1}$. The apparent half life was 5.68 ±0.14 h with MRT of 8.70 ± 0.17 h. The bioavailability was 82.42 ± 6.25%. Using MIC_{90} concentration of 1.9 $\mu g.ml^{-1}$, PFL dose of 8 mg/kg body weight administered i.m. twice daily is recommended for treatment in sheep. (Moutafchieva and Djouvinov, 1997).

Dog

Montay *et al.* (1984) reported pharmacokinetics of pefloxacin mesylate in female beagle dogs after a single oral dose of 50mg/kg. Mean peak plasma level was 27.5 ± 1.0 $\mu g.\ ml^{-1}$. Area under curve (AUC) and elimination half life ($t_{1/2}\ \beta$) were 156.4±7.2 $\mu g.ml^{-1}.h$

and 3.21 ± 0.14 h, respectively. Plasma protein binding was 19.10 ± 1.10%. Total urinary recovery of pefloxacin and metabolites was 36.3 ± 1.9% of the dose given.

Jayakumar *et al.* (1995) recorded the mean peak plasma level of pefloxacin to be 5.1 ± 0.60 (at 5 min) and 3.71 ± 0.71 μg. ml^{-1} (at 2 h) following i.v. and oral dose (20 μg/kg), respectively, in dogs. Elimination half life was 2.97 and 10.85 h after i.v. and oral dosing, respectively.

Monkey

Pefloxacin was given orally to male macaca monkeys weighing 3.2 to 4.4 kg at the rate of 25 mg/kg. The peak pefloxacin level (C_{max}) was 13.8 ± 0.6 μg.ml^{-1}. Area under curve (AUC) and the elimination half life ($t_{1/2}\beta$) was 116 ± 16 μg.ml^{-1}.h and 5.56 ± 2.25 h, respectively. The principal metabolites in plasma were pefloxacin N-oxide and norfloxacin. The urinary recovery of pefloxacin and metabolites was 26.5% of administered dose (Montay *et al.*, 1984).

Poultry

Chousalkar (1995) studied pharmacokinetics of pefloxacin in chicken following single oral administration of 50mg/kg. Mean peak serum concentration attained was 8.8 μg.ml^{-1}, Distribution half

life ($t_{1/2}$ α) and elimination half life ($t_{1/2}$ β) were 0.55 and 2.2 h, respectively. Volume of distribution (Vd) and total body clearance (Cl_B) were 2.10 $L.kg^{-1}$ and 25.2 $ml.kg^{-1}.min^{-1}$, respectively.

Rabbit

Cochereau - Massin *et al.* (1991) determined kinetics of pefloxacin after a single i.m. dose of 50 mg/kg to albino and pigmented rabbits. The mean serum AUC was 31.4±1.07 and 29.9±1.54 $\mu g.ml^{-1}.h$ in albino and pigmented rabbits, respectively. The mean peak level achieved in serum was 8.89 ± 0.65 $\mu g.ml^{-1}$ within 1 h after the injection in albino rabbits and 6.74 ± 0.17 $\mu g.ml^{-1}$ within 1 h in pigmented rabbits. The volume of distribution was significantly higher in pigmented rabbits (5.25 $L.kg^{-1}$) as compared to albino rabbits (3.81 $L.kg^{-1}$). The serum half life was significantly longer in pigmented rabbits (2.61h) as compared to albino rabbits (2.01h).

Marrakchi - Benjaafar *et al.* (1995) assessed the tolerability, kinetics and efficacy of subconjunctival pefloxacin in pigmented rabbits. The tolerability of a single subconjunctival injection of pefloxacin (0.8, 1.6, 8 or 16 mg in 0.2 ml) in the right eyes of eight pigmented rabbits was evaluated by clinical and

histopathological examination. The 0.8 mg dose was well tolerated. Pefloxacin was found in the cornea (maximum concentration, 18.13 $\mu g.ml^{-1}$, half life, 3.92 h) and in the aqueous humour (maximum concentration, 3.40 $\mu g.ml^{-1}$, half life, 2.14 h). Pefloxacin did not penetrate into the vitreous humour by this route.

Rat

Montay *et al.* (1984) observed mean peak plasma pefloxacin level in male wistar rat to be 13.0 ± 3.4 $\mu g.ml^{-1}$ following single oral administration of 50 mg/kg. AUC and $t_{1/2}$ β were 56 $\mu g. ml^{-1}.h$ and 3.3 h, respectively. Mean plasma protein binding was 20.30 ± 1.8 % and volume of distribution (V_d) was 4.25 $L.kg^{-1}$. Urinary recovery of identified metabolites was 37.8%. Biliary excretion was extensive mainly as glucuronide conjugate of the drug.

Leibovitz *et al.* (1989) studied the penetration pharmacokinetics and therapeutic efficacy of pefloxacin in rat abscess model. Peak pefloxacin concentration in serum of the infected animals was 13 ± 2.9 $\mu g.ml^{-1}$ and peak pefloxacin abscess fluid concentration after 24 h was 8.9 ± 2.2 $\mu g.ml^{-1}$ (i.e. 68% of the peak serum concentration). Abscess fluid concentration at 96 h was 4.5 ± 1.7 $\mu g.ml^{-1}$. Pefloxacin persisted significantly longer in the abscess

fluid than in the serum; but failed to sterilize the abscess following a single administration; however, after four consecutive administrations all abscesses became sterile. It was concluded that pefloxacin may be suitable for the therapy of closed space infections caused by susceptible microorganisms.

Dworkin *et al.* (1990) studied pharmacokinetics and efficacy of pefloxacin in rat model of chronic osteomyelitis. Mean peak concentration of pefloxacin in plasma and bone was 12.1 and 8.3 $\mu g.ml^{-1}$, respectively. Half life in plasma and bone was 2.8 and 3.5 h, respectively.

Mice

Pharmacokinetic study pefloxacin was conducted in male swiss mice on single oral administration at the dose rate of 50mg/kg (Montay *et al.,* 1984). The mean peak plasma concentration (C_{max}), area under curve (AUC) and the elimination half life ($t_{1/2}\ \beta$) were 5.8 $\pm$ 0.3 $\mu g.ml^{-1}$, 8.8 $\mu g.ml^{-1}.h$ and 1.9 h, respectively. Norfloxacin / pefloxacin ratio was zero and the principal compound in the urine was the unchanged drug.

Table showing pharmacokinetic parameters of pefloxacin in various species.

Species	Distribution half life ($t_{1/2}\alpha$) (h)	Elimination half life ($t_{1/2}\beta$) (h)	Volume of distribution ($L.Kg^{-1}$)	Total body clearance ($ml.kg^{-1}.min^{-1}$)	Dose (mg/kg)	Route of administration	References
Buffalo calf	0.03	2.60	4.62	20.69	5	i.v.	Prakah (2003)
Chicken	0.55	2.2	2.10	25.2	50	Oral	Chousalkar (1995)
Cow	0.15	2.53	0.68	3.67	5	i.v.	Patil et al. (1996)
Crossbred calf	0.057	2.21	1.44	7.57	5	i.v.	Srivastava et al. (2000)
Dog	-	3.21	1.48	5.43	50	Oral	Montay et al. (1984)
	-	2.97	1.84	7.3	20	i.v.	Jayakumar et al. (1995)
	-	10.85	7.5	9.7	20	Oral	Jayakumar et al. (1995)
Goat	0.97	3.39	0.41	1.41	5	i.v.	Roy et al. (1997)
	0.27	3.53	1.59	5.07	4	i.v.	Ansari et al. (2000)
	-	4.34	2.14	5.60	4	i.m.	Ansari et al. (2000)
	0.10	1.12	1.08	13.68	10	i.v.	Malik et al. (2002)
	-	2.91	-	-	20	Oral	Malik et al. 2002
Man	-	10.1	1.94	2.22	400mg as total dose	Oral	Montay et al. (1984)
	-	11.00	-	-		i.v.	Frydman et al. (1986)
	-	11.6	-	-		Oral	Webberley et al. (1987)
Monkey	-	7.11	2.21	3.33	25	Oral	Montay et al. (1984)
Mice	-	1.9	15.57	9.33	50	Oral	Montay et al. (1984)
Rabbit							
i) Albino	-	2.01	3.81	-	50	i.m.	Cochereau -Massin et al. (1991)
ii)Pigmented	-	2.61	5.25	-	50	i.m.	,,
Rat	-	3.3	4.25	14.87	50	Oral	Montay et al. (1984)
Sheep	-	6.88	-	2.93	10	i.v.	Moutafchieva and Djouvinov (1997)
	-	5.68	-	-	10	i.m.	

GENERAL PHARMACOKINETICS

Pharmacokinetics often referred as disposition kinetics, which helps in knowing absorption, distribution, metabolism and excretion of drugs (Dost, 1953). According to Wagner (1968), the aim of pharmacokinetics is to study the time concentration course of drugs and their metabolites in various body fluids, tissues and excreta and interpretation of such data based on suitable pharmacokinetic models (compartment models).

The compartment model is a hypothetical structure that can be used to characterize with reproducibility of behaviour and fate of drugs in a biological system, when administered by certain route in a particular dosage from. In pharmacokinetic studies, compartment is an entity that has a definite volume and in that concentration of a drug exists at any time. The disposition kinetics of a drug is described either by one-compartment or multi compartment open models. Body distributes the drugs in all tissues at widely varying rates and is therefore, designated as open system. An open compartment model shows free movement of drugs from one-compartment to another (i.e. blood to tissue and vice-versa).

One compartment open model

When the distribution of drug from central to peripheral compartment is very rapid, the drug is said to follow one compartment open model. Any change in drug concentration in the

blood reflects directly the quantitative changes in its tissue levels. Baggot (1974) reported that the rate of drug elimination from the body is proportional to the concentration of the drug in blood.

In one compartment open model, if the plasma concentration time profile is plotted from the peak concentration onwards on a semilogarithmic scale, a straight line is obtained (Sams, 1978) and the plasma drug levels decline according to following equation: -

$$C_p = B_e^{-\beta t} \text{ .....Eq. 1}$$

Where,

C_p = Concentration of drug plasma

B = Extrapolated zero time intercept of mono-exponential curve

β = Over all elimination rate constant

t = Time elapsed after drug administration

e = Base of natural logarithm

Baggot, (1977) reported that the one compartment open model is particularly useful in describing the time course of most drugs in plasma following extra vascular (oral/i.m/s.c.) administration.

Two compartment open model

The pharmacokinetics of most of the drugs following i.v. administration are accurately described by two compartment open model. Baggot (1974) stated that in two compartment open model, the

drug distribution is instantaneous and homogeneous into the central compartment (such as blood and other readily accessible tissues like liver and kidney) and more slowly into the peripheral compartment (comprising of less perfused organs and tissues such as muscles and fat). This indicates that distribution and elimination processes follow the first order kinetics and elimination takes place exclusively from central compartment. In two compartment open model, semilogarithmic plot of plasma drug concentration against time shows a biphasic curve. The initial steep decline in plasma drug concentration is mainly due to the distribution of drug from central to peripheral compartment. Once apparent distribution is established, the gradual decline is obtained mainly by irreversible elimination of drug from the central compartment.

The drug concentration in plasma is expressed by the following biexponential mathematical expression as a function of time: -

$$C_p = A_e^{-\alpha t} + B_e^{-\beta t} \quad \text{.....Eq. 2}$$

Where,

C_p = Plasma concentration of the drug,

A = Zero time intercept of distribution phase.

B = Zero time intercept of elimination phase

α = Distribution rate constant.

β = Elimination rate constant

e = Base of natural logarithm

t = Time elapsed after drug administration

The values of A, B, α and β are essential in calculating other kinetic rate constant (K_{12}, K_{21} and Kel) in two compartment open model. The values of these rate constants give an idea of relative contribution of distribution and elimination processes to the drug concentration time data (Baggot, 1977).

Three or multi compartment open model

The distribution kinetics of some drugs may also follow three or multiple compartment model. In three compartment open model, the semilogarithmic plot of plasma drug concentration against time shows a triphasic curve. The initial sharp decline in plasma concentration against time is due to distribution of drug from blood to highly perfused tissue compartment (Peripheral I). The gradual decline is because of distribution of drug from central to moderately blood supplied organs (Peripheral II). The drug concentration in plasma following single intravenous administration is expressed by the following triexponential mathematical formula as a function of time.

$$C_P = A_e^{-\alpha.t} + B_e^{-\beta.t} + C_e^{-r.t} \qquad \text{.....Eq. 3}$$

The additional constants C and γ are calculated by using residual methods. These constants may be employed to estimate K_{13} and K_{31} (Gibaldi and Perrier, 1975).

PHARMACOKINETICS OF CLINICAL IMPORTANCE

Clinically, the pharmacokinetic study consists of: -

(a) Calculation of various kinetic parameters following different routes of administration.

(b) Calculation/suggestion of dosage regimen in a particular species of animals

(c) Determination of drug withdrawal period of drug residues in milk and tissues of food producing animals.

SOME IMPORTANT PHARMACOKINETIC PARAMETERS

1. Absorption rate constant (Ka) and absorption half life ($t_{1/2}$ Ka)

These denote the rate of absorption (faster or slower) of a drug from its site after extra vascular (i.m./s.c./oral) administration.

2. Distribution rate constant (α) and distribution half life ($t_{1/2}\alpha$)

These parameters indicate the rate of distribution (faster or slower) of a drug from plasma to body fluids and tissues following i.v. administration.

3. Elimination rate constant (β)

Baggot (1977) and Mercer *et al.* (1977) stated that the overall elimination rate constant (β) is the most essential kinetic parameter since it is employed to determine: -

i. The elimination half-life ($t_{1/2}\beta$)

ii. The volume of distribution by area method (Vd_{area})

iii. The total body clearance (Cl_B)

iv. The drug withdrawal period for drug residues in milk and tissues of food producing animals.

4. Elimination half life ($t_{1/2}\beta$)

Gibaldi and Weintraub (1971) defined that the elimination half life is the time required to reduce the drug concentration in plasma or serum to its half during the elimination phase of the drug concentration time profile. This means that doubling the dose does not double the duration of action of drug but increase it by one half life. It is inversely proportional to the overall elimination rate constant. It is used to calculate the duration of drug action in the body. The half life of a first order process is independent of the dose of drug as well as the route of administration. Knowledge of the half life of a drug is extremely helpful in designing the rational dosage regimen.

5. Volume of distribution

The apparent volume of distribution is an important pharmacokinetic parameter used in the kinetic characterization of a drug. It is a hypothetical volume of body fluid that would be required to dissolve the total amount of the drug to attain the same concentration as that found in the blood. Riegelman *et al.* (1968) stated that the calculated value of volume of distribution is not

dependent upon the method used for its calculation if the drug distributes truly according to one compartment open model. The apparent volume of distribution indicates the amount of distribution of a drug without providing any clue, whether the drug is uniformly distributed or restricted to certain tissues (Baggot, 1977). A large volume of distribution (>1 L/kg) indicates wide distribution throughout the body or extensive tissue binding or rapid excretion of a drug or combination of all the above. A small volume of distribution indicates that the drug is restricted to certain fluid compartments like plasma, water, extra cellular fluid etc. This is due to the high protein binding or low lipid solubility of a drug.

6. Total body clearance (Cl_B)

Another important pharmacokinetic parameter is the total body clearance (Cl_B), which is the sum of the clearance of each eliminating organ, particularly liver and kidney. The half life of a drug is a complex function which depends upon the process of drug distribution, bio transformation and excretion. The parameter, body clearance, on the other hand is independent of these processes and indicates the rate of drug removal from the body. Unlike β and $t_{1/2}\ \beta$ that are hybrid constants and depends upon K_{12}, K_{21} and Kel. The total body clearance changes exactly in proportion to Kel (Jusko and Gibaldi, 1972; Rowland *et al.*, 1973).

It is reported that the various constants, namely A, α, B, β, $t_{1/2}$ α, $t_{1/2}$ β and Vd_{area} etc. change disproportionally with the magnitude of the elimination rate constant from central compartment (Kel) and hence, should not be employed individually as a direct or sole measure of a change in drug elimination or distribution (Jusko and Gibaldi, 1972).

DOSAGE REGIMEN

Dose is a quantitative term estimating the amount of drug, which must be administered to produce a particular biological response i.e. to attain optimum effective concentration of a drug in the body fluids. Maintenance of therapeutic concentration of a drug in the body requires the administration of maintenance dose at a particular dose interval after administering the priming or loading dose, so that plasma drug concentration must be above a minimum effective level and below a level producing excessive side effects and toxicity. Thus, the objective of a multiple dosage regimen is to maintain the plasma concentration of the drug within the limits of the maximum safe concentration and the minimum effective levels.

TOXICOLOGICAL STUDY OF PEFLOXACIN

Toxicological studies of pefloxacin are mainly reported in human beings and experimental animals though very few reports are

also available in domestic animal and it seems that little work has been done in goats.

Human

Chichmanian *et al.* (1992) studied dose dependent toxicity of pefloxacin in human beings. Pefloxacin gave rise to thrombocytopenia, although the responsibility of the drug could be difficult to demonstrate in infectious patients and those receiving other drugs simultaneously. Thrombocytopenia occured from 5 to 19 days after beginning of treatment and resolved between 7 and 12 days after drug withdrawal. Differential blood counts appeared to be warranted for patients at risk.

Monkey

Cukierski *et al.* (1992) reported embryotoxicity of norfloxacin in cyanomolgus monkeys. They reported norfloxacin to be embryo lethal but not teratogenic when administered to pregnant cyanomolgus monkeys prior to gestational day 36 at doses more or equal to 200 mg/kg/day.

Dog

Christ *et al.* (1988) studied some specific toxicological aspects of pefloxacin in dog. Arthropathies developed in adult dogs after 12 months of pefloxacin treatment. At higher doses pefloxacin

exerted effects on renal function. Pefloxacin caused cataracts in dog after treatment for 8-12 months.

Stahlmann *et al.* (2000) studied chondrotoxicity of ciprofloxacin in immature beagle dogs. Dogs were given orally 30 and 200 mg of ciprofloxacin/kg body weight for 5 dâys. In control dogs and in dogs treated with low dose of ciprofloxacin, no pathological changes were seen. However, cleft formation and erosions were observed in joint cartilage in two of five dogs treated with 200 mg/kg.

Sridevi *et al.* (2002) evaluated experimentally induced pefloxacin toxicity in pups. Twenty four pups were divided into four groups. Group I served as control. Group II, III and IV were given orally pefloxacin at the rate at 10, 15 and 20 mg/kg, respectively, twice daily continuously for 30 days. Two pups in groups IV died during the first week after exhibiting signs of excitability, abnormal behaviours and generalized seizures. Pups in group III and IV (those alive) exhibited slight stilted gait, reluctance to rise and inability to stand, swollen joints and arthralgia on flexion of stiffle and elbow joints. The musculoskeletal signs observed could be due to damage to articular cartilage by pefloxacin as evidenced by hind limb stiffness and recumbency. It was concluded that pefloxacin at the rate of 15 and 20 mg/kg induced observable toxic symptoms on long term (4 weeks) use while 10 mg/kg was comparatively safer.

Broiler chicken

Ashish Sachan *et al.* (2000) calculated haematological parameters in clinical toxicity study of pefloxacin in broiler chicken. Eighty broiler chicks were taken in the study and divided into four groups. Group I served as untreated control. Groups II, III and IV were given pefloxacin at the rate of 5, 10 and 40 mg/kg, respectively, *per os* dissolved in drinking water. The drug was given for the first five days of the broiler life and again repeated for five days in the third week from 21st to 25th day. Blood sample of 7th, 28th and 42nd days revealed that all the haematological parameters were in normal range and there were no significant changes observed in any of the haematological parameters.

Ashish Sachan *et al.* (2000) studied acute toxicity of pefloxacin on day old broiler chicks after an increasing dose (600, 700, 800, 900, 1000, 1100, 1300, 1500, and 1600 mg/kg) with pefloxacin 10% in 20 birds in each treatment. LD_{50} value was calculated to be 1025 mg/kg of pefloxacin. It is concluded that pefloxacin has a wide margin of safety in broiler and the proposed dose is 5-10 mg/kg body weight daily.

Ashish Sachan *et al.* (2002) evaluated safety of pefloxacin in day old broiler chicken. Forty day old broiler chicks were selected

and divided into four groups. Group I served as control and groups II, III and IV were given pefloxacin at the rate of 5, 10 and 20 mg/kg, respectively *per os* for 5 days. The levels of SGPT, SGOT, BUN and total bilirubin did not vary significantly in any of the treated groups from the control. It was concluded that pefloxacin was safe in day-old chicken without causing hepatotoxicity or nephrotoxicity.

Guinea Pigs

Horio *et al.* (1994) demonstrated phototoxicity of norfloxacin in guinea pigs. He examined experimentally phototoxicity of norfloxacin in an *in vivo* system using the administration of drug and subsequent exposure to long-wave ultraviolet (UVA) at a dose of 30 J/cm^2.

Rat

Nordman *et al.* (1989) investigated the cytotoxicity and uptake of pefloxacin in primary cultures of rat hepatocytes. As assessed by intracellular enzyme release in culture media, pefloxacin at concentration of 400 mg/ml was found to be hepatotoxic.

Pino *et al.* (1991) evaluated DNA damage induced by norfloxacin in liver and kidney of adult rats and in foetal tissues after transplacental exposure. After oral administration of single doses

ranging from 1 to 8 m mole/kg, DNA fragmentation was absent in liver and kidney both 2 and 6 h after treatment. However, when administered to pregnant rats, the highest doses produced a detectable amount of DNA damage to foetal tissues. This damage appeared to be an aspecific consequence of maternal and foetal toxicity rather than specific genotoxic effect.

Kashida and Kato (1997) examined the toxic effects of pefloxacin on the musculoskeletal system in juvenile rats. Single oral administration of 900 mg/kg pefloxacin was found to induce lesions in the muscle + fascia, tendon + sheath and synovial membrane in addition to articular cartilage in the fore and hind limbs. Among all the lesions, the ankle and elbow showed the highest incidence and severity. There was oedema of joints and increase in number of mononuclear cells, fibroblasts and macrophages. Capillary endothelial cells were hypertrophied, increased in number and stratified. These results suggest that pefloxacin have toxic potential in the muscle, tendon and synovial membrane in addition to articular cartilage and that local vascular hyper permeabilitiy may contribute to the development of these lesions.

Mice

Simonin *et al.* (1999) estimated the potentially deleterious effects of a high dose of pefloxacin (400mg/kg) on two main

constituents of cartilage in mice i.e. proteoglycans and collagen in mice. Treatment of mice with 1 day of pefloxacin treatment significantly decreased the rate of biosynthesis of proteoglycan for the first 24 h. However, no difference was observed after 48 h. On the other hand, treatment with pefloxacin for 10 days induced oxidative damage to collagen. It was concluded from the study that pefloxacin administration to mice leads to modifications in the metabolism and integrity of extracellular proteins such as collagen and proteoglycans.

Chapter - 3

Materials and Methods

MATERIALS AND METHODS

Experimental Animals

Five clinically healthy goats of non-descript breed of 1.5 to 2 years of age and weighing between 20-25 kg were used in the present study. The goats were housed in animal shed with concrete floor. The goats were maintained on paddy straw, wheat husk and greens as well as on routine grazing for at least 4-5 hours a day. Clean drinking water was supplied *ad lib.* All the goats were kept under close observation for a week prior to the start of experiment. During this period, the goats were examined for internal parasitic infestation; positive cases were treated with Albendazole, (Analgon® - Wockhardt Limited, Mumbai). The preliminary health check up was carried out in each goat prior to the experiment.

Experimental Drug And Dose

The drug used was pefloxacin (Pelox®) infusion (100 ml plastic bottle), an injectable commercial preparation manufactured by Wockhardt Limited, Mumbai which contained pefloxacin methane sulfonate dihydrate equivalent to 4 mg/ml of pefloxacin base in 5%

dextrose (anhydrous) solution. The drug was injected at the dose rate of 5 mg/kg body weight in each goat for pharmacokinetic study and 10 mg/kg body weight in each goat for toxicological study by intravenous route to carry out the present study.

Experimental Design

Pefloxacin was studied on a group of five animals for kinetic studies. Four weeks after conducting kinetic studies, the same animals were used for toxicological studies. For kinetic studies, the drug was administered by intravenous (i.v.) route in each goat. For toxicological studies, the drug was administered intravenously daily for 7 consecutive days in each goat.

KINETIC STUDY

Collection of biological fluids and their timings

The samples of blood and urine were collected post i.v. injection of pefloxacin (5 mg/kg) in each goat. The samples of blood and urine were collected at 0.042, 0.083, 0.167, 0.25, 0.333, 0.50, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 h, while the urine samples were further collected beyond 24 h at 30, 36 and 48 h post i.v. administration of pefloxacin.

(A) Blood

Before collection of blood, the sites around the jugular vein on either side of the neck of the animals were aseptically prepared. The site was sterilized prior to each collection with rectified spirit. Blood samples were collected in sterilized centrifuge tubes containing appropriate amount of sodium oxalate by venipuncture with disposable 18 G needles at various above noted time intervals after drug administration. The blood sample were centrifuged at 2500-3000 rpm for 10 min for the separation of plasma. The plasma samples were then kept in a refrigerator until assay was carried out. For the preparation of standards normal plasma prior to drug administration was also collected.

(B) Urine

The urine samples were collected for analysis by introducing a sterile Foley's balloon catheter (No. 12) lubricated with glycerine through urethra into the urinary bladder of the experimental goat with the aid of a flexible metal probe. The balloon of the catheter was inflated by injecting 20 ml of sterile water through a syringe to keep the catheter in position. The opening of the catheter was blocked with a pressure clip to check dripping of urine. Prior to

drug administration, urine sample was collected in a sterile test tube for the preparation of standards. After administration of the drug, the urine sample were collected in sterile test tubes at various above noted time intervals. The samples were kept in a refrigerator and were analysed on successive days.

Administration of Drug

The drug Pelox® infusion containing pefloxacin methane sulfonate dihydrate equivalent to 4 mg/ ml of pefloxacin base in a 5% dextrose (anhydrous) solution was injected at a dose of 5 mg / kg body weight by i.v. route in each goat.

Estimation of Pefloxacin by Microbiological Assay

Procedure adopted for the microbiological assay

(I) Sterilization of Glasswares, Needles and Porcelain Assay Cylinders :

All glasswares and porcelain assay cylinders were washed properly with detergent solution in running tap water. These were again rinsed with glass distilled water and finally air – dried. Test tubes, centrifuge tubes, vials and vial containing porcelain assay cylinders were plugged with cotton wool. Assay plates, pipettes and

syringes were wrapped with paper. All these materials were sterilized in hot air oven at 160^0 C for an hour. For administration of drug and for collection of blood, sterile disposable needles were used.

(II) Preparation of Media :

(a) Assay Agar **:** Antibiotic assay media of the following composition was used for microbiological assay of pefloxacin in blood and urine.

Sl. No.	Ingredients	Grams / Litre water
1	Peptone	6.0
2	Tryptone	4.0
3	Yeast extract	3.0
4	Beef extract	1.5
5	Dextrose	1.0
6	Agar	15.0
	Distilled water	1000 ml
	Final pH	7.9 ± 0.1

The media was heated to dissolve and the solution was transferred into a conical flask, and pH was adjusted. The month of

the flask was plugged with non – absorbable cotton wool and wrapped with aluminium foil. Wet sterilisation of media was done by autoclaving at 15 pound pressure (121^{0}C) for 20 minutes.

(b) Nutrient Broth : Nutrient broth of the following composition was prepared : -

Sl. No.	Ingredients	Grams/Litre water
1	Sodium chloride	5.0
2	Peptone	10.0
3	Beef extract	10.0
	Distilled water	1000 ml.
	Final pH	7.4 ± 0.1

The media was heated do dissolve completely and pH was adjusted. Sterilization of the broth was done by autoclaving at 15 pound pressure (121^{0}C) for 20 minutes.

(III) Preparation of Assay Agar Plates :

Twenty ml of autoclaved antibiotic assay media, while in melted condition, was poured gently into each of the sterilized special assay plate (Borosil®) with the aid of a sterile measuring cylinder. The plates were kept on a horizontally plane surface in laminar flow

to get uniform thickness of media. The plates were left at room temperature for about 1 to 2 h for solidification of agar. Afterwards, the plates were kept inside the incubator at 37^0C for 24 h to ascertain any growth, which indicates any microbial contamination. The growth free plates were then wrapped with sterile paper and stored in refrigerator until assay was carried out.

(IV) Preparation of Test Organism :

The test organism used for microbiological assay technique of pefloxacin was *Bacillus subtilis* ATCC 6633 (Montay *et al.*, 1984). The culture of *B. subtilis* ATCC 6633 was obtained from National Collection of Industrial Micro-organisms (NCIM), Division of Biochemical Sciences, National Chemical Laboratory, Pune-8. The test organism was grown on the slant of culture tube containing plain agar slants at 37^0 C for overnight. Then, it was stored under refrigeration. The organism was transferred weekly to fresh media to maintain its normal activity.

(V) Preparation of Standards in Plasma and Urine :

The drug pefloxacin was dissolved and diluted in sterile glass distilled water to have different strengths viz. 80, 40, 20, 10, 5, 2, 1 and 0.5 $\mu g.ml^{-1}$. From each standard solution 0.1 ml was added to a sterile vial containing 0.9 ml of plasma /urine collected prior to drug

administration. This yielded drug standards of 8, 4, 2, 1, 0.5, 0.2, 0.1 and 0.05 $\mu g.ml^{-1}$ in the above noted biological samples. These standards were used simultaneously with test samples in the assay plates for determination of the drug concentration in the test samples.

(VI) Assay Procedure :

The plasma and urine levels of pefloxacin were estimated by microbiological assay technique (cylinder plate diffusion method) using *Bacillus subtilis* (ATCC 6633) as the test organism (Bennet *et al.*, 1966).

The test organism was grown in nutrient broth for 2 to 3 h at 37^0C until the growth was seen (turbid by naked eye). Pefloxacin assay plates were flooded with the broth containing the organism and excess broth was drained out after sometime. The plates were dried in incubator at 37^0C for a period of about an hour. Sterile porcelain assay cylinder of uniform size were placed at appropriate distance along the circumference in the inoculated assay plates. 50 µl of standard solution of plasma and urine of various strengths as well as test samples of the drug was poured in separate porcelain cylinders in the assay plate. Such plates were left on the platform of laminar flow

for about 2 hours and then kept in the incubator at 37^0C overnight for allowing growth of the organism. The mean diameter of the bacterial zone of inhibition produced by standards as well as test samples of the drug was measured. The concentration of the drug in different test samples of a biological fluid was estimated from the standard curve plotted from the zone of inhibition versus concentration of the drug on a semilog scale.

Calculation of Pharmacokinetic Parameters

The following pharmacokinetic parameters of pefloxacin was calculated after its single i.v. administration from semi log plot of plasma drug concentration versus time curve. The experimental data was analyzed by using two compartment (for i.v. route) open model as described by Gibaldi and Perrier (1975) and Notari (1980). For a two compartment model, the concentration of the drug in plasma at any time is obtained from the formula: -

$$Cp = Ac^{-\alpha t} + Be^{-\beta t}$$

Where Cp is the drug concentration in plasma at time 't'. The description and calculation of the parameters A, B, α and β used in the above formula and other kinetic parameters are noted below.

(a) A, the zero time concentration of the drug in plasma and α, the regression coefficient (distribution rate constant) for

distribution phase were calculated by the method of residual yield.

(b) B, the zero time concentration of the drug in plasma and β, the regression coefficient (elimination rate constant) for elimination phase were calculated by the method of least squares.

(c) C_p^o, the theoretical zero time plasma concentration of drug.

$$C_p^o = A+B \text{ (two compartment model)}$$

(d) Distribution half life ($t_{1/2}\ \alpha$) and elimination half life ($t_{1/2}\ \beta$) were calculated from the following formula.

$$t_{1/2}\ \alpha = 0.693 / \alpha$$

$$t_{1/2}\ \beta = 0.693 / \beta$$

where α and β are described above.

(e) AUC, the total area under plasma drug concentration time curve (mg/L.h).

For two compartment model

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta}$$

(f) AUMC, the total area under the first moment of plasma drug concentration time curve (mg/L.h^2).

$$AUMC = \frac{A}{\alpha^2} + \frac{B}{\beta^2}$$

(g) MRT, mean residential time (h)

$$MRT = \frac{AUMC}{AUC}$$

(h) K_{21}, rate constant of transfer of drug from peripheral (tissue) compartment to the central (blood) compartment (h^{-1}):

$$K_{21} = \frac{A.\beta + B.\alpha}{C_P^o}$$

(i) Kel, the elimination rate constant of drug from central compartment (h^{-1})

$$Kel = \frac{\alpha - \beta}{K_{21}}$$

(j) K_{12}, the rate constant of transfer of drug from central to peripheral compartment (h^{-1})

$$K_{12} = \alpha + \beta - Kel - K_{21}$$

(k) Fc, the fraction of drug available for elimination from central compartment.

$$Fc = \frac{\beta}{kel}$$

(l) $T \approx P$ the approximate tissue to plasma concentration ratio.

$$T \approx P = \frac{K_{12}}{K_{21} - \beta}$$

(m) Vd_C, the volume of distribution based on distribution and elimination (L/kg)

$$Vdc = \frac{D}{C_P^o}$$

(n) Vd_B, the volume of distribution based on elimination (L/kg)

$$Vd_B = \frac{D}{B}$$

(o) Vd_{area}, the volume of distribution based on total area under curve (L/kg).

$$Vd_{area} = \frac{D}{AUC.\beta}$$

(p) Vd_{SS} the volume of distribution of steady state (L/kg)

$$Vd_{SS} = \frac{K_{12} + K_{21}}{K_{21}} \times Vdc$$

(q) Cl_B, the total body clearance (ml/kg/min)

$$Cl_B = Vd_{area} \times \beta$$

Calculation of Dosage Regimen :

Dosage regimen is generally calculated for an antimicrobial agent to maintain minimum inhibitory concentration (MIC) in plasma at desired dosage intervals. Gonzalez and Henwood (1989) reported the therapeutic plasma level (MIC) of pefloxacin to be

0.03 – 2.0 $\mu g.ml^{-1}$. But, most of the pathogens are sensitive at around 0.25 to 1.0 $\mu g.ml^{-1}$ concentration of pefloxacin. Only a few exceptional organisms are having higher MIC. So, in the present study, dosage regimen of pefloxacin were calculated at 0.25, 0.50 and 1.0 $\mu g.ml^{-1}$ levels for the dosage intervals of 8 and 12 hours using the following formulae (Saini and Srivastava, 1997) :

$$D^* = Cp^{\infty}(min).\ Vd_{area}(e^{\beta\gamma})$$

$$D_0 = Cp^{\infty}(min).\ Vd_{area}(e^{\beta\gamma} - 1)$$

Where,

D^* = Loading or Priming dose (mg/kg)

D_0 = Maintenance dose (mg/kg)

$Cp^{\infty}(min)$ = Desired minimum plasma concentration ($\mu g.ml^{-1}$).

γ = Dosage interval (h)

β and Vd_{area} are obtained from kinetic study.

TOXICOLOGICAL STUDY

Collection of Biological Fluids and their Timing :

For determining the different haematological values the blood was collected from the experimental animals from the jugular vein by venepuncture in small sterile vials having the anticoagulant

EDTA @ 1.5 mg/ml of blood. Blood was collected from the ear vein of goat for total leucocyte count (TLC) and differential leucocyte count (DLC). For blood sugar estimation, fluoride-oxalate mixture was used as anticoagulant. For other biochemical estimations like SGPT, SGOT, BUN, cholesterol, total protein and albumin, serum was required. Serum was obtained from test tube containing clotted blood. All the blood samples and serum were stored in refrigerator until estimation was completed.

The blood was collected routinely on 0, 2, 4, 8 and 10^{th} day during the study.

Administration of Drug :

The drug pefloxacin (Pelox®) containing pefloxacin sulfonate dihydrate equivalent to 4 mg/ml of pefloxacin base in 5% dextrose solution was injected @ 10 mg/kg body weight by slow i.v. route daily for 7 consecutive days in each goat.

Estimation of Toxicological Parameters :

The different toxicological parameters studied were-

(I) Haematological Parameters :

(a) Haemoglobin (Hb g %)

(b) Total leucocyte count (TLC)

(c) Differential leucocyte count (DLC)

(II) Biochemical Parameters : -

(a) Blood sugar

(b) Blood urea nitrogen (BUN)

(c) Blood cholesterol

(d) Total protein and albumin

(e) Serum glutamate pyruvate transaminase (SGPT)

(f) Serum glutamate oxaloacetate transaminase (SGOT)

(I) Estimation of Haematological Parameters :

The different haematological parameters studied on 0, 2, 4, 8 and 10^{th} day are described below.

(A) Haemoglobin (Hb in gm/dl)

The haemoglobin content 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 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 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.

(B) ***Total Leucocyte Count (TLC)***

The total leucocyte count of blood was done using improved Neubauer chamber by the method as 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 revolved. This kept 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 content of pipette was mixed first, 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.

(C) 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 spreaded evenly with a spreader slide at 30^0 angle. 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 is 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.

(II) Estimation of Biochemical Parameters :

The estimation of biochemical parameters were done using commercially available kits from Nice and Span Diagnostics. The different biochemical parameters studied on 0, 2, 4 , 8 and 10^{th} day are detailed below.

(A) Blood Sugar

The estimation of blood sugar was done by Folin and Wu method as described by Frakel *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 = $\frac{\text{O.D. of T}}{\text{O.D. of S}} \times 200$

(B) 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 a boiling water both exactly for 10 minutes and then cooled. Optical density was recorded at 540 nm (green filter) against blank.

Calculation

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

(C) Blood 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

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

(D) Blood Proteins

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

Total Protein : 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.

Albumin – 4 ml of bromocresol green reagent were added to three test tubes marked as B, S and T. 0.2 ml of distilled water was added to B whereas 0.2 ml of diluted standard (1: 10) and 0.2 ml of diluted

serum (1: 10) were added to S and T, respectively. The content of the tubes were mixed well and kept for 5 minutes at room temperature. Optical density was measured at 630 mm (red filter) against blank.

Calculation

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

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

Serum globulin in gm % = Serum total protein - Serum albumin

$$\text{A/G ratio} = \frac{\text{Albu min(gm\%)}}{\text{Globulin(gm\%)}}$$

(E) 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

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

Pyruvic acid so formed is treated with 2,4 – DNPH and the brown colour produced is measured colourimetrically 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°C for 5 minutes. Then, 0.05 ml of serum was placed in the tube, mixed and again incubated at 37°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.

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°C for 5 minutes. Then, 0.05 ml of serum was placed in the tube, mixed and again incubated at 37°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.

(F) 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 $\overset{\text{SGOT}}{\rightleftharpoons}$ Oxaloacetic acid + glutamic acid.

Oxaloacetic acid so formed is coupled with 2, 4 - dinitrophenylhydrazine (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 colourimeter.

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.

Chapter - 4

Results

RESULTS

PHARMACOKINETIC STUDY OF PEFLOXACIN

Pharmacokinetic study of pefloxacin was conducted in five healthy female goats following single intravenous (i.v.) dose of 5 mg/kg and the results are presented below.

1. Plasma levels

Concentrations of pefloxacin in plasma at various time intervals following its single i.v. administration at the dose rate of 5 mg/kg have been shown in Table 1 and Fig. 1. The mean peak plasma concentration of 17.41 ± 1.34 $\mu g.ml^{-1}$ was attained at 0.042 h. The drug was detectable upto 10 h in all animals with the mean of 0.43 ± 0.02 $\mu g.ml^{-1}$. The drug was detectable only in 2 out of 5 animals at 12 h with a mean of 0.08 ± 0.05 $\mu g.ml^{-1}$. The drug was detectable only in 2 out of 5 animals at 12 h with a mean of 0.08 ± 0.05 $\mu g.ml^{-1}$.

2. Urine Levels

Table 2 and Fig. 2 reveal urine concentrations of pefloxacin after its single i.v. administration (5 mg/kg). The drug appeared in urine of all animals at 0.042 h with a mean of 2.02 ± 0.29 $\mu g.ml^{-1}$. The mean peak urine drug concentration of 48.13 ± 1.50 $\mu g.ml^{-1}$ was achieved at 0.75 h. The drug was detectable upto 30 h in all animals with a mean of 0.87 ± 0.07 $\mu g.ml^{-1}$.

Table – 1

Plasma concentrations ($\mu g.ml^{-1}$) of pefloxacin in healthy female goat after a single intravenous dose (5 mg/kg).

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	18.50	16.25	20.50	12.80	19.00	17.41 ± 1.34
0.083	10.00	9.25	13.80	8.75	10.82	10.52 ± 0.89
0.167	8.65	8.12	10.42	7.45	9.85	8.90 ±0.55
0.25	7.65	6.95	8.14	6.45	8.14	7.47 ± 0.33
0.333	6.42	6.02	7.00	5.60	6.90	6.39 ± 0.26
0.50	5.16	4.92	5.60	4.50	5.26	5.09 ± 0.18
0.75	3.12	3.06	3.85	3.65	3.64	3.46 ± 0.16
1	2.68	2.72	3.50	3.20	3.04	3.03 ± 0.15
1.5	2.42	2.12	3.12	2.75	2.48	2.58 ± 0.17
2	2.10	2.00	2.75	2.34	2.12	2.26 ± 0.13
3	1.68	1.56	2.10	1.82	1.60	1.75 ± 0.10
4	1.52	1.42	1.65	1.28	1.25	1.42 ± 0.07
6	1.02	1.00	1.08	0.85	0.85	0.96 ± 0.05
8	0.76	0.72	0.80	0.54	0.58	0.68 ± 0.05
10	0.38	0.45	0.50	0.42	0.40	0.43 ± 0.02
12	ND	ND	0.24	0.15	ND	0.08 ± 0.05
24	--	--	ND	ND	--	--

ND = Non detectable

Fig. 1

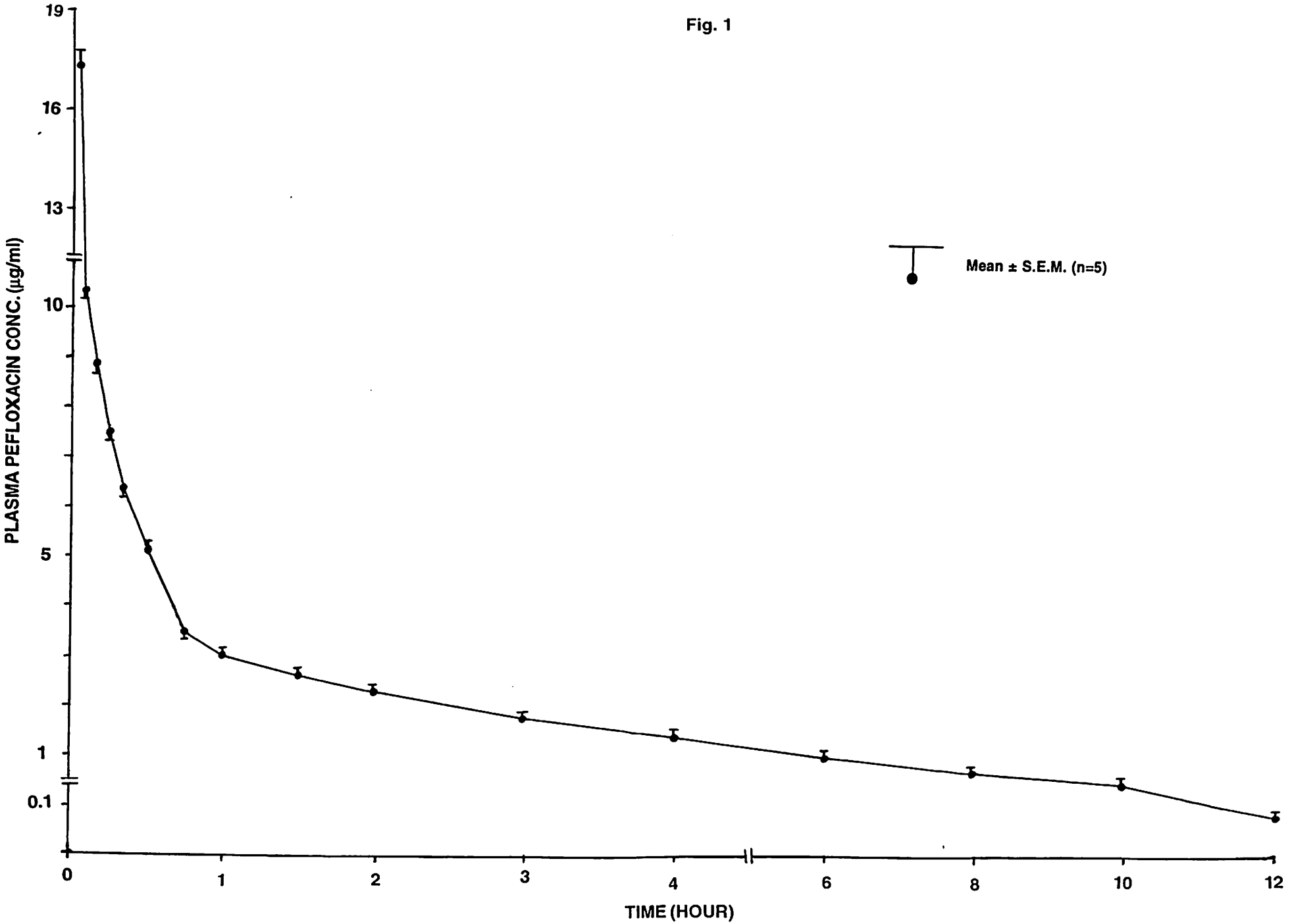
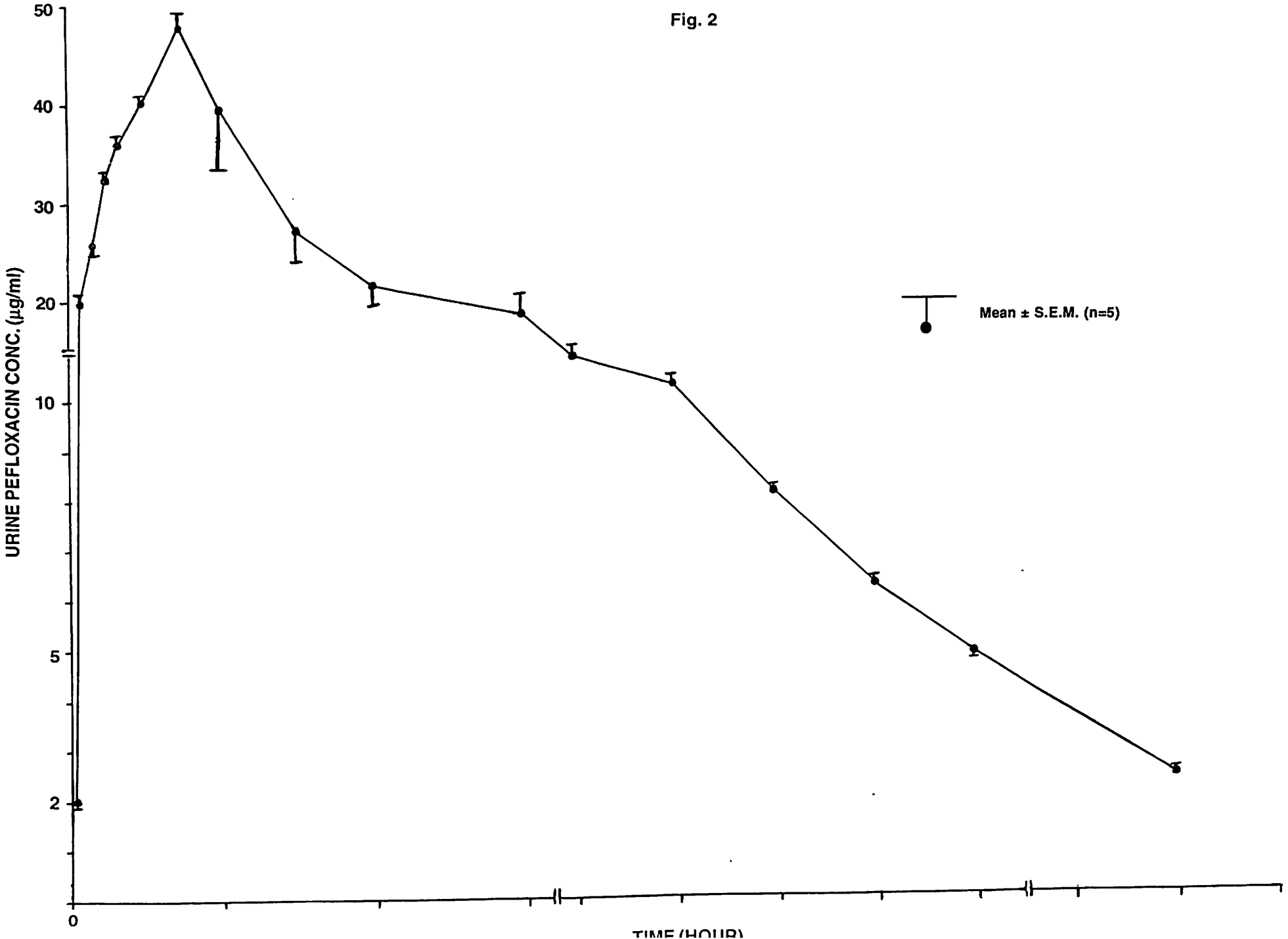

19
16
13
10
5
1
0.1
PLASMA PEFLOXACIN CONC. (μg/ml)
Mean ± S.E.M. (n=5)
0
1
2
3
4
6
8
10
12
TIME (HOUR)

Fig. 2


50
40
30
20
10
5
2
0
URINE PEFLOXACIN CONC. (μg/ml)
Mean ± S.E.M. (n=5)
TIME (HOUR)

3. Kinetic Parameters

Plasma drug concentration versus time profile has confirmed a two compartment open model for pefloxacin as depicted in Fig. 3. Table 3 presents the value of different kinetic parameters calculated by the above noted compartment model.

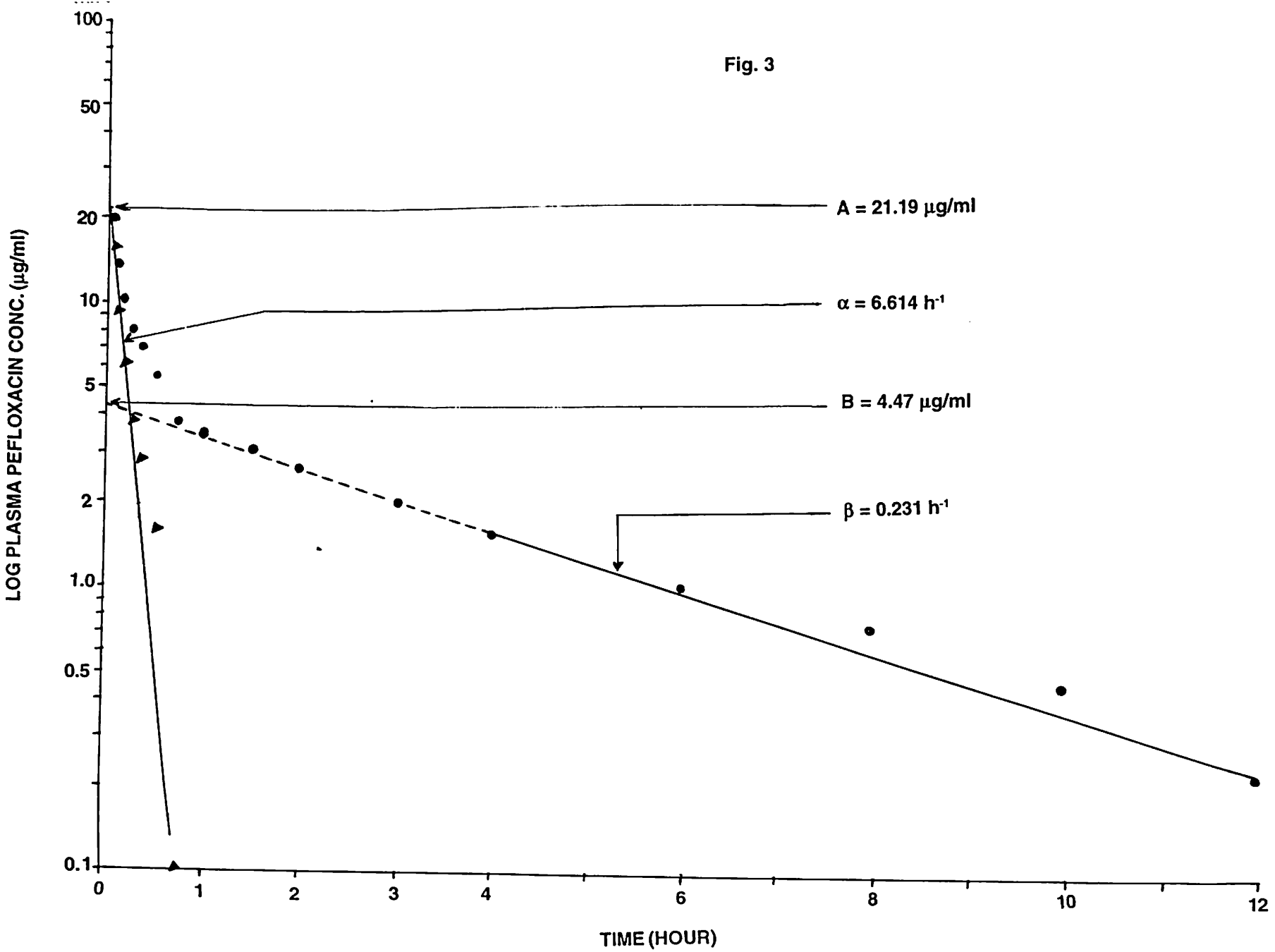
The mean extrapolated zero time concentration during distribution phase (A), elimination phase (B) and the theoretical zero time concentration (C_p^o) were noted to be 12.21 ± 2.86, 3.46 ± 0.32 and 15.67 ± 3.03 $\mu g.ml^{-1}$, respectively. The distribution rate constant (α) ranged from 2.080 to 6.614 h^{-1} with a mean value of 3.897 ± 0.880 h^{-1}, while its elimination rate constant (β) ranged from 0.178 to 0.250 h^{-1} with a mean value of 0.212 ± 0.013 h^{-1}. The mean distribution ($t_{1/2}\ \alpha$) and elimination ($t_{1/2}\ \beta$) half-life of the drug were observed to be 0.22 ± 0.05 and 3.61 ± 0.46 h, respectively. The average rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) compartment and elimination from the central compartment (Kel) were calculated to be 2.306 ± 0.682, 1.014 ± 0.123 and 0.789±0.118 h^{-1}, respectively. The fraction of drug available for elimination from central compartment (Fc) and approximate tissue to plasma concentration ration (T ≈ P) wee noted to be 0.30 ± 0.06 and 2.77 ± 0.55, respectively. The value of area under the curve (AUC), area under first moment curve (AUMC) and MRT were found to be

Table – 3

Pharmacokinetic parameters of pefloxacin in goat calculated by two compartment open model following single i.v. administration

Parameters (Unit)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
A ($\mu g.ml^{-1}$)	15.33	11.02	21.19	4.23	9.29	12.21 ± 2.86
B ($\mu g.ml^{-1}$)	3.39	2.81	4.47	3.81	2.80	3.46 ± 0.32
C_p^o ($\mu g.ml^{-1}$)	18.72	13.83	25.66	8.04	12.09	15.67 ± 3.03
α (h^{-1})	5.136	3.530	6.614	2.124	2.080	3.897 ± 0.880
$t_{1/2}\ \alpha$ (h)	0.14	0.20	0.10	0.33	0.33	0.22 ± 0.05
β (h^{-1})	0.206	0.178	0.231	0.250	0.196	0.212 ± 0.013
$t_{1/2}\ \beta$ (h)	3.36	5.35	3.00	2.78	3.54	3.61 ± 0.46
AUC ($mg.L^{-1}h$)	19.12	18.73	22.55	17.23	18.75	19.28 ± 0.88
AUMC ($mg.L^{-1}h^2$)	77.45	87.61	84.25	61.90	75.03	77.25 ± 4.45
MRT (h)	4.05	4.68	3.74	3.59	4.00	4.01 ± 0.19
K_{12} (h^{-1})	3.280	2.118	4.364	0.769	0.999	2.306 ± 0.682
K_{21} (h^{-1})	1.099	0.859	1.343	1.138	0.632	1.014 ± 0.123
Kel (h^{-1})	0.963	0.731	1.138	0.467	0.645	0.789 ± 0.118
Fc	0.21	0.24	0.20	0.54	0.30	0.30 ± 0.06
$T \approx P$	3.67	3.10	3.92	0.87	2.29	2.77 ± 0.55
Vdc ($L.Kg^{-1}$)	0.27	0.36	0.19	0.62	0.41	0.37 ± 0.07
Vd_B ($L.Kg^{-1}$)	1.47	1.78	1.12	1.31	1.79	1.49 ± 0.13
Vdarea ($L.Kg^{-1}$)	1.25	1.48	0.96	1.16	1.36	1.24 ± 0.09
Vdss ($L.Kg^{-1}$)	1.07	1.24	0.81	1.04	1.06	1.04 ± 0.07
Cl_B ($ml.Kg^{-1}.min^{-1}$)	4.29	4.39	3.70	4.83	4.44	4.33 ± 0.18

Fig. 3


LOG PLASMA PEFLOXACIN CONC. (μg/ml)
100
50
20
10
5
2
1.0
0.5
0.1
0
1
2
3
4
6
8
10
12
TIME (HOUR)
A = 21.19 μg/ml
α = 6.614 h-1
B = 4.47 μg/ml
β = 0.231 h-1

19.28 ± 0.88 $mg.L^{-1}.h$, 77.25 ± 4.45 $mg.L^{-1}.h^2$ and 4.01 ± 0.19 h, respectively. The various values of volume of distribution obtained by different methods are shown in Table 3. A mean Vd_{area} of 1.24 ± 0.09 $L.Kg^{-1}$ was noted. The total body clearance (Cl_B) value ranged from 3.70 to 4.83 with a mean of 4.33 ± 0.18 $ml.Kg^{-1}.min^{-1}$.

4. *Dosage Regimen*

The dosage regimen required to maintain the different levels of the therapeutic concentration (C_p^{∞} min = 0.25, 0.50 and 1 $\mu g.ml^{-1}$) in plasma for i.v. route in goats at different selected dosage intervals (γ) of 8 and 12 h are presented in Table 4. For maintaining C_p^{∞} min of 0.25 $\mu g.ml^{-1}$, the loading doses (D*) were calculated to be 1.69 ± 0.11 and 4.01 ± 0.47 mg/kg, while maintenance doses (D_0) were calculated to be 0.62 ± 0.04 and 1.47 ± 0.17 mg/kg at the dosage interval of 8 and 12 h, respectively.

The D*s were calculated to be 3.38 ± 0.23 and 8.03 ± 0.94 mg/kg while D_0s were found to be 1.24 ± 0.09 and 2.95 ± 0.35 mg/kg at γ of 8 and 12 h, respectively, for maintaining C_p^{∞} min of 1 $\mu g.ml^{-1}$, the D*s were calculated to be 6.77 ± 0.46 and 16.06 ± 1.87 mg/kg, while D_0s were found to be 2.49 ± 0.17 and 5.91 ± 0.69 mg/kg at γ of 8 and 12 h, respectively.

Table – 4

Dosage regimen of pefloxacin in healthy female goat following i.v. administration of pefloxacin (5 mg/kg)

C_p^{∞} min (μg/ml)	γ (h)	Dose (mg/kg)	Animal Number					Mean ± S.E.M.
			1	2	3	4	5	
0.25	8	D*	1.62	1.54	1.52	2.14	1.63	1.69 ± 0.11
		D_0	0.60	0.57	0.56	0.79	0.60	0.62 ± 0.04
	12	D*	3.70	3.13	3.84	5.83	3.57	4.01 ± 0.47
		D_0	1.36	1.15	1.41	2.14	1.31	1.47 ± 0.17
0.50	8	D*	3.25	3.07	3.05	4.29	3.26	3.38 ± 0.23
		D_0	1.19	1.13	1.12	1.58	1.20	1.24 ± 0.09
	12	D*	7.40	6.26	7.68	11.65	7.14	8.03 ± 0.94
		D_0	2.72	2.30	2.82	4.29	2.63	2.95 ± 0.35
1	8	D*	6.50	6.15	6.09	8.57	6.52	6.77 ± 0.46
		D_0	2.39	2.26	2.24	3.15	2.40	2.49 ± 0.17
	12	D*	14.81	12.53	15.35	23.30	14.29	16.06 ± 1.87
		D_0	5.45	4.61	5.65	8.57	5.26	5.91 ± 0.69

D* = Priming or loading dose

D_0 = Maintenance dose

γ = Dosage interval

C_p^{∞} min = Minimum therapeutic concentration in plasma (MIC)

TOXICITY STUDY OF PEFLOXACIN :

Toxicity study of pefloxacin was conducted in each of the five healthy female goats after i.v. administration of the drug (10 mg/kg) once daily for 7 consecutive days. The blood samples were collected on 0 (before administration of the drug), 2, 4, 8 and 10 days post drug administration. The following parameters were studied.

(i) Haemoglobin

Different values of haemoglobin (gm/dl) noted on different days (0, 2, 4, 8 and 10 days) after daily i.v. injection of pefloxacin (10 mg/kg) are presented in Table 5 and Fig. 4. The values of haemoglobin in healthy goats before drug administration varied from minimum of 8.00 gm/dl to a maximum of 9.00 gm/dl. The value slightly decreased to 8.48 ± 0.21 gm/dl on day 2 and thereafter slightly increased to 8.52 ± 0.22 and 8.52 ± 0.19 gm/dl on day 4 and 8, respectively. On day 10 the value came back to 8.50 ± 0.20 gm/dl. The data on analysis showed non-significant difference between days post i.v. injection of pefloxacin (Table 5A). This shows that pefloxacin has no effect on haemoglobin.

Table – 5

Haemoglobin content of blood of goat following repeated i.v. administration of pefloxacin (10 mg/kg) for 7 days.

Days	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
Day 0	9.0	8.1	8.0	8.9	8.5	8.50 ± 0.20
Day 2	9.1	8.0	8.1	8.8	8.4	8.48 ± 0.21
Day 4	9.0	8.0	8.0	9.0	8.6	8.52 ± 0.22
Day 8	9.0	8.0	8.2	8.9	8.5	8.52 ± 0.19
Day 10	8.9	8.1	8.0	9.0	8.5	8.50 ± 0.20

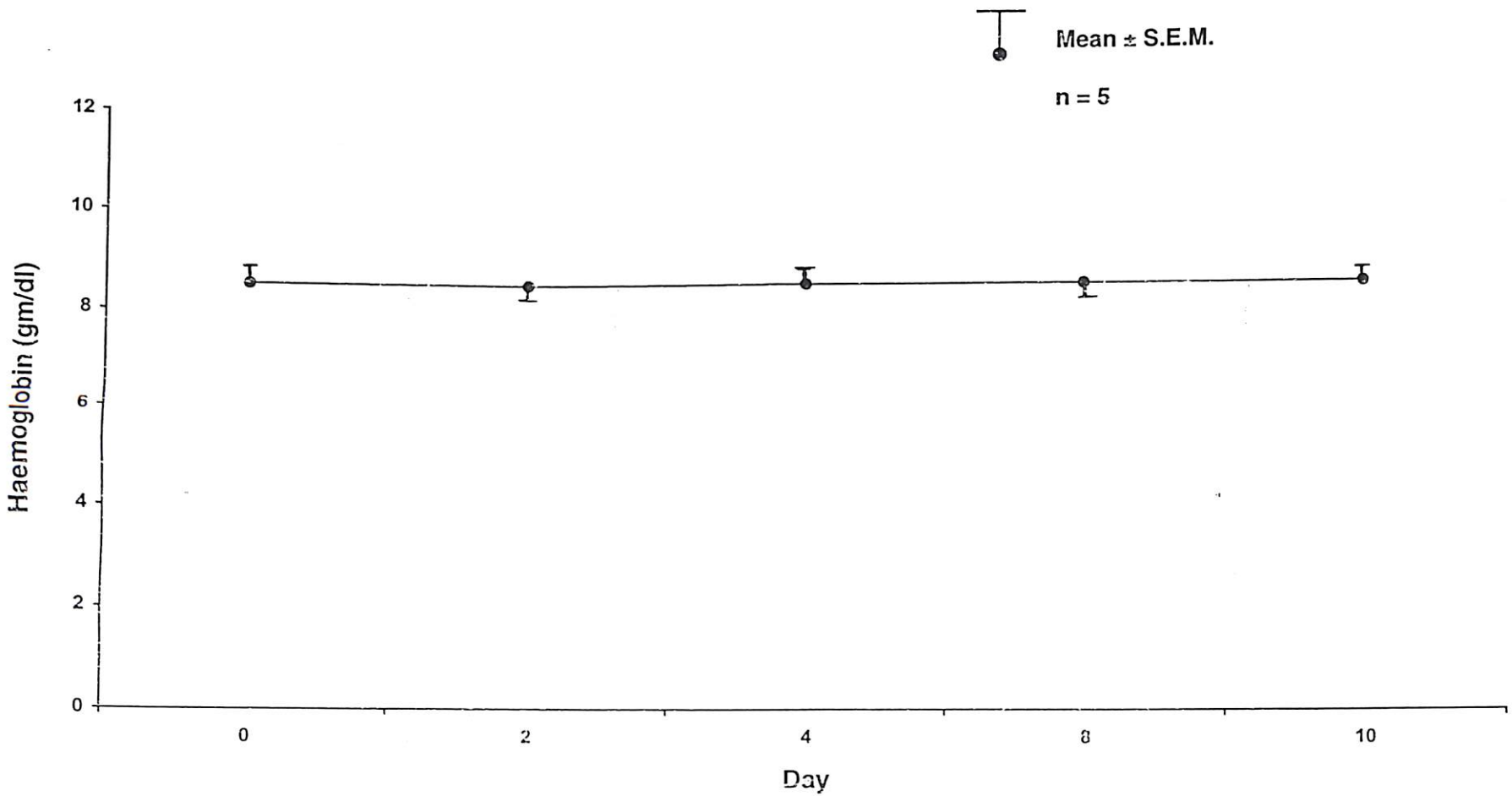
Table – 5A

Analysis of variance showing effect of pefloxacin on haemoglobin

Source of variation	D.F.	C.S.S.	M.S.	F
Between days	4	0.006	0.0015	0.007^{NS}
Error	20	4.264	0.2132	

NS = Non-significant

Fig. 4 : *Showing effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on haemoglobin in healthy female goat.*



(ii) Total Leucocyte Count (TLC)

Effect of Pefloxacin on total leucocyte count on daily i.v. administration at the dose rate of 10 mg/kg for 7 consecutive days is shown in Table 6 and Fig. 5. On day 0, the mean ± S.E.M. of TLC was observed to be 9805 ± 218.00/mm^3 of blood which significantly increased to 10,785 ± 249.45/mm^3 of blood on day 4, 11,325 ± 188.25 on day 8 and 11,300 ± 183.71 on day 10. Total leucocyte count was noted to be increased on successive days (i.e. on day 2, 4 and 8). On statistical analysis, the data showed significant difference between day 0, 2 and 8. Thus, pefloxacin has significant effect on total leucocyte count.

(iii) Differential Leucocyte Count (DLC)

Differential leucocyte count noted on different days (0, 2, 4, 8 and 10 days) after daily i.v. injection of pefloxacin (10 mg/kg) for 7 days are presented in Table 7 and Fig. 6. 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 pefloxacin on different leucocytes viz. neutorphils, lymphocytes, monocytes, eosinophils and basophils.

Table – 6

Total leucocyte count of goat following repeated i.v. administration of pefloxacin (10 mg/kg) for 7 days

Days	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0	10,200	9,225	10,400	9,700	9,500	$9,805^{a} \pm 218.00$
2	10,700	9,675	10,850	10,250	10,025	$10,300^{ab} \pm 215.75$
4	11,350	9,950	11,225	10,800	10,600	$10,785^{bc} \pm 249.45$
8	11,775	10,725	11,650	11,350	11,125	$11,325^{c} \pm 188.25$
10	11,750	10,700	11,600	11,300	11,150	$11,300^{c} \pm 183.71$

Different superscripts denote significant effect of pefloxacin on TLC ($p < 0.01$).

Table – 6A

Analysis of variance showing effect of pefloxacin on total leucocyte count.

Sources of variation	D.F.	C.S.S.	M.S.	F
Between days	4	2757895166	689473791.5	3057.53**
Error	20	4510000	225500	

** $p < 0.01$

Fig. 5 : *Showing effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on total leucocyte count (TLC) in healthy female goat.*

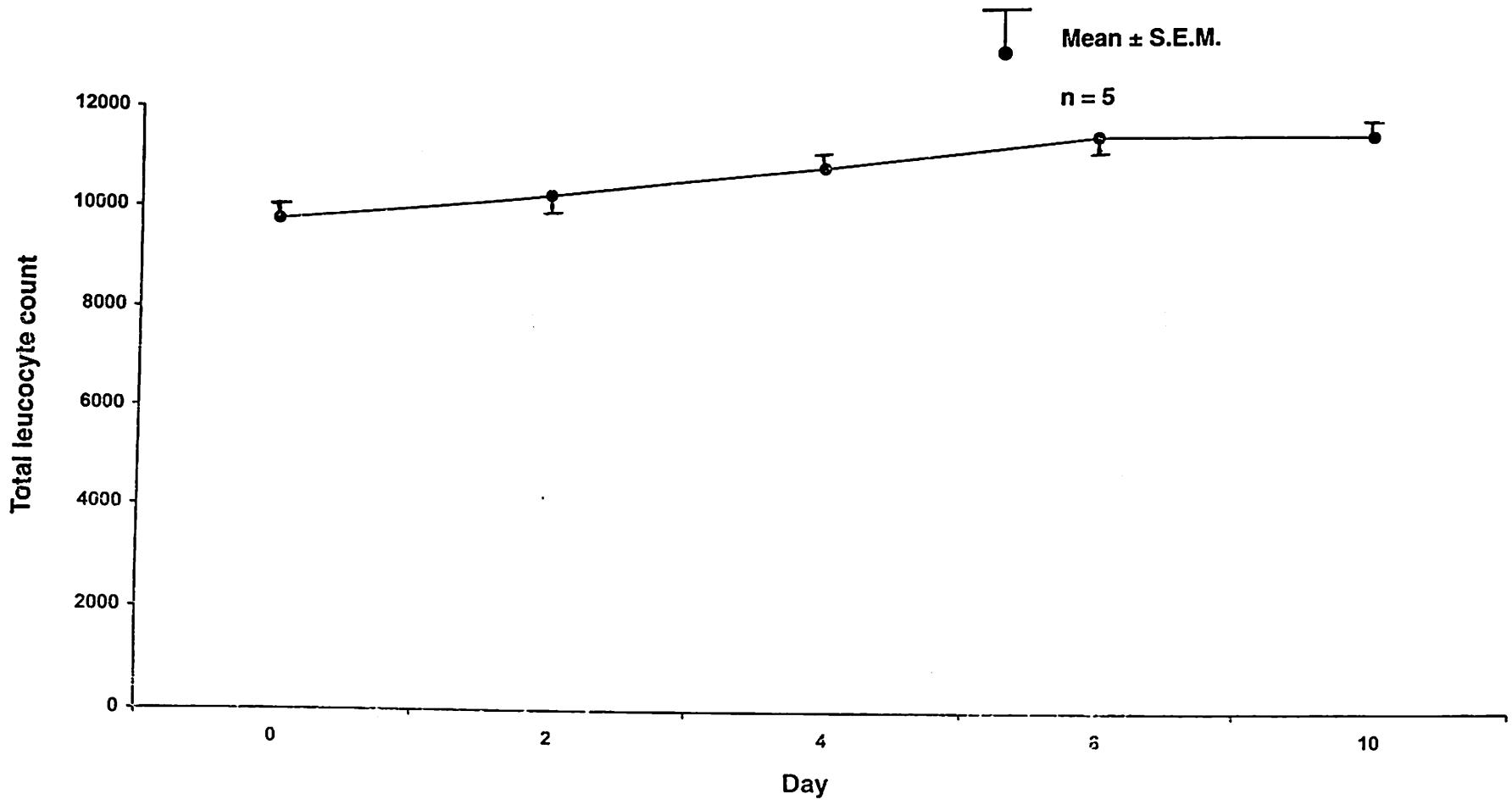


Table – 7

Differential leucocyte count (DLC) of goat following repeated i.v. administration of pefloxacin (10 mg/kg) for 7 days

Animal Number	Days																								
	0					2					4					8					10				
	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	39.8% (39.11)	49.5% (44.71)	7.1% (15.45)	2.7% (9.46)	0.9% (5.44)	39.9% (39.17)	49% (44.43)	6.7% (15.00)	4.8% (12.66)	0% (0)	40 % (39.23)	52% (46.15)	6% (14.18)	2 % (8.13)	0 %(0)	40.2 % (39.35)	52% (46.15)	5 % (12.92)	2.8% (9.63)	0% (0)	38% (38.06)	53% (46.72)	6% (14.18)	2.2% (8.53)	0.85% (5.13)
2	39% (38.65)	53% (46.72)	3% (9.98)	5% (12.92)	0% (0)	38% (38.06)	51% (45.57)	3% (9.98)	8% (16.43)	0% (0)	39.4% (38.88)	52.6% (46.49)	3% (9.98)	5% (12.92)	0% (0)	39% (38.65)	53% (46.72)	2% (8.13)	6% (14.18)	0% (0)	40% (39.23)	51% (45.57)	4% (11.54)	5% (12.92)	0% (0)
3	38% (38.06)	54% (47.29)	4.2% (11.83)	3.1% (10.14)	0.7% (4.8)	39% (38.65)	54.2% (47.41)	3.4% (10.63)	2.8% (9.63)	0.6% (4.48)	40.3% (39.11)	53.7% (47.12)	4.1% (11.68)	1.9% (7.92)	0% (0)	40.1% (39.29)	53.5% (47.01)	3.8% (11.24)	2.6% (9.28)	0% (0)	39.6% (39.00)	55% (47.87)	3.4% (10.63)	2.0% (8.13)	0% (0)
4	41% (39.82)	54% (47.29)	3% (9.98)	2% (8.13)	0% (0)	40.2% (39.35)	53.8% (47.18)	4% (11.54)	2% (8.13)	0% (0)	39.8% (39.11)	53.8% (47.18)	3.6% (10.94)	2.4% (8.91)	0.4% (3.63)	40% (39.23)	53% (46.72)	4% (11.54)	2.5% (9.1)	0.5% (4.05)	41.5% (40.11)	53.6% (47.06)	3.5% (10.78)	1.4% (6.8)	0% (0)
5	40.1% (39.29)	52% (46.15)	5% (12.92)	2.9% (9.81)	0% (0)	40.3% (39.41)	52.7% (46.55)	4% (11.54)	2.9% (9.81)	0% (0)	40.5% (39.52)	53% (46.72)	3.8% (11.24)	2.7% (9.46)	0% (0)	39% (38.65)	54% (47.29)	4.2% (11.83)	2.8% (9.63)	0% (0)	39.7% (39.06)	52% (46.15)	5% (12.92)	3% (9.98)	0.3% (3.14)
Mean ± S.E.M.	38.99 ± 0.30	46.43 ± 0.48	12.03 ± 1.02	10.09 ± 0.79	2.05 ± 1.26	38.93 ± 0.25	46.23 ± 0.55	11.74 ± 0.87	11.33 ± 1.47	0.89 ± 0.89	39.23 ± 0.11	46.73 ± 0.19	11.60 ± 0.70	9.47 ± 0.91	0.73 ± 0.73	39.03 ± 0.16	46.78 ± 0.19	11.13 ± 0.80	10.36 ± 0.96	0.81 ± 0.81	39.09 ± 0.33	46.67 ± 0.39	12.01 ± 0.68	9.27 ± 1.04	1.65 ± 1.06
Geom. mean	39.57 %	52.47%	4.22%	3.0%	0.79% (n=2)	39.47 %	52.10 %	4.05%	3.62%	0.6% (n =1)	40.0%	53.02 %	3.99%	2.62%	0.4% (n =1)	39.66 %	53.10 %	3.64%	3.14%	0.5% (n=1)	39.74 %	52.90 %	4.28%	2.47%	0.497 % (n = 2)

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

The data in parenthesis indicate Arc sin value

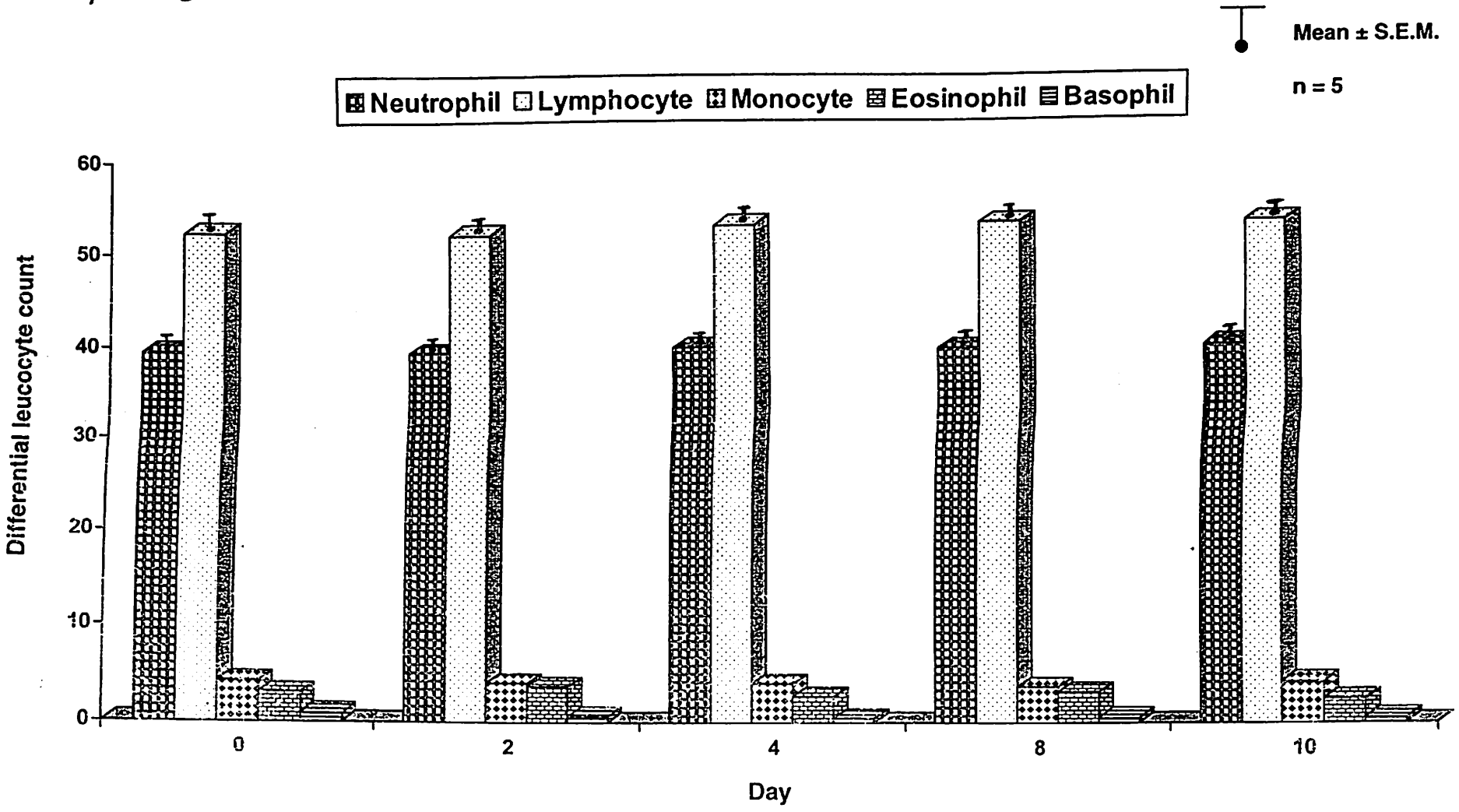
Table - 7A

Analysis of variance showing effect of pefloxacin (10 mg/kg) i.v. daily for 7 days on differential leucocyte count (DLC)

Leucocytes	Sources of variation	D.F.	C.S.S.	M.S.	F
Neutrophil	Between days	4	0.267	0.067	0.225 NS
	Error	20	5.955	0.298	
Lymphocyte	Between days	4	1.082	0.271	0.356 NS
	Error	20	15.214	0.761	
Monocyte	Between days	4	2.698	0.675	0.199 NS
	Error	20	67.873	3.394	
Eosinophil	Between days	4	13.362	3.341	0.596 NS
	Error	20	112.149	5.607	
Basophil	Between days	4	6.981	1.745	0.373 NS
	Error	20	93.594	4.680	

NS = Non-significant

Fig. 6 : *Showing effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on differential leucocyte count (DLC) in healthy female goat.*



BIOCHEMICAL PARAMETERS

(i) Blood Sugar

Mean ± S.E.M. of blood sugar values noted on different days after daily i.v. injection of pefloxacin (10 mg/kg) for 7 consecutive days are presented in Table 8 and Fig. 7. The blood sugar value on day 0 (before injection of pefloxacin) varied from 57.10 to 61.41 mg/dl with a mean of 59.34 ± 0.87 mg/dl. On i.v. administration of pefloxacin, the value slightly decreased on day 2 (59.13 ± 2.17 mg/dl) and subsequently slightly increased on day 4 (63.35 ± 1.42 mg/dl). Again, the values slightly decreased on day 8 (62.20 ± 0.83 mg/dl) while the value slightly increased on day 10 (62.90 ± 0.67 mg/dl). On statistical analysis, these values do not differ significantly from day 0 and between them also. Thus, it seems clear that pefloxacin has no effect on sugar metabolism.

(ii) Blood Urea Nitrogen (BUN)

Table 9 and Fig. 8 present the values of blood urea nitrogen (BUN) before (0 day) and after i.v. injection of pefloxacin (10 mg/kg) in each of the five female goats. On day 0, the mean ± S.E.M. of BUN was observed to be 17.7 ± 0.76 mg/dl which slightly decreased on day 2 (17.40 ± 0.94 mg/dl). Thereafter, the values increased slightly on day 4 (18.11 ± 1.06 mg/dl) and day 8 (18.47 ± 0.99 mg/dl). The

Table – 8

Effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on blood sugar (mg/dl)

Days	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0	59.83	57.10	57.50	60.87	61.41	59.34 ± 0.87
2	54.54	58.40	56.83	58.54	67.33	59.13 ± 2.17
4	62.85	61.25	63.61	68.57	60.47	63.35 ± 1.42
8	63.15	64.00	61.33	63.15	59.36	62.20 ± 0.83
10	61.43	62.30	63.21	65.33	62.25	62.90 ± 0.67

Table - 8A

Analysis of variance showing effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on blood sugar

Sources of variation	D.F.	C.S.S.	M.S.	F
Between days	4	80.49	20.12	2.33^{NS}
Error	20	172.83	8.64	

NS = Non-significant

Fig. 7 : *Showing effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on blood sugar (mg/dl) in healthy female goat.*

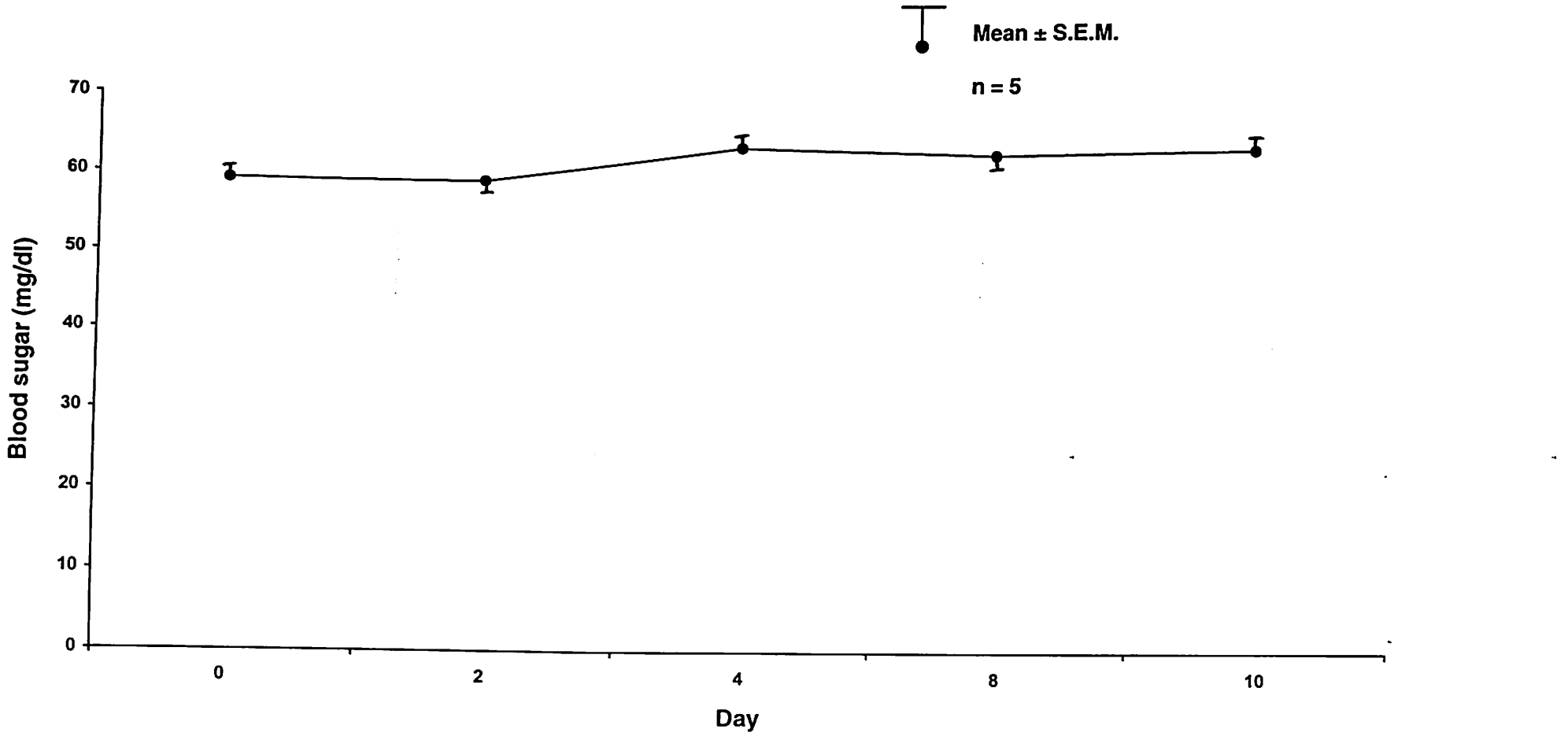


Table – 9

Effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on blood urea nitrogen (mg/dl)

Days	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0	18.93	16.94	17.51	19.92	15.55	17.7 ± 0.76
2	17.53	17.65	17.25	20.32	14.32	17.40 ± 0.94
4	19.64	15.83	18.35	21.07	15.64	18.11 ± 1.06
8	20.50	18.54	17.70	20.44	15.17	18.47 ± 0.99
10	19.33	17.24	16.94	19.47	16.12	17.82 ± 0.67

Table - 9A

Analysis of variance showing effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on blood urea nitrogen.

Sources of variation	D.F.	C.S.S.	M.S.	F
Between days	4	3.13	0.78	0.19^{NS}
Error	20	80.72	4.04	

NS = Non-significant

Fig. 8 : *Showing effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on blood urea nitrogen (mg/dl) in healthy female goat.*

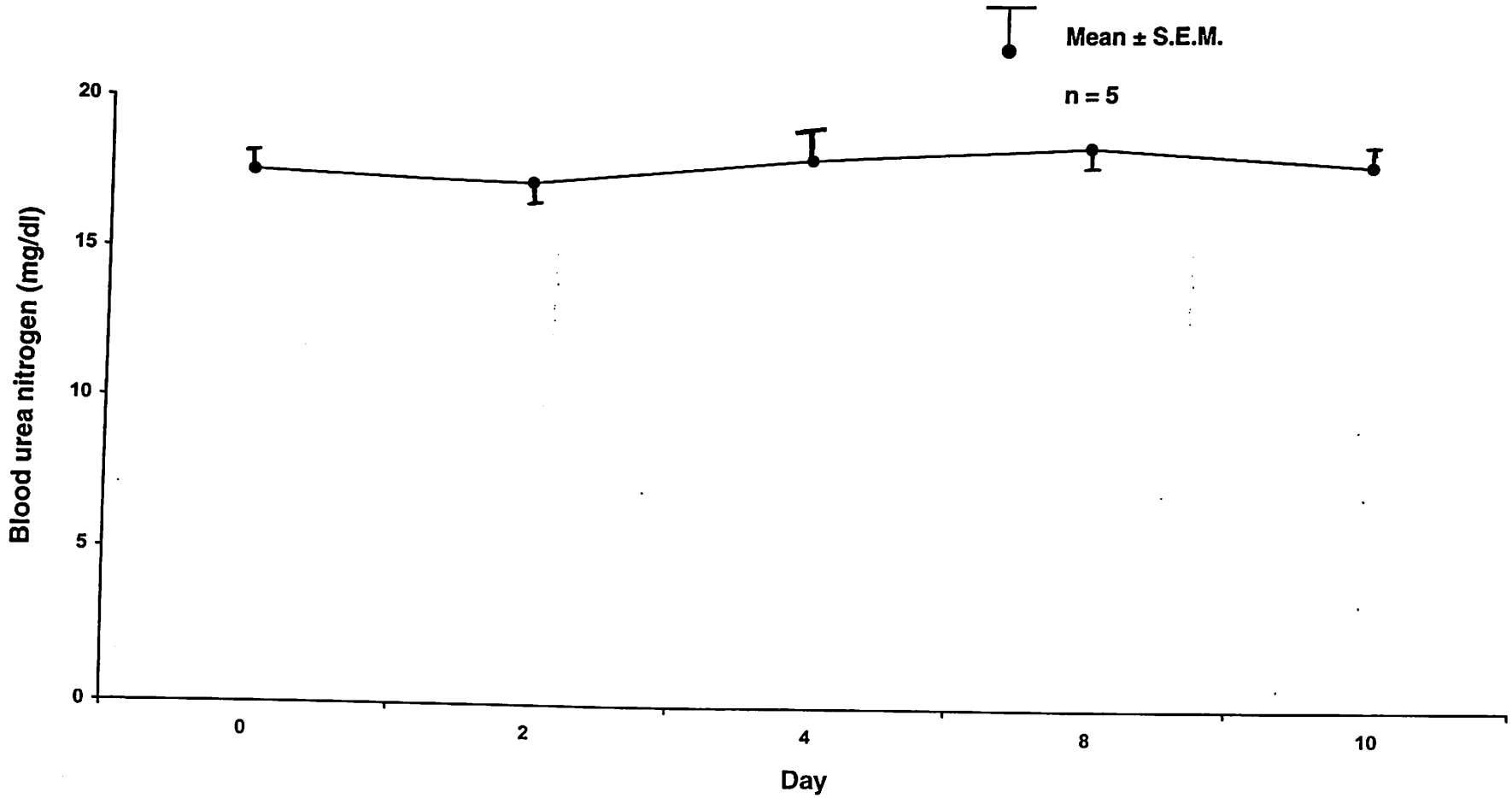
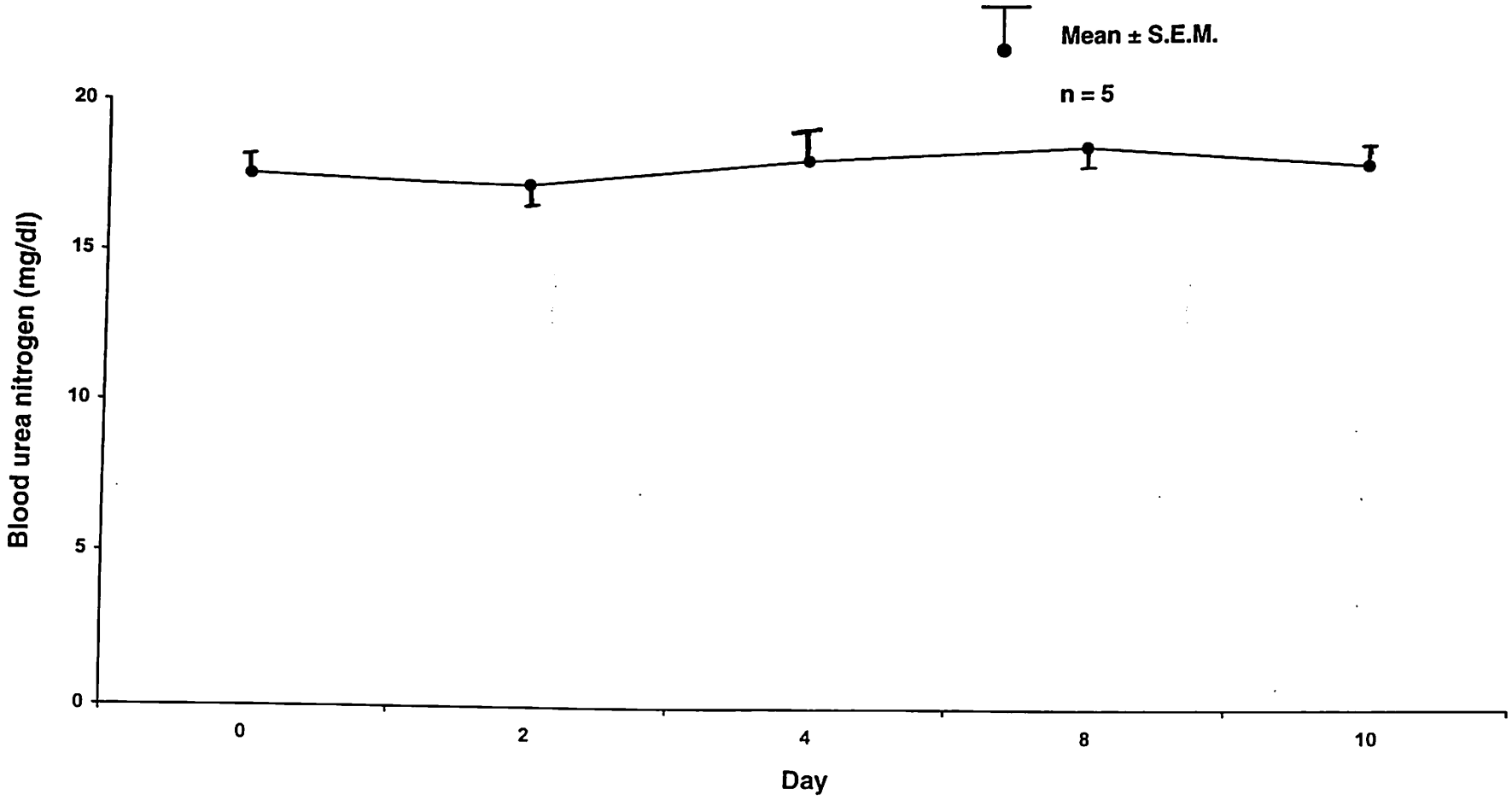


Fig. 8 : *Showing effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on blood urea nitrogen (mg/dl) in healthy female goat.*



value again slightly decreased on day 10 (17.82 ± 0.67 mg/dl). On statistical analysis as shown in Table 9A, the values are noted to differ non-significantly. This shows that pefloxacin has no effect on BUN.

(iii) Blood Cholesterol

Table 10 and Fig. 9 present the values of blood cholesterol before (day 0) and after daily i.v. injection of pefloxacin (10 mg/kg) in each of the five female goats. On day 0, the value of blood cholesterol ranged from 74.62 to 90.90 mg/dl with a mean of 81.92 ± 2.86 mg/dl. The value slightly decreased on day 2 (7.26 ± 2.51 mg/dl) and then slightly increased on day 4 (78.03 ± 4.50 mg/dl) and day 8 (80.01 ± 4.44 mg/dl). On day 10, the value again decreased to 79.44 ± 3.76 mg/dl. On statistical analysis, these values did not differ significantly from day 0 and among days also (Table 10A). Thus it is evident that pefloxacin has no effect on blood cholesterol.

(iv) Total Blood Protein and Albumin

Table 11 and Fig. 10 present the values of total protein before (day 0) and after i.v. injection of pefloxacin (10 mg/kg) in each of five female goats. On day 0, mean ± S.E.M. value of total protein was observed to be 7.22 ± 0.08 gm/dl. The value slightly decreased on day 2 (7.19 ± 0.06 gm/dl), day 4 (7.16 ± 0.06 gm/dl) and

Table - 10

Effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on blood cholesterol (mg/dl)

Days	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0	85.00	77.42	90.90	81.66	74.62	81.92 ± 2.86
2	74.54	70.18	85.45	78.57	77.55	77.26 ± 2.51
4	90.90	66.25	83.47	79.78	69.77	78.03 ± 4.50
8	80.86	63.41	91.36	84.66	79.56	80.01 ± 4.44
10	71.42	73.43	92.56	82.10	77.67	79.44 ± 3.76

Table - 10A

Analysis of variance showing effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on blood cholesterol.

Sources of variation	D.F.	C.S.S.	M.S.	F
Between days	4	65.77	16.44	0.24^{NS}
Error	20	1373.14	68.66	

NS = Non-significant

Fig. 9 : *Showing effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on serum cholesterol (mg/dl) in healthy female goat.*

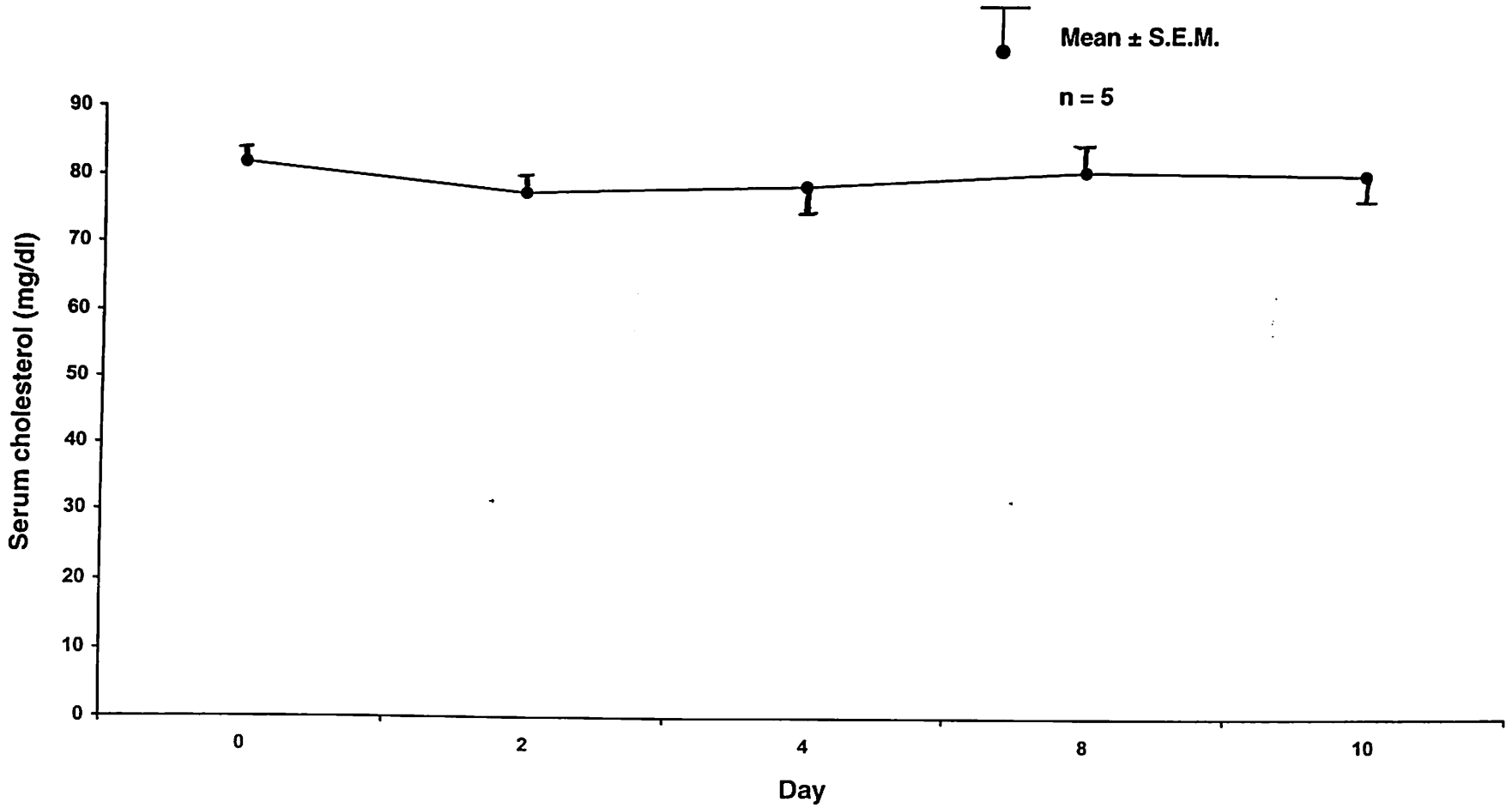


Table - 11

Effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on total protein, albumin, globulin and A/G ratio.

Animal Number	Total Protein (g/dl)					Albumin (g/dl)					Globulin (g/dl)					A/G ratio				
	Days					Days					Days					Days				
	0	2	4	8	10	0	2	4	8	10	0	2	4	8	10	0	2	4	8	10
1	7.22	7.24	7.26	7.22	7.30	2.33	2.29	2.55	2.41	2.37	4.39	4.95	4.71	4.85	4.93	0.476 (43.62)	0.467 (42.88)	0.541 (47.35)	0.500 (45.00)	0.481 (43.91)
2	7.25	7.17	7.02	7.00	7.07	2.50	2.44	2.35	2.30	2.38	4.75	4.73	4.67	4.70	4.69	0.526 (46.49)	0.516 (45.92)	0.503 (45.17)	0.489 (44.37)	0.507 (45.40)
3	7.11	7.03	7.04	7.14	7.15	2.28	2.23	2.25	2.31	2.32	4.83	4.80	4.79	4.83	4.83	0.472 (43.36)	0.465 (42.99)	0.470 (43.28)	0.478 (43.74)	0.480 (43.85)
4	7.48	7.36	7.32	7.30	7.41	2.90	2.72	2.68	2.64	2.80	4.58	4.64	4.64	4.66	4.61	0.633 (52.71)	0.586 (49.95)	0.578 (49.49)	0.567 (48.85)	0.607 (51.18)
5	7.04	7.13	7.18	7.16	7.23	2.14	2.22	2.18	2.16	2.19	4.90	4.91	5.00	5.00	5.04	0.436 (41.32)	0.452 (42.25)	0.436 (41.32)	0.430 (40.98)	0.435 (41.27)
Mean ± S.E.M.	7.22 ± 0.08	7.19±0 .06	7.16±0 .05	7.16±0 .06	7.23±0 .13	2.43±0 .09	2.38±0 .08	2.40±0 .10	2.36±0 .06	2.41±0 .06	4.79±0 .06	4.81±0 .06	4.76±0 .06	4.81±0 .06	4.82±0 .08	45.51± 1.98	44.80± 1.44	45.32± 1.44	44.59± 1.27	45.12± 1.65
Geometric Mean																0.504	0.494	0.503	0.491	0.499

Note – The data in parenthesis denote Arc sin value

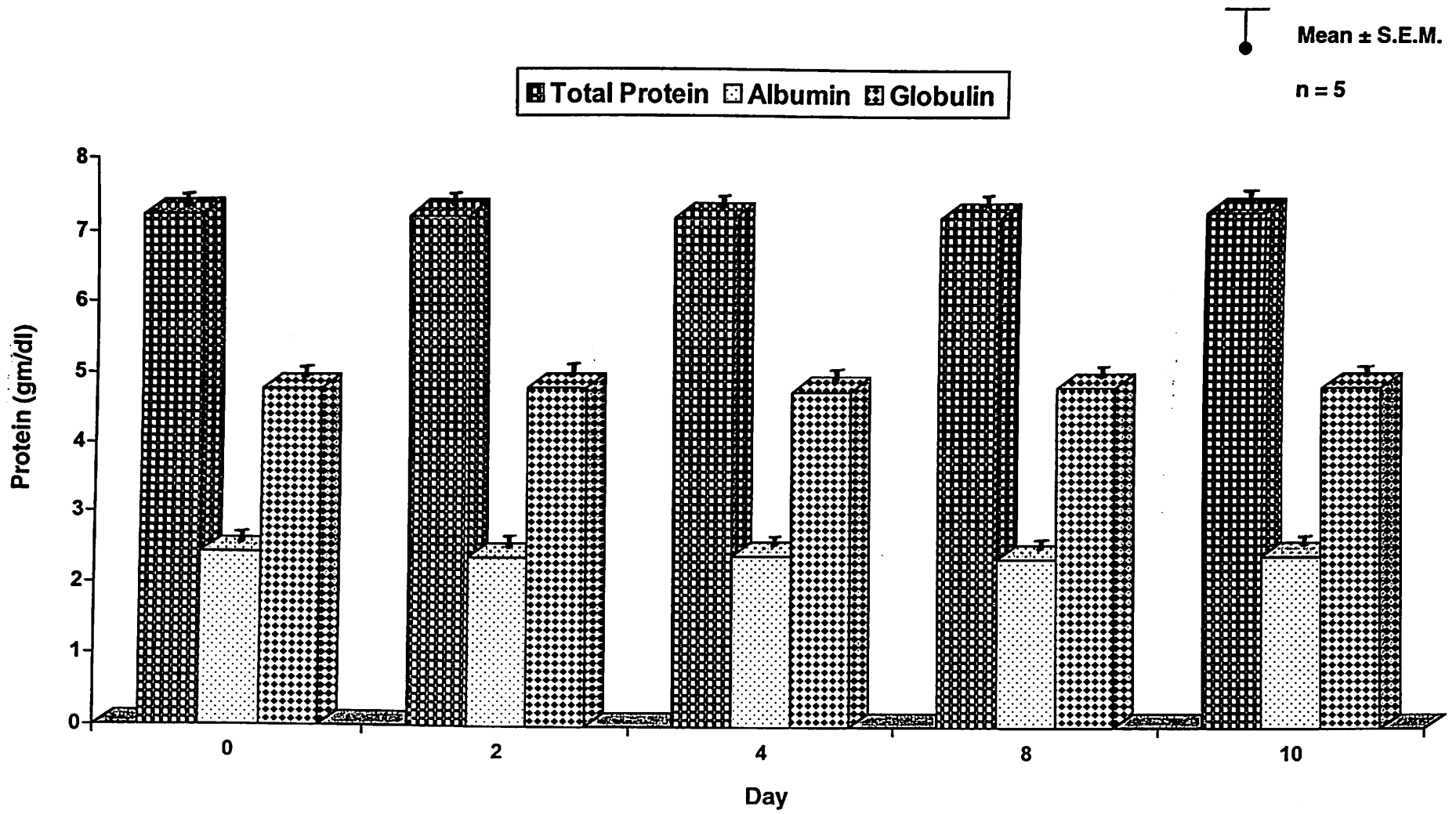
Table - 11A

Analysis of variance showing effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on total protein, albumin, globulin and A/G ratio

Parameters	Sources of variation	D.F.	C.S.S.	M.S.	F
Total protein (g/dl)	Between days	4	0.02	0.005	0.275^{NS}
	Error	20	0.36	0.018	
Albumin (g/dl)	Between days	4	0.01	0.003	0.066 ^{NS}
	Error	20	1.03	0.052	
Globulin (g/dl)	Between days	4	0.01	0.003	0.120 ^{NS}
	Error	20	0.41	0.021	
A/G ratio	Between days	4	2.81	0.703	0.057 ^{NS}
	Error	20	248.18	12.409	

NS = Non-significant

Fig. 10 : *Showing effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on protein in healthy female goat.*



day 8 (7.16 ± 0.05 gm/dl). On day 10, the value increased to 7.23 ± 0.06 gm/dl. On statistical analysis (Table 11A), the data shows no significant difference between days. Thus, it seems clear that pefloxacin has no effect on total blood protein.

Mean ± S.E.M. values of albumin noted on different days after i.v. injection of pefloxacin (10 mg/kg) for 7 consecutive days are presented in Table 11 and Fig. 10. On day 0, the value of albumin was observed to be 2.43 ± 0.13 gm/dl. The values on day 2, 4, 8 and 10 were found to be 2.38 ± 0.09, 2.40 ± 0.09, 2.36 ± 0.08 and 2.41 ± 0.10 gm/dl. On statistical analysis (Table 11A) the data shows non-significant difference between days indicating that pefloxacin has not any effect on albumin.

Mean ± S.E.M. values of globulin noted on different days after daily i.v. injection of pefloxacin (10 mg/kg) for 7 days are presented in Table 11 and Fig. 10. On day 0, the mean ± S.E.M. value of globulin was observed to be 4.79 ± 0.06 gm/dl, which slightly increased on day 2 (4.81 ± 0.06 gm/dl) while slightly decreased on day 4 (4.76 ± 0.06 gm/dl). On day 8 and day 10, the value again slightly increased upto 4.81 ± 0.06 and 4.82 ± 0.08 gm/dl, respectively. On statistical analysis the data showed non significant difference between days indicating that pefloxacin has no effect on serum globulin level.

Mean ± S.E.M. values of albumin globulin ratio (A/G ratio) noted on different days after daily i.v. injection of pefloxacin (10 mg/kg) for 7 days are presented in Table 11 and Figure 10. In parenthesis, the original values that have been converted to Arc sin values are shown for proper statistical analysis. Geometric mean of A/G ratio on day 0, 2, 4, 8 and 10 were 0.504, 0.494, 0.503, 0.191 and 0.499 respectively. The data on different days (Table 11A) do differ significantly indicating that pefloxacin has no any effect on albumin globulin ratio.

(v) Serum Glutamate Pyruvate Transaminase (SGPT) Alanine Transaminase (ALT) :

Table 12 and Fig. 11 present the values of SGPT before (day 0) and after i.v. injection of pefloxacin (10 mg/kg) for 7 consecutive days in each of the five female goats. On day 0, mean ± S.E.M. value of SGPT was observed to be 11.80 ± 0.56 IU/L which significantly increased to 15.40 ± 0.66 IU/L on day 2. On day 4 and 8 also, the values significantly increased to 19.6 ± 0.76 and 26.8 ± 0.66 IU/L, respectively. On statistical analysis (Table 12A) the data showed significant difference between days. Only, on day 10 (25.6 ± 0.43 IU/L) SGPT level was found to be decreased and the difference between day 8 and 10 was non-significant. The data indicates that pefloxacin has some effect on SGPT.

Table – 12

Effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on serum glutamate pyruvate transaminase (SGPT) (IU/L)

Days	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0	13.5	12.0	11.5	10.0	12.0	$11.80^{a} \pm 0.56$
2	17.0	15.5	16.0	13.0	15.5	$15.4^{b} \pm 0.66$
4	20.5	18.0	21.0	17.5	21.0	$19.6^{c} \pm 0.76$
8	27.0	24.5	28.5	26.5	27.5	$26.8^{d} \pm 0.66$
10	26.0	24.0	26.5	25.5	26.0	$25.6^{d} \pm 0.43$

Different superscripts differ significantly at $p < 0.01$.

Table - 12A

Analysis of variance showing effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on SGPT.

Sources of variation	D.F.	C.S.S.	M.S.	F
Between days	4	830.16	207.54	105.9**
Error	20	39.20	1.96	

** $p < 0.01$

Fig. 11 : *Showing effect of pefloxacfin (10 mg/kg i.v. daily for 7 days) on serum glutamate pyruvate transaminase (SGPT) in IU/L in healthy female goat.*

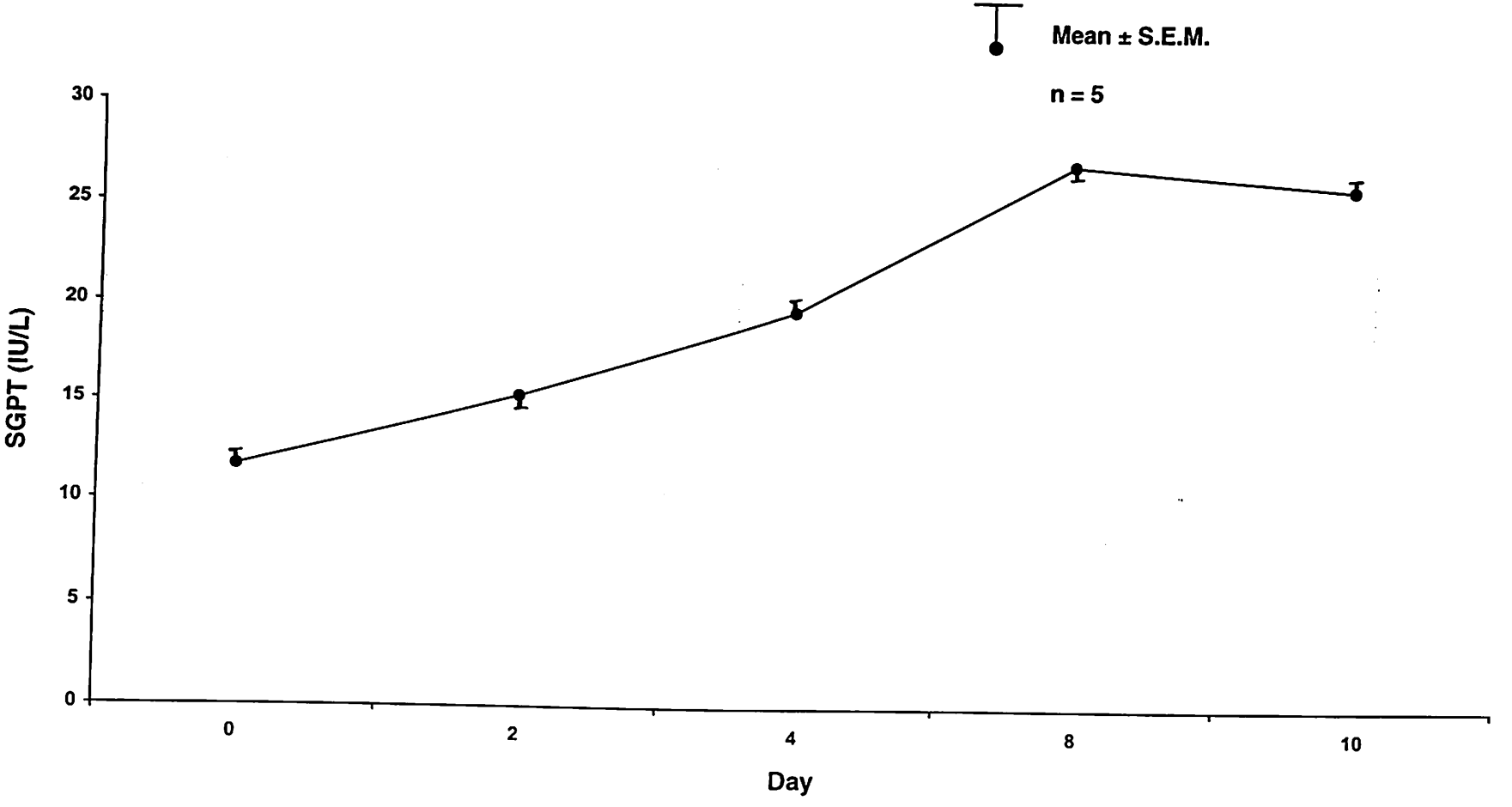


Table – 13

Effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on serum glutamate oxaloacetate transaminase (SGOT) (IU/L)

Days	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0	56.0	62.5	71.5	75.0	69.0	$66.80^{a} \pm 3.39$
2	73.5	79.0	90.0	94.5	87.5	$84.90^{b} \pm 3.80$
4	100.5	106.5	117.5	120.0	116.0	$112.10^{c} \pm 3.69$
8	139.0	145.0	152.0	162.5	153.5	$150.40^{d} \pm 3.99$
10	136.5	139.0	148.5	158.0	150 0	$146.4^{d} \pm 3.90$

Different superscripts differ significantly at $p < 0.01$.

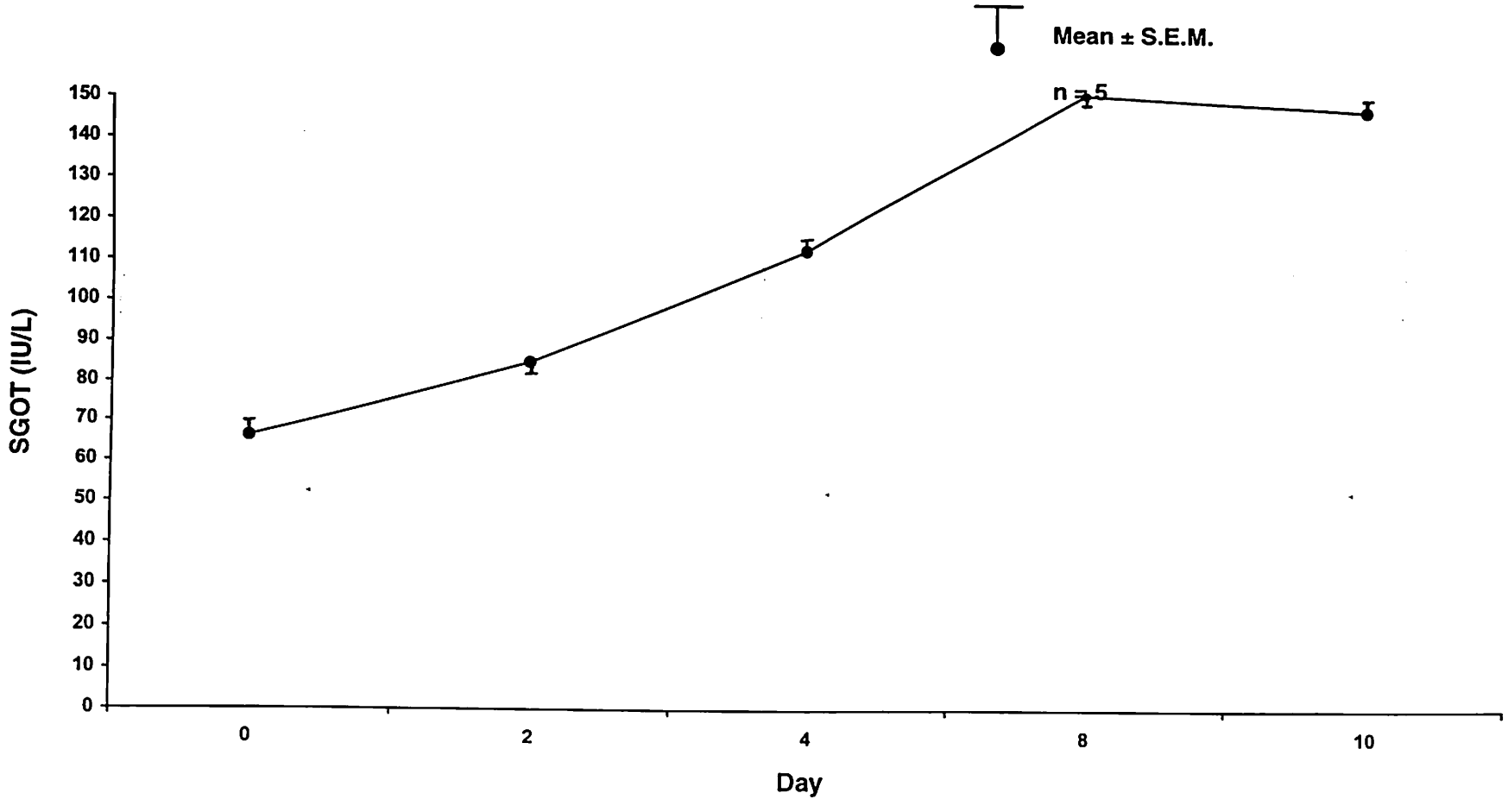
Table - 13A

Analysis of variance showing effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on SGOT.

Sources of variation	D.F.	C.S.S.	M.S.	F
Between days	4	2717654	6794.14	96.08**
Error	20	1414.1	70.71	

** $p < 0.01$

Fig. 12 : *Showing effect of pefloxacin (10 mg/kg i.v. daily for 7 days) on serum glutamate oxaloacetate transaminase (SGOT) in IU/L in healthy female goat.*



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

Table 13 and fig. 12 present the SGOT values before (0 day) and after i.v. injection of pefloxacin (10 mg/kg) for 7 consecutive days in each of the five female goats. On day 0, the mean ± S.E.M. value of SGOT was observed to be 66.80 ± 3.39 IU/L. The values on day 2, 4 and 8 were subsequently found to increase significantly i.e. 84.90 ± 3.80 IU/L on day 2, 112.10 ± 3.69 IU/L on day 4 and 150.40 ± 3.99 IU/L on day 8. On day 10, the value slightly decreased to 146.4 ± 3.90 IU/L, but the difference was non-significant. On statistical analysis of data (Table 13 A) it is observed that pefloxacin has marked effect on SGOT level.

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

Table 13 and fig. 12 present the SGOT values before (0 day) and after i.v. injection of pefloxacin (10 mg/kg) for 7 consecutive days in each of the five female goats. On day 0, the mean ± S.E.M. value of SGOT was observed to be 66.80 ± 3.39 IU/L. The values on day 2, 4 and 8 were subsequently found to increase significantly i.e. 84.90 ± 3.80 IU/L on day 2, 112.10 ± 3.69 IU/L on day 4 and 150.40 ± 3.99 IU/L on day 8. On day 10, the value slightly decreased to 146.4 ± 3.90 IU/L, but the difference was non-significant. On statistical analysis of data (Table 13 A) it is observed that pefloxacin has marked effect on SGOT level.

Chapter - 5

Discussion

DISCUSSION

Pefloxacin, a recent fluoroquinolone being widely used in human is also becoming popular in veterinary practice as well, due to its better absorption after oral administration, good penetration into tissues (Contrepois *et al.*, 1984), longer half-life of 6.2 to 12.4 h in man (Bressole *et al.*, 1994), wider distribution in tissues as well as biological fluids, increased antibacterial activity and little toxicity.

Since the drug is required to be administered for a longer period of time approximately a week or more, it is essential to study the adverse effects of the drug on its prolonged administration so that therapeutic effect of the drug in comparison to its adverse effects could be assessed. Though, few kinetic studies were carried out in animals, no literature is available on toxicity study in goats. Keeping this fact in view, the pharmacokinetic and toxicity study of pefloxacin were carried out in goats.

PHARMACOKINETIC STUDY OF PEFLOXACIN :

(A) Distribution in Biological Fluids

Pefloxacin was present at 0.042 h with a mean plasma concentration of 17.41 ± 1.34 $\mu g.ml^{-1}$. The drug continuously declined with time and was traced upto 10 h in all the animals on its post i.v.

administration @ 5 mg/kg. In urine, at therapeutic concentration the drug was detectable at 0.042 h. Mean peak urine concentration of 48.13 ± 1.50 $\mu g.ml^{-1}$ was detected at 0.75 h. Thereafter, the drug declined with time and was detectable in all animals upto 30 h with a mean of 0.87 ± 0.07 $\mu g.ml^{-1}$ (Table 2).

The mean therapeutic concentration (≥ 0.25 $\mu g.ml^{-1}$) was maintained from 0.042 to 10 h in plasma and from 0.042 to 30 h in urine. Roy *et al.* (1997) observed that therapeutic concentration of pefloxacin in plasma was maintained upto 6 h after i.v. administration of the drug (5mg/kg) in goat. Ansari *et al.* (2000) reported that the mean therapeutic concentration of pefloxacin in goat (≥ 0.12 $\mu g.ml^{-1}$) post i.v. administration (4 mg/kg) was maintained upto 8, 8 and 30 h in plasma, milk and urine, respectively; whereas Malik *et al.* (2000) obtained pefloxacin concentration ≥ 0.25 $\mu g.ml^{-1}$ in plasma upto 6 and 10 h after i.v. (10 mg/kg) or oral (20 mg/kg) administration of drug in goat, respectively.

(B) Kinetic Parameters

In the present study, the high mean value of 12.21 ± 2.86 $\mu g.ml^{-1}$ for zero time concentration during distribution phase (A) while low mean value of 3.46 ± 0.32 $\mu g.ml^{-1}$ for zero time

concentration during elimination phase (B) and mean value of 15.67 ± 3.03 $\mu g.ml^{-1}$ for theoretical zero time concentration (C_p^0 = A + B) were obtained after single i.v. administration of pefloxacin (5 mg/kg). Distribution rate constant (α) of 3.897 ± 0.880 h^{-1} and distribution half-life ($t_{1/2}\alpha$) of 0.22 ± 0.05 h were noted in goat in the present study. Lower $t_{1/2}$ α of 0.10 ± 0.05 h in man (Barre *et al.*, 1984), 0.1536 ± 0.0036 h in cow (Patil *et al.*, 1996) and 0.147 ± 0.010 h in goat (Jare, 1996) were recorded. However, $t_{1/2}\alpha$ of 0.55 h in chicken (Chousalkar, 1995) was found to be higher than that of goat. The mean elimination half-life ($t_{1/2}$ β) of 3.61 ± 0.46 h were noted in the present study (Table 3). More or less similar $t_{1/2}$ β values of 3.30 and 3.21 h in rat and dog, respectively (Montay *et al.*, 1984), 3.25 and 4.26 h in goat after oral and i.v. administration, respectively (Jare 1996) were recorded. However, lower $t_{1/2}\beta$ values of 1.9 h in mouse (Montay *et al.*, 1984), 2.8 h in rat (Dworkin *et al.*, 1990), 2.01 h in albino rat (Cochereau *et al.*, 1991), 2.54 ± 0.10 h in cow (Patil *et al.*, 1994), 2.2 h in chicken (Chousalkar, 1995) and 2.97 h in dog (Jayakumar *et al.*, 1995) were observed. However, higher $t_{1/2}$ β of 7.11 h in monkey (Montay *et al.*, 1984) and 7.2 to 13 h in man (Barre *et al.*, 1984; Danan *et al.*, 1985; Cardey *et al.*, 1987; Sharma *et al.*, 1994) were recorded. The difference in observed half life value in the present study as

compared to other species may be due to differences in biotransformation and excretion pathways between species. Pefloxacin is metabolised to norfloxacin to a greater extent and also undergoes phase – II metabolism.

The mean values of 2.306 ± 0.682 and 1.014 ± 0.123 h^{-1} were noted in the present study for the rate constant of drug transfer from central to peripheral (K_{12}) and peripheral to central (K_{21}) compartment, respectively (Table 3). The mean values of 0.0643 ± 0.0015 and 0.0226 ± 0.0008 min^{-1} and 3.216 ± 0.343 and 1.079 ± 0.014 min^{-1} were observed for K_{12} and K_{21}, respectively in cow (Patil *et al.*, 1996) and goat (Jare, 1996). However, higher mean values of 7.35 ± 0.54 and 1.149 ± 0.094 h^{-1} for K_{12} and K_{21}, respectively were reported in man (Bare *et al.*, 1984). The mean value of 0.708 ± 0.052 h^{-1} for the rate constant of elimination of drug from central compartment (Kel) was calculated for goat in the present study (Table 3). Kel value of 0.9075 ± 0.0070 min^{-1} in cow (Patil *et al.*, 1996) and 0.768 ± 0.040 min^{-1} in goat (Jare, 1996) were estimated.

The volume of distribution based on total area under curve (Vd_{area}) was calculated to be 1.24 ± 0.09 L. Kg^{-1} in goat in the present investigation. More or less similar volume of distribution of 1.48 and 2.21 $L.Kg^{-1}$ in dog and monkey, respectively (Montay *et al.*, 1984), respectively, 1.84 $L.Kg^{-1}$ in dog (Jayakumar *et al.*, 1995), 2.10

$L.Kg^{-1}$ in chicken (Chousalkar, 1995) and 1.7 to 1.9 $L.Kg^{-1}$ in man (Danan *et al.*, 1985; Frydman *et al.*, 1986; Cardey *et al.*, 1987; Hoffler *et al.*, 1988; Sultan *et al.*, 1988) wee estimated. However, lower Vd_{area} of 0.685 ± 0.038 $L.Kg^{-1}$ in cow (Patil *et al.*, 1996) and very high Vd_{area} values of 4.25 and 15.57 $L.Kg^{-1}$ in rat and mouse, respectively (Montay *et al.*, 1984) and 7.35 $L.Kg^{-1}$ in goat (Jare, 1996) were observed. A high value of Vd_{area} obtained in the present study may be attributed to wide distribution of pefloxacin in the body because of its amphoteric nature (Vancutsem *et al.*, *loc. cit.*) and high lipophilicity (Prescott and Baggot, 1994; Cochereau *et al.*, 1991). This statement is further supported by higher approximate tissue to plasma concentration ratio (T ≈ P) value of 2.77 ± 0.55 observed in the present study (Table 3).

In the present study, total body clearance (Cl_B) value of 4.33 ± 0.18 $ml.Kg^{-1}.min^{-1}$ was noted. More or less similar Cl_B values of 4.349 (Jare, 1996) and 5.07 ± 1.15 higher Cl_B values of 9.33 and 14.87 $ml.Kg^{-1}.\ min^{-1}$ in mouse and rat, respectively (Montay *et al.*, 1984), 9.7 $ml.Kg^{-1}.\ min^{-1}$ in dog (Jayakumar *et al.*, 1995) and 25.2 $ml.Kg^{-1}.min^{-1}$ in chicken (Chousalkar, 1995) were reported. On the other hand, lower Cl_B value of 3.62 $ml.Kg^{-1}.min^{-1}$ in cow (Patil *et al.*, 1996) was recorded.

(C) Dosage Regimen

The main purpose of conducting kinetic study is to compute rational dosage regimen for treating various diseased states. For treating mild systemic infections or highly susceptible microbes [C_p^{∞} min (MIC) = 0.25 μg/ml], loading dose (D*) and maintenance dose (D_0) of 8.93 ± 0.94 and 2.95 ± 0.35 mg/kg at dosage interval (γ) of 12 h can be effectively used. Similarly, for treating moderate systemic infections or moderately susceptible microbes [C_p^{∞} min (MIC) = 0.50 μg/ml], D* and D_0 of 8.03 ± 0.94 and 2.95 ± 0.35 mg/kg at γ of 12 h can be successfully used, but against severe systemic infections or less susceptible microbes [C_p^{∞} min (MIC) = 1 μg/ml], D* and D_0 of 6.77 ± 0.46 and 2.49 ± 0.17 mg/kg at shorter γ of 8 h can be effectively used.

The drug maintains its therapeutic concentration of ≥ 0.25 $\mu g.ml^{-1}$ for more than 30 h when given @ 5 mg/kg i.v. (Table 2) the drug can be administered daily @ 5 mg/kg parenterally or even 30 hourly against urinary tract infections in goats caused by drug sensitive organisms.

TOXICITY STUDY OF PEFLOXACIN

(A) Haematological Parameters

In the present study, the effect of pefloxacin on haematological parameters namely haemoglobin, total leucocyte

count (TLC) and differential leucocyte count (DLC) after its i.v. administration (10 mg/kg) daily for 7 consecutive days were estimated. The study revealed that there is no significant change in haemoglobin (gm/dl) and the value remained around 8.50 gm/dl during the study. Similarly, there was no significant effect on differential leucocyte count from 0 to 10 days. In contrast, the DLC significantly increased from day 0 (9.805 ± 218.00) to 10,300 ± 215.78 (day 2), 10.785 ± 249.45 (day 4), 11.325 ± 128.25 (day 8) and 11,300 ± 183.71 (day 10) (Table 6). There is marked significant increase in the trend. But, the DLC values remained within the normal range of 8000 – 12000/ mm^3 of blood. The results were in concurrence with the findings of Ashish Sachan *et al.* (2000) who reported that there was no significant change in any of the haematological parameters when pefloxacin was administered to broiler chicken at different doses of 5, 10 and 40 mg/kg orally. The results also confirmed the findings of Gary (1995) who reported no untoward effect on haematological parameters on patients receiving a fluoroquinolone pefloxacin. The results were also in accordance with the findings of Pallavicini *et al.* (1989) who reported that pefloxacin in concentrations of 0.5 to 50 mg/ml did not induce inhibition of myelopoiesis.

(B) Biochemical Parameters

The present study reveals that the value of blood sugar noted before the injection of pefloxacin (day 0) was found to be 59.34 ± 0.87 mg/dl which differed non-significantly on day 2 (59.13 ± 2.17 mg/dl), day 4 (63.35 ± 1.42 mg/dl), day 8 (62.20 ± 0.83 mg/dl) and day 10 (62.90 ± 0.67 mg/dl). This clearly indicates that pefloxacin has no effect on blood sugar metabolism. Similarly pefloxacin was found to have no significant effect on blood urea nitrogen (BUN) and serum cholesterol and values on different days differed non-significantly from the value noted before the injection (day 0). This clearly indicates that pefloxacin has no effect on cholesterol metabolism. It can also be concluded that drug is safe in goats with regard to urinary system.

Pefloxacin was found to have non-significant effect on total protein, albumin, globulin and A/G ratio and the values of total protein, albumin, globulin and A/G ratio remained around normal on different days during the study. It can be concluded that Pefloxacin has no effect on protein metabolism.

The values of serum glutamate pyruvate transaminase (SGPT) before injection of pefloxacin (0 day) was noted to be 11.80 ± 0.56 IU/L which increased significantly on day 2 (26.8 ± 0.66 IU/L),

day 4 (19.6 ± 0.76 IU/L), and day 8 (26.8 ± 0.66 IU/L). This shows that pefloxacin has some hepatotoxic effect, but the effect is very little as the increase in SGPT values on different days are not so pronounced. Similar trend was noted with serum glutamate oxaloacetate transaminase (SGOT) (Table 13). The value of SGOT was noted to be 66.80 ± 3.39 IU/L on day 0 which increased significantly on day 2 (84.90 ± 3.90 IU/L), day 4 (112.10 ± 3.69 IU/L) and day 8 (150.40 ± 3.99 IU/L). This indicates that pefloxacin has some effect on SGOT level. It can be concluded that increased levels of SGOT may be due to some muscle damaging effects of pefloxacin like other fluoroquinolones. Since the increase in SGOT level is not very pronounced, it can be evaluated as a relatively safer drug. Ashish Sachan *et al.* (2002) evaluated that pefloxacin when administered orally @ 5, 10 and 20 mg/kg for 5 days, the levels of SGPT, SGOT, BUN and total bilirubin did not vary significantly in any of the treated groups from the control and it was concluded that pefloxacin was safe in day old chicks without causing hepatotoxicity or nephrotoxicity. Ashish Sachan *et al.*, (2000) calculated LD_{50} value of pefloxacin to 1025 mg/kg orally in broiler chicken, and it was concluded that pefloxacin had a wide margin of safety in broiler. Sridevi *et al.*, (2002) concluded that pefloxacin at the rate of 15 and

20 mg/kg b.wt. induced observable toxic symptoms in pups on long term (4 weeks) use while 10 mg/kg b.wt. was comparatively safer. However chondrotoxicity, athropathy, muscle damage etc. are reported on prolonged administration of pefloxacin by several authors, the drug is considered to be a safer one in clinical use of shorter duration.

Chapter - 6

Summary

SUMMARY

Detailed pharmacokinetic and toxicological studies of pefloxacin were conducted in five healthy female goats of non-descript breed weighing between 20-22 kg. Concentrations of pefloxacin in plasma and urine as well as kinetic parameters were calculated by two-compartment open model in healthy goats following i.v. administration (5 mg/kg). Further, toxicological study was conducted after i.v. injection of pefloxacin (10 mg/kg) once daily for 7 consecutive days. Various haematological (haemoglobin, total leucocyte count and differential leucocyte count) and biochemical parameters (blood sugar, serum cholesterol, BUN, total protein, SGPT and SGOT) were estimated on 0, 2, 4, 8 and 10^{th} day post i.v. injection of pefloxacin. The following salient findings were obtained :

KINETIC STUDY

(1) Following single i.v. administration, the mean peak plasma concentration of 17.41 ± 1.34 $\mu g.ml^{-1}$ and mean peak urine concentration of 48.13 ± 1.50 $\mu g.ml^{-1}$ were found at 0.043 and 0.75 h, respectively.

(2) The drug was detectable in all animals upto 10 h in plasma and 30 h in urine.

(3) The mean extrapolated zero time concentration during distribution phase (A), elimination phase (B) and theoretical zero time concentration (C_p^0) were noted to be 12.21 ± 2.86, 3.46 ± 0.32 and 15.67 ± 3.03 $\mu g.ml^{-1}$, respectively.

(4) A high value for distribution rate constant (α) of 3.897 ± 0.880 h^{-1} and shorter value of distribution half-life ($t_{1/2}$ α) of 0.22 ± 0.05 denote that the drug is distributed to peripheral tissues at a faster rate.

(5) A moderate elimination half-life ($t_{1/2}$ β) of 3.61 ± 0.46 h denotes that the drug is removed at a moderate rate as compared to other antimicrobials.

This has led to the maintenance of drug concentration for a moderate period in the body as shown by moderate value of mean residential time (MRT) of 4.01±0.19 h.

(6) The high tissue to plasma concentration (T ≈ P) ratio of 2.77 ± 0.55 indicates the better penetration capacity of pefloxacin into various tissues. This is further supported by higher Vd_{area} of 1.24 ± 0.09 $L.Kg^{-1}$.

TOXICITY STUDY

(I) ***Haematological parameters***

(i) Haemoglobin values between 0 day (pre-treatment) and 2, 4, 8 and 10 days after drug administration did not show any significant change.

(ii) The total leucocyte count (TLC) before treatment was noted to be 9808 ± 218.00 which showed significant increase after treatment (2, 4, 8 and 10 days). But the increase is within the physiological limit. However, no significant difference was noted with pretreatment (0 day) and post-treatment with pefloxacin on differential leucocyte count (DLC).

(II) ***Biochemical parameters***

(i) Blood sugar value of 59.34 ± 0.87 mg/dl was noted before treatment (0 day) which did not differ significantly as compared to day 2, 4, 8 and 10 post-treatment values.

(ii) In case of serum cholesterol also, the values noted on pretreatment (0 day) did not differ significantly from those of day 2, 4, 8 and 10 post treatment.

(iii) The value of blood urea nitrogen (BUN) before treatment (17.77 ± 0.76 mg/dl) also did not differ significantly with day 2, 4, 8 and 10 post treatment.

(iv) Total protein, albumin, globulin as well A/G ratio values also before treatment did not differ significantly with those of day 2, 4, 8 and 10 post treatment.

(v) The value of serum glutamate oxaloacetate transaminase (SGOT) noted on day 0 (66.80 ± 3.39 IU/L) increased significantly with increasing days of post-treatment with pefloxacin and the values were noted to be 84.90 ± 3.80, 112.10 ± 3.69, 150.40 ± 3.99 and 146.4 ± 3.90 IU/L on day 2, 4, 8 and 10 post-treatment, respectively.

(vi) The values of serum glutamate pyruvate transaminase (SGPT) also increased significantly like SGOT values. The SGPT value before treatment (day 0) was 11.80 ± 0.56 IU/L which increased to 15.4 ± 0.66, 19.6 ± 0.76, 26.8 ± 0.66 and 25.6 ± 0.43 IU/L on day 2, 4, 8 and 10 post treatment respectively.

From the above noted observations of the present study, it can be concluded that shorter distribution half-life (0.22 ± 0.05 h) and moderate elimination half-life (3.61 ± 0.46 h) denote faster distribution and moderate rate of elimination of pefloxacin as compared to other antimicrobials. High tissue to plasma (T ≈ P) ratio of 2.77 ± 0.55 indicates the penetration capacity of pefloxacin into

'arious tissues and which is further supported by higher Vd_{area} of 1.24 ± 0.09 $L.kg^{-1}$. This may be due to its low molecular weight and strong ipophilicity (Cochereau *et al.*, 1991). The good tissue penetration of)efloxacin may be of clinical utility for the treatment of various)acterial infections prevailing in goat tissues. On the basis of)harmacokinetic parameters and maintenance of therapeutic :oncentration in plasma, the drug may be given at the dose rate of 5 ng/kg body weight parenterally every 12 hourly for treating nfections caused by drug sensitive bacteria.

The toxicological study of pefloxacin did not show any ;ignificant difference in haemoglobin values post i.v. injection (10 ng/kg daily for 7 consecutive days) from pre-treatment value. This :learly indicates that in higher doses also, the drug may not affect the)lood forming system, and hence may not induce anaemia. However, significant rise in TLC was noted on all days post-treatment (2, 4, 8 and 10) as compared to pre-treatment (9805 ± 218.0). The maximum value was noted on day 8 (11,325 ± 188.25) which falls within the normal range of 8000-12000 (Dukes, 1970).

There is no significant effect of drug on blood sugar, ;erum cholesterol, blood protein and blood urea nitrogen levels as :ompared to their pre-treatment values (Table 8, 9 and 10). From the

above fact, it can be assumed that the drug may not have much toxicity on kidney, but it should be used carefully when animals suffer from kidney diseases.

There is significant rise in level of SGOT and SGPT values post i.v. injection of pefloxacin as compared to pre-treatment values. 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 diseases whereas SGPT increases 3000 times more in liver and 400 times more in heart in diseased condition (Vasudevan and Kumari, 1998). The significant increase in SGOT and SGPT level noted in the present study after injection of pefloxacin is within the physiological limit. The normal value in goat for SGOT and SGPT varies from 43 to 132 IU/L and 7 to 24 IU/L, respectively (Kaneko *et al.*, 1997). Hence, the drug should be used carefully in case of liver and muscular disorders.

Hence, by taking into account of various advantages and its least toxicity as noted above, the drug can be used safely in animals, particularly in goat. Precaution may be taken before prescribing drug in animals, which have the history of kidney, liver and heart diseases.

Bibliography

BIBLIOGRAPHY

Abd El-Aty, A.M., and Goudah, A. 2002. Some pharmacokinetic parameters of pefloxacin in lactating goats. *Vet. Res. Commun.* **26** : 553-61.

Aldridge, K.E., Schiro,D.D. and Sanders, C.V. 1986. Pefloxacin : An *in vitro* comparision with other oral antimicrobial agents. *Current Therap. Res.* **40** : 1103-1113.

Andriole, V.T. 1988. The quinolones. New york: Acadmic Press Inc.

Ansari, M.K., Sinha, S.P., Jayachandaran, C. and Singh, M.K., 2000. Pharacokinetics and distribution of pefloxacin in biological fluids of lactating goats after intravenous administration. *Ind. J. Anim. Res.* **34** : 95 – 99.

Arpi, M., Hensen, B.G., Sogaard, P. and Bentzon, M. W. 1987. Comparative *in vitro* activities of pefloxacin, ofloxacin, enoxacin and ciprofloxacin against 256 clinical isolates. *Acta Pathological Microbiologia et Immunologica Scandinavica.* Section B. **95** : 141 – 46.

Ashish Sachan, Jayakumar, K., Honnegowda, Gowda, R.N.S. and Narayana, K. 2000. The effect of pefloxacin on haematological parameters in clinical toxicity study in broiler chickens. *Ind. Vet. J.*, **77** : 307 – 309.

Ashish Sachan, Jayakumar, K., Honnegowda, Narayanaswamy, M. and Sachan, A. 2000. Acute toxicity study of pefloxacin, a fluoroquinolone compound in day old broiler chicks. *Ind. Vet. J.* **77** : 547 – 548.

Ashish Sachan, Jayakumar, K., Honnegowda, Umesh M.H., Venkatesha Udupa and Narayana, K. 2002. Safety evaluation of pefloxacin in day old broilder chicken. *Ind. Vet. J.* **79** : 387- 388.

Auckenthaler, R., Hamzenpour, M.M. and Pechere, J.C. 1986. *In vitro* activity of newer quinolones against aerobic bacteria. *J. Antimicr. Chem.* **17** : 29- 39.

Baggot, J.D. 1977. Principles of drug disposition in domestic animals. The basis of veterinary clinical pharmacology, 1t edn., W.B., Saunders compnay, Philadelphia., London Toranto.

Baggot., J.D. 1974. Principles of drug distribution. *Aust. Vet. J.* **50** : 111-119.

Barlow, A.M. 1963. Nalidixic acid in infection of urinary tract. Laboratory and clinical investigations. *Bri. Med. J.* **2**: 1308-10.

Barre, J., Houine, G. and Tillment, J.P. 1984. Dose dependent pharmacokinetic study of pefloxacin, a new antimicrobial agent in human. *J. Pharmac. Sci.* **73** : 1379 – 1382.

Bennet, J.,V., Broide, J.L., Banner, E.J., and Kirby, W.M.M., 1966. Simplified accurate method for antibiotics assay of clinical specimens. *Appl. Microbiol.* **14** : 170-77.

Boddie and Goe, F. 1962. Diagnostic Methods in Veterinary Medicine. 5th edn. Oliver and Boyd, Edinburgh and London 335-351.

Bressole, R., Goncalves, F., Gouby, A. and Galtier, M. 1994 Pefloxacin clinical pharmacokinetics. Laboratorie de pharmacocin etique, faculte de pharmacie, Mout pellier, France.

Cahal, D.A. 1965. Reaction to nalidixic acid. *Bri. Med. J.* **2** : 590.

Cardey, J., Silvain, C., Bougquet, S., Breux, J. P., Becq Giraudan, B., Fourtillan J.P. and Beauchant, M. 1987. Oral pharmacokinetics and ascitic fluid penetration of pefloxacin in cirrhosis. *Eur. J. Clin. Pharmacol.* **33** : 469 – 472.

Chichmanian, R.M., Spreux, A., Bernard, E., Garraffo, R. and Fuzibet, J.G., 1992. [Thrombocytopenia due to pefloxacin (peflacine) : dose dependent toxicity ?]. *Therapie.* **47** : 419-21.

Chousalkar, M.S. 1995. Pharmacokinetic studies of pefloxacin and its residue studies in tissues and eggs of chicken. M.V.Sc. Thesis submittes to Konkan Krishi Vidyapeeth. Dapoli, Maharashtra. India

Christ, W., Lehnert, T. and Ulbrich B., 1988. Specific toxicological aspects of the quinolones. *Rev. Infects. Dis.* **10** : 5141-6.

Cochereau – Massin, I., Bauchet, J., Faurisson, F., Vallois, J.M., Lacombe, P. and Pocidalo, J.J. 1991. Ocular Kinetics of pefloxacin after intramuscular administration in albino and pigmented rabbits. *Antimic. Agents. Chemoth* **35** : 1112-1115.

Contrepois, A., Daldoss, C., Pangon, B., Garad, J.J., Keir, M., Sarrazin, C., Vallois, J.M. and Carbon, C. 1984. Pefloxacin in rabbits : protein binding, extravascular diffusion, urinary excretion and bactericidal effect in experimental endocarditis. *J. Antimicrob. Chemother.* **14** : 51-57.

Corret, G., Flandrois, J.P. and Lobry, J.R. 1991. Biphasic kinetics of bacterial killing by quinolone. *J. Antimicr. Chemother.* **27** : 319-27.

Cukierski, M.A., Hendrickx, A.G., Prahalada, S., Tarantal, A.F., Hess, D.L., Lasley, B.L., Peter, C.P., Tarara, R. and Roberston, R.T. 1992. Embryotoxicity studies of norfloxacin in cynomolgus monkeys II. Role of progesterone. *Teratology*. **46** : 429-38.

Danan, G., Montay, G., Cunci, R. and Erlinger, S. 1985. : Pefloxacin kinetics in cirrhosis. *Clinical Pharamcology and Therapeutics* **38** : 439- 442.

Davis, R. and Bryson, H.M. 1994. Levofloxacin : A review of its antibacterial activity pharmacokinetics and therapeutic efficacy. *Drugs*. **47** : 677- 700.

Dost, F. H. 1953. Der Blustspiegel Kinetics der Konentration Sablaug in der Fries louff ussig keit George Thiem Lepzig (cited by Verma 1980).

Dukes, H.H. 1970. Duke's Physiology of Domestic Animals. 8th edn. M.J., Swenson (ed.). Comstock Ithaak., New York.

Dworkin, R., Modin, G., Kunz, S., Rich R., Żak, O. and Sande, M. 1990. Comparative efficacies of ciprofloxacin, pefloxacin, and vancomycin in combination with rifampin in a rat model of methicillin resistant *Staphylococcus aureus* chromic ostemyelitis. *Antimicrob Agents Chemother* **34** : 1014-16.

Felmingham, D., OHarre, M.D., Robbins, M.J., Wall, R.A. and Williams, A.H. 1985. Comparative *in vitro* studies with 4-quinolone antimicrobials. Drugs under experimental and clinical research. **11**: 317- 329.

Frankel, S., Reitman, S., and Sonnerwirtha, A.C. 1970. Gradiwhol's Clinical Laboratory Methods and Diagnosis. Vol. I. pp. 82-83. The C.V. Inospby Co., St. Louis.

Frydman, A.M., Le Rank, R., Lefebre, M.A., Djebbar, F. and Fourtillan, J. 1986. B. pharmacokinetics of pefloxacin after repeated intravenous and oral administration (400 mg bid) in young healthy volunters. *J. Antimicrob., Chemother.* **17**: 65-79.

Gary, E.S. 1995. *Drugs.* **49** : 497.

Gibaldi, M. and Perrier, D. 1975 Pharmacokinetics. Marcel Dekkar, New York.

Gibaldi, M. and Weintraub, H. 1971. Some considerations as to determination and significance of biological half life. *J. Pharma. Sci.* **60** : 624 - 626.

Gonzalez, J.P. and Henwood, J.M. 1989. Pefloxacin : A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. *Drugs.* **37** : 628- 68.

Goueffon, Y., Montay, G., Roquet, F. and Pesson, M. 1981. A new synthetic antimicrobial 1-ethyl-6-fluoro –7- (4-methyl –1-piperazinyl) –4- oxo-dihydroquinolone –3- carboxylic acid. *C.R. Acad. Sci.* **292** : 37- 40.

Harold, C.N., 1987. Ciprofloxacin : An overview and prospective appraisal. *Am. J. Med.* **82** : 395-404.

Higgins, N.P., Peebles, C.L., and Sugino, A 1978 Purification of subunits of *Escherichia coli* DNA gyrase and reconstitution of enzymic activity. *Proc. Natl. Acad. Sci.* **75** : 1773.

Hoffler, D., Schafer, I., Koeppe, P. and Sorgel. F. 1988. Pharmacokinetics of pefloxacin in normal and impaired renal function. *Arzncimittel Forschung*. **38** : 739-743.

Horio, T., Miyauchi, H., Asada, Y. Aoki, Y. and Harada, M., 1994. Phototoxicity and photoallergenicity of quinolones in guinea pigs. *J. Dermatol. Sci.* **7** : 130 – 5.

Jare, N.M. 1996. Pharmacokinetic profile and safety assessment of pefloxacin injection in lactating goats. M.V.Sc. Thesis submitted to Konkan Krishi Vidyapeeth, Dapoli, Maharashta, India.

Jayakumar, K., Shashi Prakash, T. R., Jagdish Kumar, B. and Modyastha, K.M. 1995. Pharmacokinetics and metabolism of pefloxacin in the dog. Cited in II NCVPT conference and national symposium on "quality drugs for assured productivity in animals". Mumbai, India, Dec., 12, 1995.

Jusko, W.J. and Gibaldi, M. 1972. Effect of change in estimation of various parameters of the two compartment open model. *J. Pharm. Sci.* **61** : 1270- 1273.

Kaneko, J.J., Harvey, J.W. and Bruss., M.L. 1997. Clinical Biochemistry of Domestic Animals. 5th edn. Academic Press, California.

Kashida, Y. and Kato, M. 1997. Toxic effects of quinolone antibacterial agents on the musculskeletal system in juvenile rats. *Toxicol. Pathol.* **25** : 635-43.

Leibovitz, E., Keren, G., Shabtai, M., Barzilai, A. and Rubinstein, E. 1989. The pharmacokinetics and therapeutic efficacy of fleroxacin and pefloxacin in a rat abscess model. J. *Antimicrob. Chemother.* **24** : 375-85.

Lesher, G.Y., Froelich, EJ. and Gruett, M.D. 1962. 1,8-napthyridine derivative. A new class of chemotherapeutic agents. *J. Med. Pharmacol. Chem.* **5** : 1063-8.

Lightvoet, E.J. and Meningio, W.T. 1985. *In vitro* activity of pefloxacin compared with six other quinolones. *J. Antimicrob. Chemother.* **16** : 485-90.

Machida, M., Kusajima, H., Aijima., H., Maeda, A., Ishida, R. and Uchida, H. 1990. Toxicokinetic study of norfloxacin induced arthropathy in juvenile animals. *Toxicol. Appl. Pharmacol.* **105** : 403-12.

Malik, J. K., Rao, G.S., Ramesh, S., Muruganandan, S. Tripathi, H.C. and Shukla, D.C. 2002. Pharmacokinetics of pefloxacin in goats after intravenous or oral administration. *Vet. Res., Commun.* **26** : 141-9.

Marrakchi –Benjaafar, S., Cochereau, I., D'Hermies, F. and Pocidalo, J.J. 1995. Tolerability, kinetics and efficacy of subconjunctival pefloxacin in pigmented rabbits. *Antimicrob. Agents. Chemother.* **39** : 834-8.

Mercer, H.D., Baggot, J.D. and Sams, R.A. 1977. Application of pharmacokinetic method of the drug residue profile. *J. Toxicol. Env. Hith.* **2** : 787 - 801.

Montay, G., Blain Y., Roquet, F. and LeHir, A. 1983. High performance liquid chromatography of pefloxacin and its main active metabolites in biological fluids. *J. Chromatogr.* **272** : 359-65.

Montay, G., Goueffon, Y. and Roquet, F. 1984. Absorption distribution metabolic fate, and elimination of pefloxacin mesylate in mice, rats dogs, monkeys, and humans. *Antimicrob. Agents. Chemother.* **25** : 463-72.

Moutafchieva, R. and Djouvinov, D. 1997. Pharmacokinetics of pefloxacin in sheep. *J. Vet. Pharmacol. Ther.* **20** : 405-407.

Neu, H.C. 1992. Quinolone antimicrobial agents. *Ann. Rev. Med.* **43** : 465-86.

Nordmann, P., Pechinot, A. and Kazmierczak, A. 1989. Cytotoxicity and uptake of pefloxacin, ciprofloxacin and ofloxacin in primary cultures of rat hepatocytes. *J. Antimicrob. Chemother.* **24** : 355- 63.

Notari, R.E. 1980. Biopharmaceutics and clinical pharmacokinetics. 3rd edn., Marcel Dekker Inc., New York.

Pallavicini, F., Antinori, A., Federico G., Fantomi, M. and Nervo, P. 1989. Influence of two quinolones, ofloxacin and pefloxacin, on human myelopoiesis *in vitro Antimicrob. Agents Chemother.* **33** : 122 - 123.

Patil, R.V., Gatne, M.M., Somkuwar, A.P. and Ranade, V.V 1996. Pharmacokinetics and milk concentration of pefloxacin injection (Pelwin) in lactating cows. *Ind. Vet.J.* **73** : 1130-1132.

Pedrini, A. 1979. Nalidixic acid. In : Hahn FE, ed. Antibiotics. Berlin : Springer Verlag.

Pino, A., Maura., A., Villa, F. and Masciangelo, L. 1991. Evaluation of DNA damage induced by norfloxacin in liver and kidney of adult rats and in foetal tissues after transplacental exposure. *Mutat. Res.* **264** : 81-5.

Prakash, N., 2003. Pharmacokinetics of pefloxacin after single intravenous administration in buffalo calves. *Ind. Vet. J.* **80**: 746-748.

Prescott, J.F. and Baggot J.D. 1994. Antimicrobial Therapy in Veterinary Medicine, 2nd end. Int. Book Distribution Co., New Delhi.

Quantin R., Koubao, N., Cattier B., Garignet, M. and Goudeau, A. 1988. *In vitro* activities of five new quinolones against 88 genital and neonatal *Haemophilus* isolates. *Antimicrob. Agents Chemother.* **32** : 147 – 49.

Reinhold, J.G. 1953. Total proteins, albumins and globulins. In standard methods of clinical chemistry. Reine, M., (ed). Vol. I. pp. 88 academic Press, New York.

Reitman, S. and Frankel, S. 1957 *Am. J. Clin. Path* **28** : 56.

Riegelman, S., Loo, J. and Rowland, M. 1968. Concept of a volume of distribution and possible errors in evaluation of this parameter. *J. Pharm. Sci.* **57** : 128 - 133.

Rowland, M., Bennet, L.Z. and Graham, G.C. 1973. Clearance concept in pharmacokinetics. J. *Pharmacokinet. Ani. Sci.* **67** : 471 - 73.

Roy, B.K., Pandey, S.N., Sinha, K.P., Thakur, D.K. and Singh, K.K. 1997. Pharmacokinetics of pefloxacin in goats. *Ind. J. Pharmacol.* **29** : 435 – 436.

Saini, S.P.S. and Srivastava, A.K. 1997. Pharmacokinetics and dosage regimen of amikacin in febrile cow calves. *Ind. J. Anim. Sci.* **67** : 471-473.

Sams, R. 1978. Pharmacokinetics and metabolic consideration as they apply to clinical pharmacology. In proceedings of Second Equine Pharmacology Symposium (Oct 15- 17). Edited by Powers. J.D. and Powers, T.E. *Am. Assoc. Equine., Practitioners.* 120 - 129.

Schalm, O.W., Jain N.C. and Caroll, E.J. 1975. Veterinary Haematology 3rd edn. Lea and Febiger, Philadelphia.

Sharma, A.K., Khosla, R., Kela, A.K., and Mehta, V.L., 1994. Fluoroquinolones: Antimicrobial agents of the 90's *Indian Journal of pharmacology*. **26** : 249 - 261.

Simonin, M.A., Gegout – Pottie, P., Minn, A., Gillet., P., Netter, P. and Terlain, B. 1999. Proteoglycan and collagen biochemical variations during fluoroquinolone induced chondrotoxicity in mice. *Antimicrob. Agents Chemother.* **43** : 2915-21.

Smith, J.T. and Levin, C.S 1988. Chemistry and mechanisms of action of the 4- quinolone antibacterials. In : Andriole VT, ed. the quinolones. London. : Academic Press. 23 – 81.

Snedecor, W.G., and Cochran, W.G. 1976. Statistical Methods. 6th edn., Calcutta. ; Oxford and IBH Publishing Company.

Sridevi, V., Reddy K.S., Kalakumar, B. and Gopala Reddy. 2002. Evaluation of experimentally induced pefloxacin toxicity in pups. *Ind. Vet. J.*. **79** : 385 – 386.

Srivastava, A.K, Dumka, V.K. and Deol, S.S. 2000. Disposition kinetics and urinary excretion of pefloxacin after intravenous injection in crossbred calves. *Vet. Res. Commun.* **24** : 189-96.

Stahlmann, R., Kuhner, S., Shakibaa, M., Schuabe, R., Flores, J., Evander, S.A., and Van Sickle, D.C., 2000. Chondrotoxicity of ciprofloxacin in immature beagle dogs: immunohistochemistry, electron microscopy and drug plasma concentrations. *Arch. Toxicol.* **73** : 564-72.

Sultan, E., Richard, C., Pezzano., M., Auzepy, P. and Singlas, E. 1988. Pharmacokinetics of pefloxacin and amikacin administerod simultanesouly to intensive care patients. *Eur. J. Clin. Pharmacol.* **34** : 637- 643.

Vancutsem, P.M., Babish, J. H. and Schwork, K.W.S. 1988. *Cornell. Vet.* **50** : 173.

Vasudevan, D.M. and Kumari, S.S. 1998. Text book of biochemistry 2nd end Jaypee Brothers Medical Publishers (P) Ltd., New Delhi.

Wagner, J.G. 1968. Pharmacokinetics. *Ann. Rev. Pharmacol.* **8** : 67-94.

Wall, R.A., Mabey, D.C.W., Bellow, C.S.S. and Felmingham, D. 1985. The comparative *in vitro* activity of twelve 4- quinolone antimicrobials against *Haemophilus ducreyi. J. Antimicrob. Chemother.* **16** : 165 – 68.

Wang, J.C. 1974. Interactions between DNAs and enzymes. The effect of superhelical turns. *J. Med. Bio.* **87** : 797.

Wang, J.C. 1985. DNA topoisomerases. *Ann. Rev. Biochem.* **54** : 665.

Webberley, J.M., Andrews, J.M., Ashby, J.P., McLeod, A and Wise, R. 1987. Pharmacokinetics and tissue penetration of orally administered pefloxacin. *Eur. J. Clin. Microbiol.* **6** : 521-4.

Wolfson, J.S. and Hevpev, D.C. 1985. The fluoroquinolones : Structures, mechanism of action, resistance and spectra of activity in vitro. *Antimic. Agents Chemother.* **28** : 581-6.

Wootton, J.D.P. 1964 Microanalysis in Medical Biochemistry. 4th edn. pp. 83-84, 92-93, 101-105. J. & A. Churchill Ltd., London.

Appendix

APPENDIX - I

Calculation of Kinetic Parameters

Kinetic parameters were calculated from log plasma drug concentration versus time profile. An example is noted below from the data of goat no. 4 obtained after single i.v. injection of pefloxacin (5 mg/kg). The data showed a biphasic curve and hence, fits well into a two compartment open model.

Sl. No.	Time (h) X	X^2	Plasma drug concentration (Y) $\mu g.ml^{-1}$	Log Y	XY
1	4	16	1.28	0.1072	0.4288
2	6	36	0.85	-0.0706	- 0.4236
3	8	64	0.54	-0.2676	- 2.1408
4	10	100	0.42	-0.3768	-3.7680
5	12	144	0.15	-0.8239	-98868
Σn = 5	$\Sigma X = 40$ $\overline{X} = 8.0$	ΣX^2 = 360		$\Sigma \log Y$ = -1.4317 $\overline{Y} = -0.2863$	ΣXY = -15.7904

b, slope of line $= \frac{n.\sum x.y - \sum x.\sum y}{n.\sum x^2 - (\sum x^2)^2}$

$= \frac{(5x - 15.7904) - (40 \times -1.43147)}{(5 \times 360) - (40 \times 40)}$

$= \frac{-78.952 - (-57.268)}{1800 - 1600}$

$= \frac{-21.684}{200} = -0.1084$

β, elimination rate constant $= b \times (-2.303)$

$= -0.1084 \times -2.303$

$= 0.250\ h^{-1}$

B, zero time concentration during elimination phase can be obtained from the formula $\overline{Y} = a + b\overline{X}$.

where $\overline{Y}$ = mean log drug concentration

$\overline{X}$ = mean time

b = slope of line

a = zero time concentration

Therefore,

$a = \overline{Y} - b.\overline{X}$

$= -0.2863 - (-001084 \times 8.0)$

$= \log 0.5809$

Zero time concentration (B) = antilog of 0.5809 = 3.81μg.ml^{-1}

Similarly , the theoretical plasma concentration (Y) can be calculated by putting the value of the time (X) in the above equation during the time intervals of distribution phase (Y = a + bX)

Subtracting the theoretical values from observed values, a series of residual concentrations were obtained and slope of line in natural log (distribution rate constant, α) and the zero time intercept (zero time concentration during distribution phases, A) can be calculated as per method adopted for calculation of B and β. The value of A is 4.23 μg.ml^{-1} and α is 2.124 h^{-1}.

C_p^0, the theoretical plasma concentration at time zero

$$C_p^0 = A + B = 4.23 + 3.81 = 8.04 \ \mu g.ml^{-1}$$

Distribution half life ($t_{1/2}\ \alpha$) $= \frac{0.693}{\alpha}$

$$= \frac{0.693}{2.124} = 0.334h$$

Elimination half life ($t_{1/2}\ \beta$) $= \frac{0.693}{\beta} = \frac{0.693}{0.250} = 2.78h$

Area under curve, AUC

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta} = \frac{4.23}{2.124} + \frac{3.81}{0.250} = 1.99 + 15.24 = 17.23\ mg.L^{-1}.h$$

Area under first moment curve, AUMC

$$AUMC = \frac{A}{\alpha^2} + \frac{B}{\beta^2}$$

$$= \frac{4.23}{(2.124)^2} + \frac{3.81}{(0.250)^2}$$

$$= \frac{4.23}{4.511} + \frac{3.81}{0.0625}$$

$$= 0.94 + 60.96$$

$$= 61.90 \text{ mg.L}^{-1}.\text{h}^2$$

Mean residential time, MRT

$$MRT = \frac{AUMC}{AUC} = \frac{61.90}{17.23} = 3.59\text{h}$$

Rate constant drug transfer from peripheral to central compartment, K_{21}

$$K_{21} = \frac{A.\beta + B.\alpha}{C_p^0}$$

$$= \frac{(4.23 \times 0.250) + (3.81 \times 2.124)}{8.04}$$

$$= \frac{1.0575 + 8.0924}{8.04}$$

$$= \frac{9.1499}{8.04}$$

$$= 1.138 \text{ h}^{-1}$$

Elimination rate constant of the drug from central compartment, Kel

$$Kel = \frac{\alpha.\beta}{K_{21}} = \frac{2.124 \times 0.250}{1.138} = \frac{0.531}{1.138} = 0.467\ h^{-1}$$

Rate constant of drug transfer from central to peripheral compartment, K_{12}

$$K_{12} = \alpha + \beta - K_{21} - Kel$$

$$= 2.124 + 0.250 - 1.138 - 0.467$$

$$= 0.769\ h^{-1}$$

The fraction of drug available for elimination from central compartment, Fc

$$Fc = \frac{\beta}{Kel} = \frac{0.250}{0.467} = 0.54$$

Approximate tissue to plasma concentration ratio, $T \approx P$

$$T \approx P = \frac{K_{12}}{K_{21} - \beta} = \frac{0.769}{1.138 - 0.250}$$

$$= \frac{0.769}{0.888} = 0.87$$

Volume of distribution based on both distribution and elimination, V_{dc}

$$V_{dc} = \frac{D}{C_p^o} = \frac{5}{8.04} = 0.62 L.Kg^{-1}\ L./kg$$

where, D = Dose (5 mg/kg)

The volume of distribution based on elimination, Vd_B

$$Vd_B = \frac{D}{B} = \frac{5}{3.81} = 1.31 \text{ L.Kg}^{-1}$$

The volume of distribution based on total area under curve, Vd_{area}

$$Vd_{area} = \frac{D}{AUC.\beta} = \frac{5}{17.23 \times 0.250} = \frac{5}{4.3075} = 1.16 \text{ L.Kg}^{-1}$$

The volume of distribution at steady state, Vd_{SS}

$$Vd_{SS} = \frac{K_{12} + K_{21}}{K_{21}} \times Vd_C$$

$$= \frac{0.769 + 1.138}{0.138} \times 0.62$$

$$= \frac{1.907}{1.138} \times 0.62 = 1.04 \text{ L.Kg}^{-1}$$

The total body clearance, Cl_B,

$$Cl_B = Vd_{area} \times \beta.$$

$$= 1.16 \times 0.250$$

$$= 0.29 \text{ ml.Kg}^{-1}.\text{min}^{-1}.$$