PHARMACOKINETIC STUDY OF OFLOXACIN IN HEALTHY AND FEBRILE GOATS (*Capra hircus*)

THESIS

SUBMITTED TO

RAJENDRA AGRICULTURAL UNIVERSITY

(BIHAR)

In partial fulfilment of the requirements

FOR THE DEGREE OF

Master of Veterinary Science

IN

PHARMACOLOGY & TOXICOLOGY

By

Shrawan Kumar

Registered No. M/VPT/69/1998 99

Department of Pharmacology & Toxicology

BIHAR VETERINARY COLLEGE

PATNA

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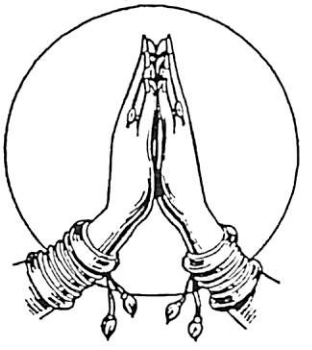
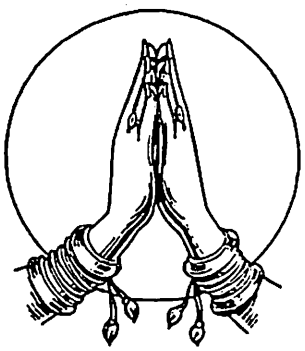


DEDICATED

to

MY

Respected Parents



DEDICATED

to

MY

Respected Parents

DEPARTMENT OF VETERINARY PHARMACOLOGY & TOXICOLOGY
Bihar Veterinary College, Patna – 800 014
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Dr. C. Jayachandran
Ph.D.
Associate Professor

CERTIFICATE – I

This is to certify that the thesis entitled "**PHARMACOKINETIC STUDY OF OFLOXACIN IN HEALTHY AND FEBRILE GOATS**" submitted in partial fulfilment of the requirements for the degree of **Master of Veterinary Science** (**Veterinary Pharmacology & Toxicology**) of the faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar, Pusa, is the record of bonafide research carried out by **Dr. Shrawan 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.

Jayachandran 31/01/07

(C. Jayachandran)
Major Advisor

Endorsed :

31/01/07

Chairman/Head of the Department

CERTIFICATE – II

We, the undersigned members of the Advisory Committee of **Dr. Shrawan 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 "**PHARMACOKINETIC STUDY OF OFLOXACIN IN HEALTHY AND FEBRILE GOATS**" may be submitted by **Dr. Shrawan Kumar** in partial fulfilment of the requirements for the degree.

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CERTIFICATE – III

This is to certify that the thesis entitled **"PHARMACOKINETIC STUDY OF OFLOXACIN IN HEALTHY AND FEBRILE GOATS"** submitted by **Dr. Sharawan Kumar** in partial fulfilment of the requirements 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 ..... 2001.

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Shrawan Kumar
31.1.2004

(Shrawan Kumar)

LIST OF TABLES

Introduction

INTRODUCTION

Antimicrobial agents play a major role in medical and veterinary practices in combating various microbial infections. Quinolones are remarkably free from toxicity in animals. The fluoroquinolones are a class of antimicrobials that are frequently used in veterinary practices to treat a variety of infections (Greene and Budsberg, 1993). They possess broad spectrum with bactericidal activities (Wolfson and Hooper, 1985; Vancutsem *et al.*, 1990; Chu, 1996).

Ofloxacin, one of the latest flouroquinolones, is currently used in human therapy due to its wide spectrum of antimicrobial activity, excellent distribution in different tissues and lower toxicity. It is more potent against *E. coli* and various species of *Salmonella, Shigella, Enterobacter, Campylobacter* and *Neissaria* (Sanders, 1988). Minimum inhibitory concentrations of ofloxacin for 90% strains (MIC 90) are usually less than 0.2 μg/ml (Norris and Mandell, 1988). It is also very effective against Staphylococci including methicillin resistant strains (MIC_{90} = 0.1 to 2μg/ml). For Mycobacterium spps its MIC values range from 0.5 to 3 μg/ml (Hooper, 1995).

In India, goat rearing is most popular among small, marginal and landless farmers. India ranks first among the country in

the world in goat population (Sahani, 1982). Goat contributes to about 35% of the total meat and 3% of the total milk production in India (Chawala *et al.*, 1981). Besides, by export of goatskin, casin and hair, valuable foreign exchange is earned. The manure produced from dropping enriches the soil. Pashmina and mohair production is another important contribution by the same species of goat. This species of animal contributes to the livelihood of a large population of small and marginal farmers and landless labourers. Keeping in view the major contribution of goats in natural economy and employment avenues, its proper and effective health coverage is essential by achieving the new dimensions through ofloxacin therapy.

To be effective in febrile and other diseased conditions, the drug should reach the target organ in effective concentration. The quantitative estimation of antimicrobials like ofloxacin in plasma, milk and urine following parenteral administration will be highly helpful in achieving rational therapy for treating local and systemic infections. Contamination of milk with antimicrobials is a public health hazard and required withdrawal of milk for human consumption for sufficient period of time after cessation of therapy.

Before using a drug in therapy, it is essential to conduct detailed pharmacokinetic studies. Pharmacokinetic studies are generally carried out in healthy animals and appropriate dosage

regimen is derived for effective treatment. Now, it is well established that the kinetic parameters of a drug may change during febrile and other diseased conditions resulting into sharp change in dosage regimen.

As such a need is felt to undertake this study for derivation of suitable dosage regimen of ofloxacin for effective treatment in goats during febrile and other diseased conditions. On the basis of available literature, it seems that no kinetic study has been conducted in goats particularly in febrile state.

Keeping in view of the above mentioned facts, the present investigation was carried out with following objectives.

1. Distribution of ofloxacin in different body fluids in healthy and febrile goats after i.v. and i.m. administration.
2. Determination of pharmacokinetic parameters of this drug in healthy and febrile goats.
3. Calculation of dosage regimen of this drug in healthy and febrile goats.


Review
of
Literature

Review of Literature

Quinolones, carboxilic acid derivatives are synthetic antimicrobial agents that are becoming more popular in medical and veterinary practices. Initially, nalidixic acid was introduced in clinical practice in 1963. Nalidixic acid possesses narrow spectrum of activity (mostly gram negative organisms) and mainly used for treating urinary tract infections caused by gram negative organisms. Due to narrow spectrum of activity and rapid development of resistance of nalidixic acid, systematic search was carried out to synthesise agents possessing wide spectrum of antimicrobial activity for systemic use.

Introduction of 6-fluorine atom into the basic nucleus of quinolones in fluoroquinolone produced racemic mixture in which one isomer was more active than the other which possess extended gram positive activity. Further advancement in the quinolone field came with the synthesis of norfloxacin which because of its 6-fluorine and 7-piperazine group, had enhanced antibacterial activity. Similarly, a number of other newer fluoroquinolones have been synthesized viz enrofloxacin, ofloxacin, ciprofloxacin, pefloxacin etc. (Harold, 1987) and some of them are effectively used in veterinary practice also for the treatment of various bacterial infections (Goldstein and Citron, 1993).

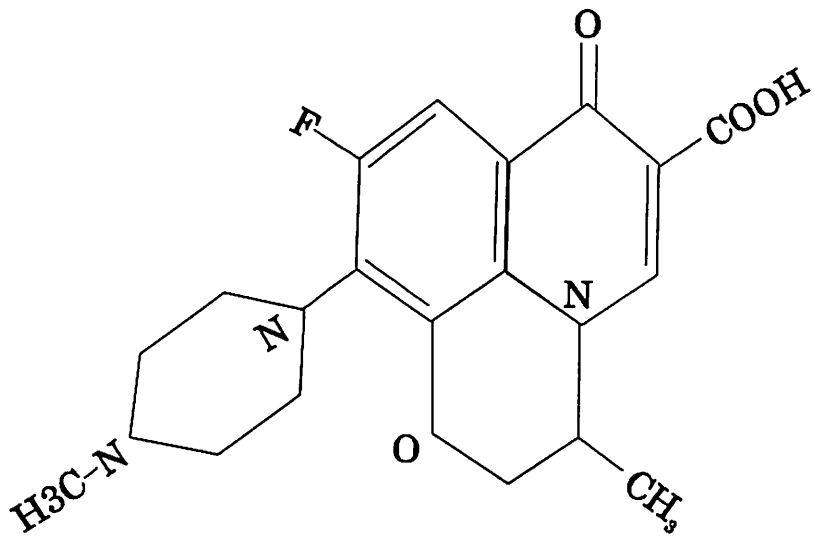
OFLOXACIN

Chemistry

The fluoroquinolones that are currently available for clinical use are 4-quinolones that all contain a carboxilic acid moiety in 3 position of the basic ring structure. The newer fluoroquinolones also contain a fluorine substitute at position 6 and many at position 7.

The chemical structure of ofloxacin is shown in Fig.-1.

Fig. - 1



Mechanism of Action

Ofloxacin is a bactericidal agent. The target site for bactericidal action is the enzyme "gyrase" bacterial type-II topoisomerase (Vancutsem *et al.*, 1990). Ofloxacin penetrates the cell nucleus of bacteria and acts by inducing irreversible inhibition of DNA gyrase, a bacterial enzyme responsible for vital function of

bacteria. The inhibition of gyrase by ofloxacin stops the replication and supercoiling of DNA within a very short time and there by kills the bacteria (Crumplin *et al.*, 1984; Bahri and Blouin, 1991).

Antimicrobial activity

Ofloxacin is a broad spectrum antimicrobial with bactericidal action. It is effective against both gram positive and gram negative as well as mycoplasma. In addition, some of the anaerobic pathogens are also susceptible. Development of resistance to ofloxacin is lower than other quinolones. Hence, it is effective against microorganisms that are resistant to β lactum antibiotics, tetracycline, aminoglycosides or macrolids and has a special role in the therapy of multi drug resistant infections. Further, it is not having immuno-supressive properties, as it does not effect the DNA of the host cells.

The MIC of ofloxacin for most of the organism is 0.2 to 2 μg/ml. In case of *Staphalococci* infection its MIC is 0.1 to 1 μg/ml. In *M. tuberculosis*, *M. kansaii* and *M. fertuitum* MIC is 0.5 to 3 μg/ml (Hooper, 1995).

GENERAL PHARMACOKINETICS

Pharmacokinetics often referred to as disposition kinetics, 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 characterise with reproducibility of behaviour and fate of drugs in a biological system, when administered by certain route in a particular dosage form. 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 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 drug 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 change in its tissue level. 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 level declines according to following equation :-

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

where,

C_p = Concentration of drug in 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 extravascular (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 instataneous 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:

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

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 constants (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 disposition 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:

$$C_p = A_e^{-\alpha t} + B_e^{-\beta t} + C_e^{-\gamma t} \quad \text{---------- Eq. 3}$$

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 studies consist of :

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

(b) Estimation of drug dosage regimen in a particular species of animal.

(c) Determination of drug withdrawal period for 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 extravascular (im/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 ($t_{1/2}$ β)

(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 ($t_{1/2}$ β)

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 increases 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 truely 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, extracellular 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, biotransformation 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 $t_{1/2}$ β that are hybrid constants and depend upon K_{12}, K_{21} and K_{el}, the total body clearance changes exactly in proportion to K_{el} (Jusko and Gibaldi, 1972; Rowland *et al.*, 1973).

7. Bioavailability

When a drug is administered intravenously, the peak concentration in blood is attained quickly and whole administered drug is available for distribution, metabolism and excretion. The peak plasma level following extravascular administration is some what delayed and its magnitude decreases. The bioavailability of a drug indicates the rate of drug absorption as well as the amount of absorption of a drug in pharmacologically active form. The extent of absorption (F) is generally known as bioavailability and is calculated experimentally by the ratio of the area under the plasma concentration time curve after extravascular and intravenous administration (Baggot, 1977; Sams, 1978).

8. Protein binding

Some drugs have tendency to get bound with plasma protein mainly with albumin. Binding of drugs with plasma protein affects drug distribution (high molecular weight

of plasma protein prevents bound drug from diffusing out of capillaries into tissues), drug effects (free drug fraction is alone pharmacologically active, since it can penetrate to the region of target organ) and drug elimination (free drug is alone filtered at the glomerulus and also excreted into saliva, milk etc). The protein bound drug also acts as a reservoir.

It is reported that the various constants, namely A, α, B, β, $t_{1/2}\,\alpha$, $t_{1/2}\,\beta$ 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 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.

KINETIC STUDY OF OFLOXACIN

Pharmacokinetic studies of ofloxacin were carried out in man as well as in different species of animals but no such study was carried out in goat so far. Reports on kinetic studies in man and various species of animals are noted below.

Man

Warlich *et al.* (1990) conducted pharmacokinetic study of ofloxacin in the healthy volunteers and noted the terminal half lives ($t_{1/2}\beta$) in serum blister fluid (7.0 h) and cantherides blister fluid (6.3 h) to be in accordance with serum half life (6.6 h). Ofloxacin levels in the skin are well above the MIC for 90% of strains tested. Loade *et al.* (1987) noted $t_{1/2}\beta$ between 231 to 267 min (3.85 – 4.45 h) after oral and i.v. administration of ofloxacin. They also noted high volume of distribution (1.2 to 1.4 L/Kg) which denoted effective distribution in extravascular space. Verho *et al.*(1985) showed $t_{1/2}\beta$ of 5.6 – 6.4 h after oral administration of ofloxacin in man.

Rabbit

Perkins *et al.* (1994) recorded the penetration into vitrous humor of six albino rabbits to be 32.6 ± 2.12% with $t_{1/2}\beta$ values of 3.21

and 2.39 h respectively, for vitrous humour and serum. In rabbits following single dose (20 and 40 mg/Kg) by i.v. and s.c. routes, the area under concentration time curve (AUC) changed proportionally with the dose while the half life was unaltered and ranged from 1.5-1.9 h (Marangos *et al.*, 1997).

Bucks

Takawale *et al.* (2000a) carried out the pharmacokinetic profile of ofloxacin following its i.v. injection at the dose rate of 5 mg/Kg in adult barberi bucks. The highest serum concentration of ofloxacin was 9.14 ± 0.22 μg/ml at 2.5 min while lowest concentration was 0.55 ± 0.04 μg/ml at 72 h with a secondary peak of 1.29 ± 0.08 μg/ml at 36 h. The $t_{1/2}\alpha$, $t_{1/2}\beta$, $t_{1/2}\gamma$ and $t_{1/2}$ terminal were 0.22 ± 0.05, 0.30 ± 0.07, 3.27 ± 0.88 and 21.50 ± 0.88 h, respectively. The Vd_{area}, AUC and Fc were 1.49 ± 0.20 L/Kg, 14.50 ± 2.04 μg/ml h and 0.19 ± 0.02 while Cl_B was 380.40 ± 53.10 ml/Kg/h and the value of K_{12}/K_{21} and K_{13}/K_{31} ratio were 2.61 ± 0.11 and 0.88 ± 0.21, respectively. MIC value of 0.55 ± 0.04 μg/ml was considered as therapeutically satisfactory concentration against most of the ofloxacin sensitive organism.

Rams

Takawale *et al.* (2000b) conducted the disposition kinetics of ofloxacin following a single i.v. injection at the dose rate of 5 mg/Kg

in adult rams. The highest concentration of ofloxacin in serum was 8.17 ± 0.41 μg/ml at 2.5 min while the lowest concentration was 0.33 ± 0.17 μg/ml at 72 h. In between 0 and 72 h, a secondary peak of 0.68 ± 0.10 μg/ml was observed at 24 h. The $t_{1/2}\alpha$, $t_{1/2}\beta$, $t_{1/2}\delta$ and $t_{1/2}$ terminal were 0.14 ± 0.01, 0.42 ± 0.11, 2.69 ± 0.45 and 31.77±3.68 h, respectively. The Vd_{area}, AUC and Fc were 1.61 ± 0.24 L/Kg, 12.00 ± 0.35 μg/ml.h and 0.31 ± 0.12 while Cl_B was 480.40 ± 13.20 ml.Kg.h^{-1} and the values of K_{12}/K_{21} and K_{13}/K_{31} ratio were 2.83 ± 0.21 and 1.53 ± 0.21, respectively.

Dog

Yoshida *et al.* (1998) conducted kinetics study and distribution of ofloxacin in synovial fluid condylar drug concentrations in immature (3 months old) and mature (18 months old) male beagle dogs. They noted longer $t_{1/2}$ β of 4.5 ± 0.3 h in mature dog as compared to short $t_{1/2}\beta$ 3.8 ± 0.3 h in immature dogs after single oral dose of ofloxacin (20 mg/Kg). Mean concentrations (2.9-5.1 μg/ml) of the joint synovial fluid of immature dogs did not differ from those (5.2-6.6 μg/ml) of mature dogs, while articular cartilage concentrations (humrus : 14.0-14.4 μg/g and femur : 12.3-14.0 μg/g) of immature dogs were significantly lower than those (27.1-34.4 μg/ml and 22.0-30.0 μg/g, respectively) of mature dogs.


Materials
and
Methods

MATERIALS AND METHODS

Experimental Animals

The present study was conducted on six clinically healthy lactating goats of non-descript breed between 1.5 to 2 yrs. of age and 20 to 25 kg body weight. The goats were housed in the animal shed with concrete floor. The goats were maintained on korai, chunni and greens as well as on routine grazing of at least 4-5 hours a days. Clean water was supplied *ad. lib*. The goats were provided ear tags for proper identification. 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 appropriate drugs. The preliminary health check up was carried out in each goat prior to the experiment.

Experimental Drug

The drug ofloxacin (Tarivid®) injection (100 ml) an injectable commercial preparation manufactured by Hoechst-Marion Roussel Limited, India which contains 2 mg/ml ofloxacin base in water was used. The drug was injected at the dose rate of 4mg/kg body weight in each goat by i.v. as well as i.m. route in healthy and febrile goats to carry out the present study.

Experimental Design

The drug (ofloxacin) was studied on a group of six goats. A gap of 2 weeks was allowed to lapse before administration of the next dose. The drug was administered by intravenous (i.v.) and intramuscular (i.m.) routes in each healthy goat initially. After inducing febrile state, the drug was administered by i.v. as well as i.m. routes to predict the variation in distribution of the drug in different biological fluids as well as pharmacokinetic parameters.

Induction of Febrile State

Three clinically healthy lactating goats were used initially for standardizing the febrile condition. Rectal temperature was noted in each goat at a particular time for three consecutive days. When the temperature was noted to be similar for all the days, the initial trial was carried out. Lipopolysaccharide of *E. coli* (055-B5) of Difco Laboratories, USA was dissolved in sterile distilled water to make a solution of 2 μg/ml. The lipopolysaccharide was injected i.v. at a dose of 0.25, 0.50, 1.0 and 2.0 μg/ml in three goats of each dose level and rise of temperature was noted every half an hour. A rise of temperature of 1.5-2.5^{0}F was noted after ½ -1 hour post injection at the dose of 1.0 μg/kg body weight. The temperature was maintained for about 6-8 hours. The drug was administered after the rise of temperature i.e. ½ – 1 hour after the injection of *E. coli* toxin. The

temperature was recorded at every ½ hour up to 8 hour post injection of drug.

Collection of blood, milk & urine and their timings

The samples of blood, milk and urine were collected post i.v. and i.m. injection in healthy and febrile goats. The samples were collected at 0.042, 0.083, 0.167, 0.25, 0.333, 0.50, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h post i.v. and i.m. administration of ofloxacin for estimating ofloxacin levels.

A. Blood Sampling

Hairs around the jugular vein on either side of neck of the goats were shaved by shaving blade and the area was cleaned with ether. The site of prick was properly sterilized prior to each collection with rectified spirit Blood samples were collected in sterilized centrifuge tubes containing appropriate amount of sodium oxalate from jugular vein by venipuncture at the above noted various time intervals following drug administration. The blood samples were centrifuged at 2500 rpm for 10 minutes for the separation of plasma. The plasma samples thus obtained were kept in a refrigerator. For preparation of plasma standards of the drug, plasma collected prior to drug administration was used.

B. Milk Sampling

The udder of the goat was washed with soap water and dried with clean soft towel. The milk was collected in sterile test tubes by hand milking. The milk samples were collected at various time intervals as noted above following administration of the drug. The samples thus collected were estimated on following days. For preparation of milk standards of the drug, milk collected prior to drug administration was used.

C. Urine Sampling

On the day of experiment, a sterile Foley's balloon catheter (No–12) lubricated with glycerine was introduced 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 25-30 ml of sterile water through a syringe to keep the catheter in position.

The catheter was fixed with a pressure clip to check dripping of urine. After administration of the drug, the urine samples were collected in sterile test tubes at various times intervals as noted above. The samples were kept in a refrigerator and the drug concentrations were estimated on following days. For preparation of urine standards of the drug, urine collected prior to drug administration was used.

Administration of Drug

The drug ofloxacin (Tarivid) containing 2 mg/ml of free drug as ofloxacin base in water was injected through i.v. and i.m. route separately in each goat under healthy condition as well as after inducing febrile state. The biological samples of blood, urine and milk were collected before and after injection of the drug at different time intervals as mentioned above for estimating the concentrations of ofloxacin in the respective biological fluid.

Procedure adopted for the micro-biological assay

Strerlization of glasswares, needles and porcelin assay cylinders :

All glasswares, needles and porcelin assay cylinders were properly washed with detergent solution in running tap water. These were again rinsed with glass distilled water and finally air dried. Test tubes, centrifuge tubes, vials, porcelin assay cylinders placed in vials and needles put in test tubes were plugged with cotton wool. Assay plates, pipettes and syringes were wrapped by paper. All these materials were sterilized in hot air oven at 160^0C for an hour.

Preparation of media

(A) *Assay Agar* :- Antibiotic assay agar media with the following composition was used for microbiological assay of ofloxacin in blood, milk and urine after i.v. and i.m. administration in goat.

Sl. No.	Ingredients	Grams/Liter
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, which was plugged with cotton wool. Wet sterlization of media was done by autoclaving at 15 pound pressure (121^{0}C) for 20 min.

(B) Nutrient Broth :

The composition of nutrient broth is shown below

Sl. No.	Ingredients	Grams/Liter
1.	Sodium Chloride	5.0
2.	Peptone	10.0
3.	Beef extract	10.0
	Distilled water	1000 ml
	pH	7.4 (approximately)

Sterlization of the broth was done as mentioned above.

Preparation of assay agar plates

20 ml of melted antibiotic assay media was poured gently with the aid of a sterile measuring cylinder into each of the sterile special assay plate (Borosil) kept on a horizontally plane surface to get uniform thickness of media. The plates were left at room temperature for about 1 to 2 hours for solidification of agar. The plates were kept inside the incubator at 37^{0}C for 24 hour to ascertain any growth that indicates any microbial contamination. The growth free plates were then stored in refrigerator until assay was carried out.

Preparation of test organism

The test organism used for the microbiological assay technique of ofloxacin was *E. coli* (ATCC 25922). The culture of *E. coli* was obtained from National Collection of Industrial Micro-organism (NCIM), Division of Bio-chemical Sciences, National Chemical Laboratory, Poona-8. The organism was grown on the slant of culture tube containing nutrient 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.

Preparation of standards in biological samples

The drug (ofloxacin) was dissolved and diluted in glass distilled water to have different strengths viz., 100μg/ml, 50μg/ml, 20μg/ml, 5μg/ml, 2μg/ml, 1μg/ml and 0.5μg/ml. From each standard

solution 0.1ml was added to a sterile vial containing 0.9 ml of plasma, milk and urine collected prior to drug administration. This yielded drug standards of 10μg/ml 5μg/ml, 2μg/ml, 1μg/ml, 0.5μg/ml, 0.2μg/ml, 0.1μg/ml and 0.05μg/ml in the above noted biological fluid. These standards were used simultaneously with test samples in the assay plates for determination of the drug concentration in the test samples.

ASSAY PROCEDURE

The plasma, milk and urine levels of ofloxacin were estimated by microbiological assay technique (cylinder plate diffusion method) using *E. coli* (ATCC 25922) as the test organism. The test organism was grown in nutrient broth for ½ to 1 hour at 37°C until the growth was seen (turbid by naked eye). Ofloxacin assay plates were flooded with the broth containing the organism and excess broth was drained out after some time. The plates were dried in the incubator at 37°C for a period of about an hour. Sterile porcelin assay cylinders of uniform size were placed at appropriate distance along the circumference in the inoculated assay plates. 50 microlitres of standard solution of various strengths as well as test samples of the drug was poured in separate porcelin cylinder in the assay plate. Such plates were left on the table for about 2 hours and then kept in the incubator at 37°C for overnight in order to allow the growth of

organism. The mean diameter of the bacterial zone of inhibition produced by the 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 THE PHARMACOKINETIC PARAMETERS

The following pharmacokinetic parameters of ofloxacin following a single i.v. and i.m. administration were calculated from semilog plot of plasma drug concentration versus time curve. The experimental data was analysed using one compartment (for i.m. route) or two compartment (for i.v. route) open model.The concentration of the drugs in plasma at any time is obtained by the following formula.

(i) $C_p = B_e^{-\beta t}$ ..... (1-compartment model)

(ii) $C_p = A_c^{-\alpha t} + B_e^{-\beta t}$ ..... (2- compartment model)

C_p = Plasma drug concentration at time 't'

A = Zero time concentration of the drug in plasma during distribution/absorption phase.

α = Regression coefficient for distribution phase (distribution rate constant calculated by the method of residual yield)

B = Zero time concentration of the drug in plasma during elimination phase.

β = Regression co-efficient for elimination phase (elimination rate constant calculated by the method of least squares).

e = Base of natural logarithm.

Ka = Regression co-efficient for absorption rate constant after i.m. administration of the drug (calculated by the method of residual yield)

(a) Zero time theoretical plasma concentration of drug (C_o^p)

$C_o^p = A + B$ (2-compartment model) theoretical plasma concentration of drug at zero time.

(b) $t_{1/2}\, K_a = 0.693/Ka$

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

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

Where – $t_{1/2}Ka$ = Absorption half life.

$t_{1/2}\, \alpha$ = Distribution half life.

$t_{1/2}\, \beta$ = Elimination half life.

Ka, α , and β are described above.

(c) The total area under the curve (AUC)

(i) For 2- compartment model

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

(ii) For 1-compartment model

$$AUC = \frac{B}{\beta} - \frac{A}{\alpha}$$ (Rischell, 1976)

(d) Rate constant of transfer of drug from peripheral (tissue) compartment to the central (blood) compartment (K_{21})

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

(e) The Elimination rate constant of drug from central compartment (Kel).

$$K_{el} = \frac{\alpha \times \beta}{K_{21}}$$

(f) The rate constant of transfer of drug form central to peripheral compartment (K_{12})

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

(g) The fraction of drug available for elimination from central compartment (F_c)

$$F_c = \frac{\beta}{Kel}$$

(h) The approximate tissue to plasma concentration ratio ($T \approx P$)

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

(i) The volume of distribution based on distribution and elimination (V_d)

$$V_d = \frac{D}{C_o^p}$$

(j) The volume of distribution based on elimination (V_{dB})

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

(k) The volume of distribution based on total area under curve (Vd_{area})

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

(l) The volume of distribution at steady state (Vd_{ss})

$$Vd_{s.s} = \frac{K_{12} + K_{21}}{K_{21}} \times Vd$$

(m) The total body clearance (Cl_B)

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

CALCULATION OF DOSAGE REGIMEN

Edlstein *et al.* (1996) reported that 90% strains of clinical *Legenella* isolates were inhibits by a concentration of 0.032 μg/ml of ofloxacin. The MIC values of enrofloxacin (a close congener of ofloxacin) for different species of micro-organisms ranges between 0.01 to 1 μg/ml in veterinary practice (Mevius *et al.*, 1990; Prescott and Yielding, 1990). Sudha Kumari (1998) and Uday Kumar (2000) has taken 0.12 μg/ml as MIC value for calculating dosage regimen of enrofloxacin. Similarly, various workers (Singh, 1998; Srivastava, 1987) have taken 0.12 μg/ml as MIC for calculating dosage regimen of ciprofloxacin, another closely related congener of ofloxacin. Hence, in the present study, dosage regimen of ofloxacin were calculated at 0.12 μg/ml level for the dosage interval of 8 and 12 h using the formulae (Saini and Srivastava, 1997).


Results

RESULTS

I. PHARMACOKINETIC STUDY OF OFLOXACIN IN HEALTHY GOATS AFTER A SINGLE I.V. ADMINISTRATION

1. Plasma levels

The plasma drug concentration profile at various time intervals after a single i.v. dose (4mg/Kg) of ofloxacin in healthy goats has been shown in Table-1 and Fig-1. The mean plasma concentration of the drug at 0.042 h (2.5 min) was found to be 7.64 ± 0.34 μg/ml. The drug was detectable in plasma samples of all the goats up to 6 h with mean plasma concentration of 0.19 ± 0.04 μg/ml. The drug was detectable in five out of six animals at 8 h and none of the animals at 10 h. The mean therapeutic concentration (≥0.12 μg/ml) of the drug was maintained from 2.5 min to 6 h.

2. Milk levels

The concentrations of ofloxacin in milk following i.v. administration (4mg/kg) are depicted in Table–2 and Fig–2. The drug appeared in milk samples of all the goats at 1.5 h with the value of 0.62 ± 0.14 μg/ml. The peak milk drug concentration of 1.45 ± 0.08 μg/ml was attained at 3 h. The drug was detectable up to 5 h in all goats with mean concentration of 0.68 ± 0.21 μg/ml. The drug was detectable at five, four, three and one animal at 6, 8, 10 and 12 h, respectively. The mean therapeutic concentration (≥ 0.12 μg/ml) was attained at 0.75 h and was maintained up to 10 h.

Table – 1 Plasma concentrations (μg/ml) of ofloxacin in healthy goats after a single i.v. dose.

Time (h) ↓	Animal Number						Mean ± S.E. ↓
	1	2	3	4	5	6	
0.042	7.83	8.12	7.50	6.98	8.85	6.55	7.64 ± 0.34
0.083	4.25	6.15	5.82	5.15	7.82	5.20	5.73 ± 0.50
0.167	1.99	5.80	4.64	4.00	6.10	2.85	4.23 ± 0.66
0.25	1.70	4.15	3.88	3.10	4.58	2.00	3.24 ± 0.48
0.333	1.46	3.20	3.00	2.62	3.80	1.65	2.62 ± 0.37
0.50	1.25	2.40	2.50	2.00	2.55	1.38	2.01 ± 0.24
0.75	0.93	1.80	1.92	1.40	1.92	1.20	1.53 ± 0.17
1	0.68	1.15	1.25	0.94	1.76	0.82	1.10 ± 0.16
1.5	0.50	0.96	0.90	0.75	1.15	0.66	0.82 ± 0.09
2	0.37	0.90	0.78	0.68	0.96	0.50	0.70 ± 0.09
3	0.27	0.62	0.56	0.52	0.70	0.35	0.50 ± 0.07
4	0.08	0.50	0.36	0.40	0.52	0.20	0.34 ± 0.07
5	0.06	0.38	0.30	0.28	0.36	0.14	0.25 ± 0.05
6	0.05	0.26	0.22	0.20	0.30	0.08	0.19 ± 0.04
8	ND	0.16	0.10	N.D	0.16	N.D	0.07 ± 0.03
10	-	N.D	N.D	-	N.D	-	-

N. D. = Non-Detectable

Fig.-1

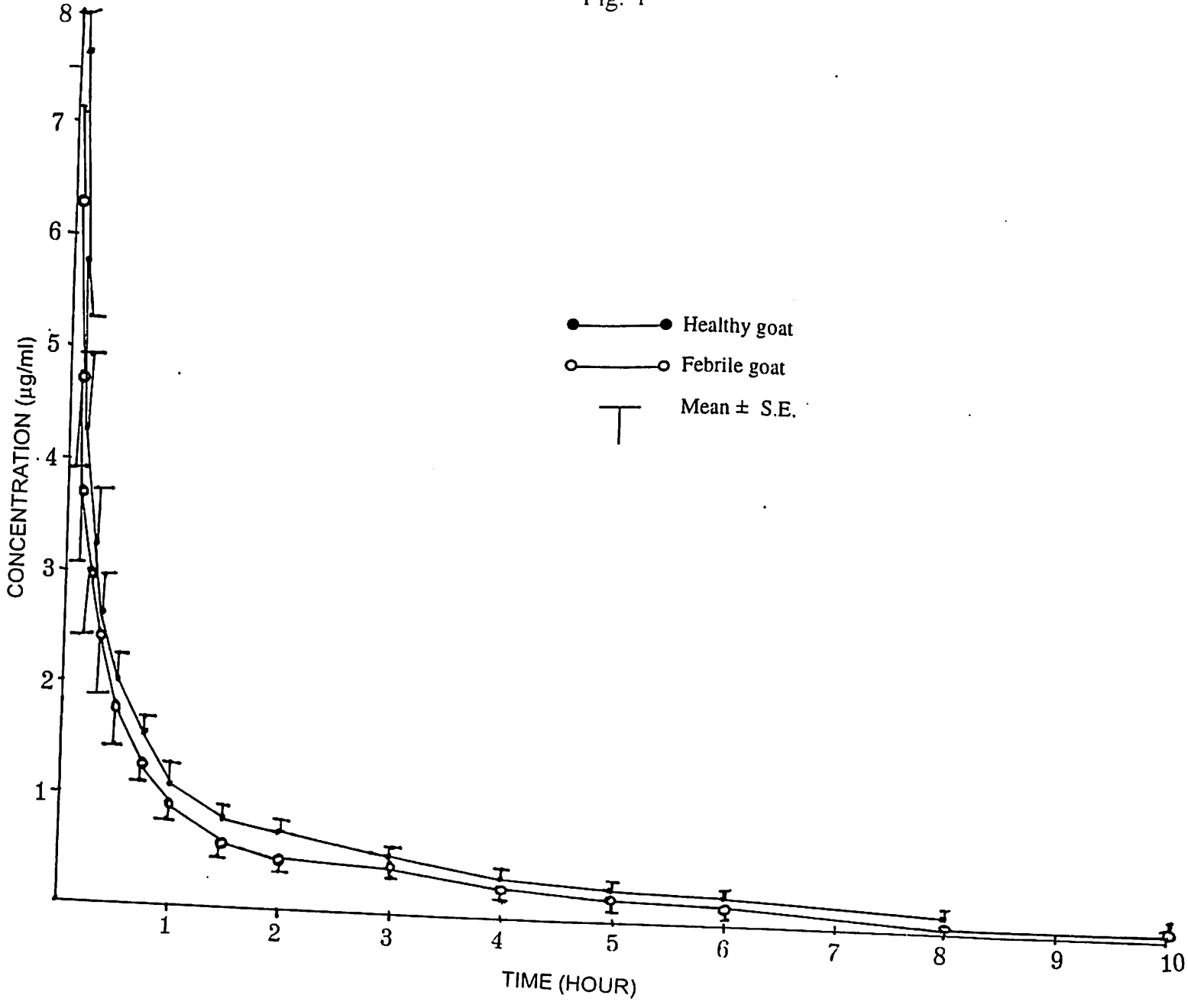
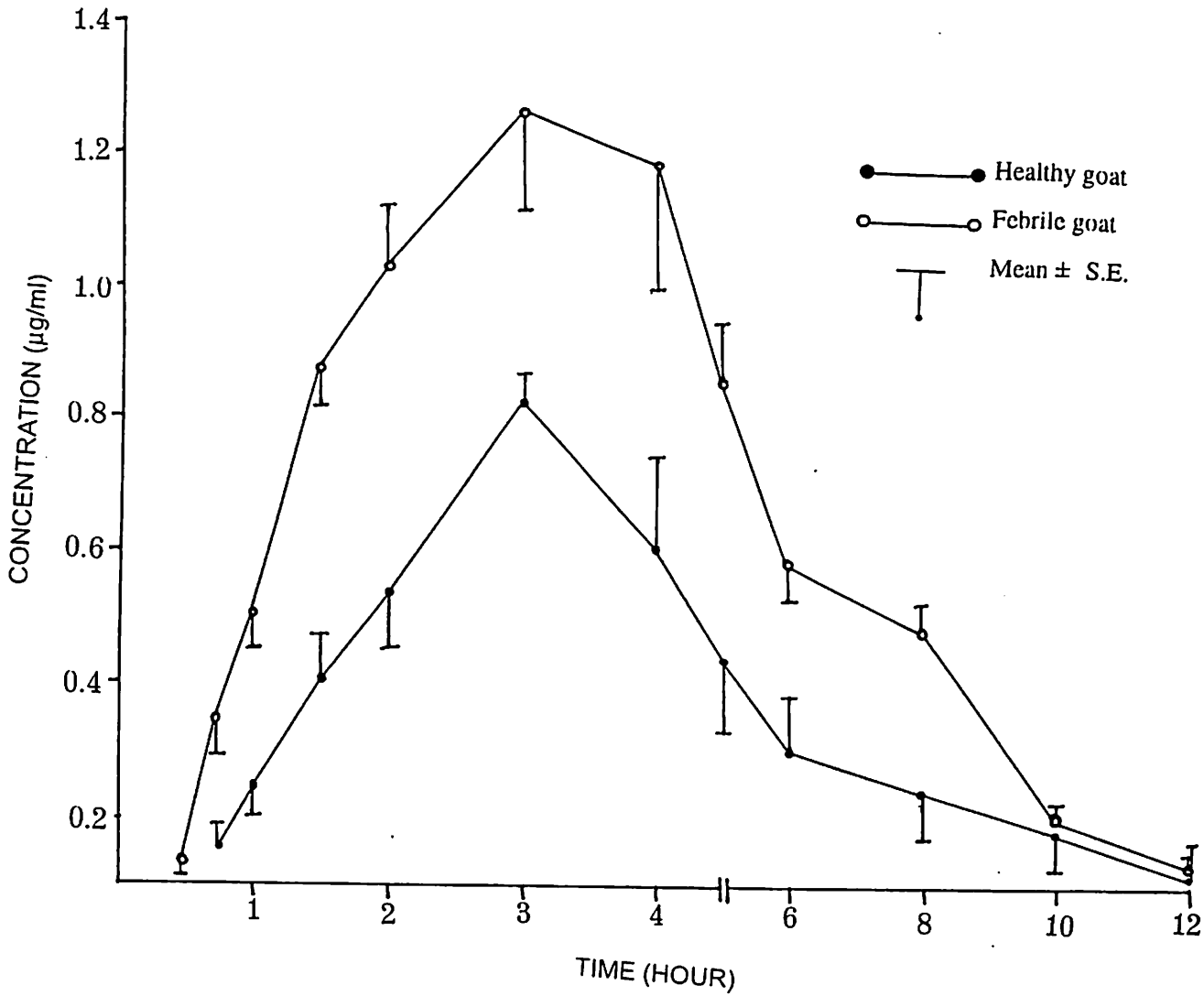

CONCENTRATION (µg/ml)
TIME (HOUR)
Healthy goat
Febrile goat
Mean ± S.E.

Table – 2 Milk concentrations (μg/ml) of ofloxacin in healthy goats after a single i.v. dose.

Time (h) ↓	Animal Number						Mean ± S.E. ↓
	1	2	3	4	5	6	
0.042							
0.083							
0.167							
0.25							
0.333							
0.50							
0.75	N.D	N.D	0.42	N.D	0.18	0.10	0.12 ± 0.07
1	0.00	0.32	0.54	0.28	0.50	0.15	0.30 ± 0.08
1.5	0.17	0.45	0.97	0.85	0.92	0.38	0.62 ± 0.14
2	0.25	0.65	1.23	1.15	1.25	0.75	0.88 ± 0.16
3	1.15	1.58	1.56	1.50	1.65	1.28	1.45 ± 0.08
4	0.36	0.45	1.75	0.65	1.90	1.00	1.02 ± 0.27
5	0.17	0.22	1.56	0.48	0.95	0.68	0.68 ± 0.21
6	N.D	0.05	0.97	0.22	0.78	0.52	0.42 ± 0.16
8		N.D	0.86	0.12	0.42	0.32	0.29 ± 0.13
10			0.68	N.D	0.18	0.15	0.17 ± 0.11
12			0.24		N.D		0.04 ± 0.04
24			N.D	-	-	-	

N. D. = Non-Detectable

Fig.-2


1.4
1.2
1.0
0.8
0.6
0.4
0.2
CONCENTRATION (µg/ml)
1
2
3
4
6
8
10
12
TIME (HOUR)
Healthy goat
Febrile goat
Mean ± S.E.

3. *Urine levels*

The concentrations of the drug in urine post i.v. administration (4mg/Kg) have been presented in Table – 3 and Fig – 3. The drug appeared in urine samples of all the goats at 0.042 h with mean concentration of 20.05 ± 11.40 μg/ml. The mean peak urine concentration of 499.5 ± 74.87 μg/ml was obtained at 0.75 h. The drug was detectable up to 24 h in all goats (0.19 ± 0.02 μg/ml). The drug appeared in effective concentration (≥0.12 μg/ml) at 2.5 min and was maintained up to 24 h.

4. *Kinetic parameters*

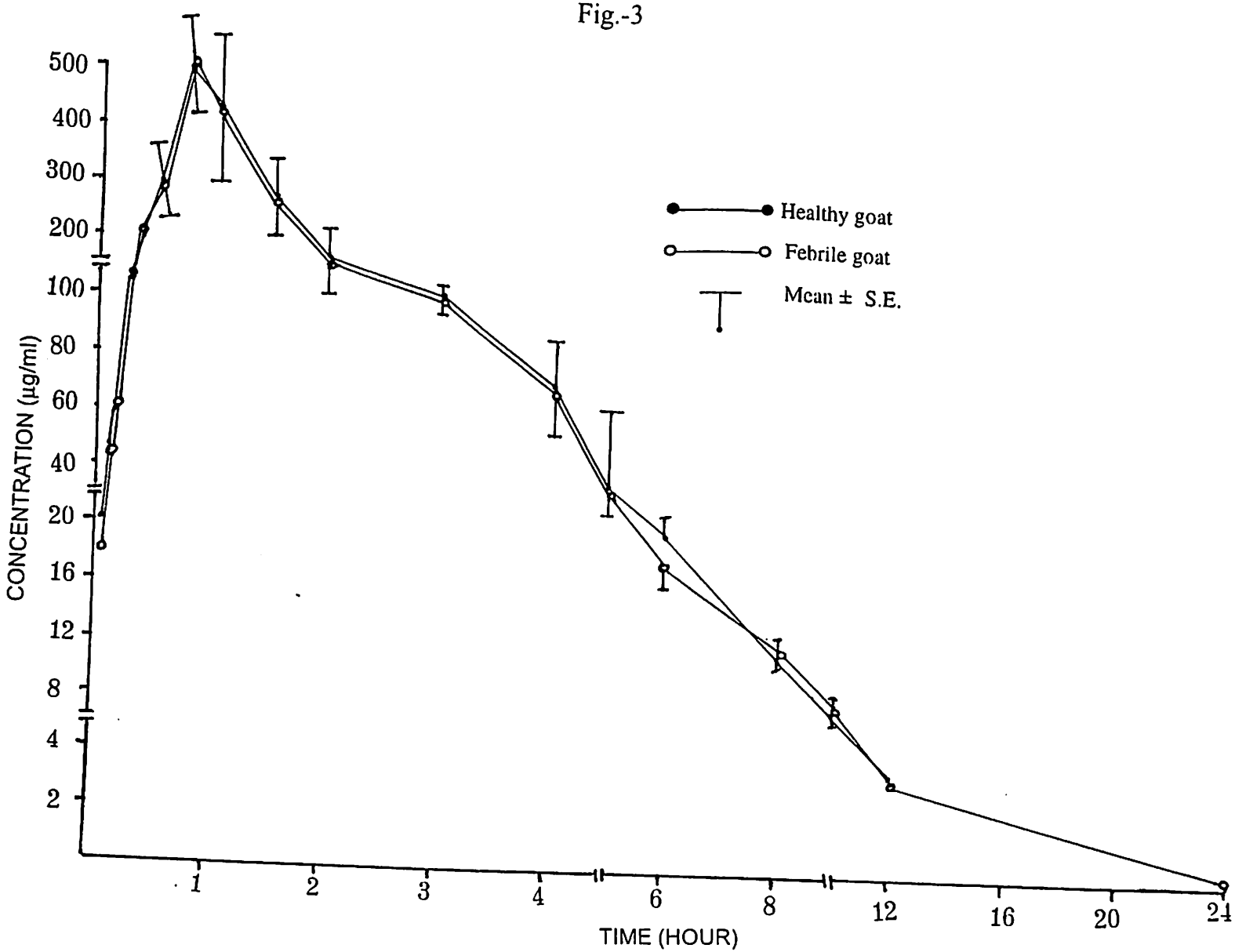
The plasma drug concentration versus time profile has confirmed the 2 compartment open model as shown in Table – 4 and Fig – 4. Table - 4 presents the values of different pharmacokinetic parameters calculated by the above compartment model. The mean extrapolated zero time concentration of the drug in plasma during distribution phase (A) elimination phase (B) and the theoretical zero time concentration (C_o^p) were noted to be 5.37 ± 0.58, 1.42 ± 0.08 and 6.79 ± 0.65 μg/ml, respectively. The distribution constant (∝) ranged from 3.033 to 4.810 h^{-1} with a mean value of 3.718 ± 0.273 h while its elimination rate constant (β) ranged from 0.291 to 0.595 h^{-1} with a mean value of 0.379 ± 0.050 h^{-1}. The mean distribution ($t_{1/2}\alpha$) and elimination ($t_{1/2}\beta$) half-life of the drug were obtained to be 0.23 ± 0.05 and 1.96 ± 0.20 h, respectively. The average rate of transfer of drug

Table – 3 Urine concentrations (μg/ml) of ofloxacin in healthy goats after a single i.v. dose (4mg/kg).

Time (h) ↓	Animal Number						Mean ± S.E. ↓
	1	2	3	4	5	6	
0.042	74.5	6.50	5.85	4.12	3.82	25.50	20.05 ± 11.40
0.083	112.5	27.9	19.5	17.85	16.55	85.85	46.70 ± 17.03
0.167	134.4	39.6	22.5	20.15	20.80	120.6	59.67 ± 21.72
0.25	191.1	113.4	95.1	90.20	85.85	185.6	126.9 ± 19.83
0.333	386.7	135.3	169.0	140.6	150.2	225.2	201.2 ± 39.43
0.50	550.2	161.4	226.2	208.5	222.0	420.8	296.2 ± 62.26
0.75	782.7	229.2	402.2	550.8	480.2	550.6	499.5 ± 74.87
1	920.7	192.3	226.2	320.2	280.6	650.8	431.8 ± 62.69
1.5	550.2	161.4	195.9	210.8	185.8	380.5	280.8 ± 62.69
2	386.7	128.2	89.9	110.6	115.2	195.8	171.1 ± 45.57
3	216.2	107.6	63.42	85.85	90.55	128.2	115.3 ± 22.04
4	152.0	75.6	35.65	55.64	52.20	68.60	73.28 ± 16.73
5	82.45	27.94	15.00	32.65	23.20	42.55	37.30 ± 29.80
6	26.09	18.85	9.35	20.20	16.45	16.80	17.97 ± 2.23
8	15.38	12.24	6.40	14.18	9.85	12.25	11.72 ± 1.31
10	9.06	8.86	4.25	9.15	6.20	8.12	7.61 ± 0.81
12	2.25	4.46	3.6	4.18	4.00	2.55	3.51 ± 0.37
24	0.18	0.24	0.12	0.20	0.25	0.16	0.19 ± 0.02

N. D. = Non-Detectable

Fig.-3


500
400
300
200
100
80
60
40
20
16
12
8
4
2
CONCENTRATION (µg/ml)
Healthy goat
Febrile goat
Mean ± S.E.
1
2
3
4
6
8
12
16
20
24
TIME (HOUR)

from central to peripheral (K_{12}) peripheral to central (K_{21}) compartment and elimination from central compartment (Kel) were calculated to 1.861 ± 0.200, 1.110 ± 0.145 and 1.269 ± 0.094 h^{-1}, respectively. The fraction of drug available for elimination from central compartment (Fc) and approximate tissue to plasma concentration ratio (T≈P) were noted to be 0.28 ± 0.03 and 2.53 ± 0.20, respectively. The values of area under curve in plasma (AUC), area under first moment Curve (AUMC) and mean residential time (MRT) were noted to be 5.63 ± 0.82 mg/L.h , 13.07 ± 2.51 mg/ $L.h^2$ and 2.14 ± 0.21 h. The various values of volume distribution calculated by different methods are shown in Table – 4. The mean value of Vd_{area} was calculated to be 2.30 ± 0.13 L/Kg. The total body clearance (Cl_B) ranged from 0.50 to 1.43 with a mean value of 0.82 ± 0.15 ml/Kg/min.

5. Calculated dosage regimen

Table 5 shows the calculated dosage regimen of ofloxacin for iv route in healthy goats.For treating systemic infections (C_B^{∞} min = 0.12 μg/ml) at the dosage interval (γ) of 8 h, the loading (D*) and maintenance (D_o) doses were calculated to be 9.24 ± 5.06 and 8.99 ± 5.05 mg/Kg, respectively, while at γ of 12 h, D* and D_o were calculated to be 78.17 ± 57.84 and 77.92 ± 57.83 mg/Kg, respectively.

Table – 4 Kinetic parameters of ofloxacin in healthy goats after a single i.v. dose. (4mg/kg).

Parameters (Unit) ↓	Animal Number						Mean ± S.E. ↓
	1	2	3	4	5	6	
A (μg/ml)	3.52	6.74	5.97	5.40	6.79	3.80	5.37 ± 0.58
B (μg/ml)	1.23	1.51	1.50	1.24	1.75	1.30	1.42 ± 0.08
(μg/ml)	4.75	8.25	7.47	6.64	8.54	5.10	6.79 ± 0.65
α (h^{-1})	4.810	3.418	3.274	3.546	3.033	4.227	3.718 ± 0.273
β (h^{-1})	0.595	0.291	0.332	0.298	0.301	0.458	0.379 ± 0.050
$t_{1/2}$ α (h)	0.14	0.47	0.21	0.20	0.21	0.16	0.23 ± 0.05
$t_{1/2}$ β (h)	0.16	2.38	2.09	2.33	2.30	1.51	1.96 ± 0.20
AUC (mg/L.h.)	2.80	7.16	6.34	5.68	8.05	3.74	5.63 ± 0.82
AUMC (mg/L.h^2)	5.00	18.41	14.16	14.39	20.05	6.41	13.07 ± 2.51
MRT (h)	1.295	2.57	2.23	2.53	2.49	1.71	2.14 ± 0.21
K_{12} (h^{-1})	2.021	1.693	1.506	1.771	1.413	2.764	1.861 ± 0.200
K_{21} (h^{-1})	1.686	0.863	0.922	0.905	0.861	1.423	1.110 ± 0.145
Kel (h^{-1})	1.697	1.153	1.178	1.168	1.060	1.360	1.269 ± 0.094
c	0.35	0.25	0.28	0.18	0.28	0.34	0.28 ± 0.03
≈P	1.85	2.96	2.04	2.92	2.52	2.86	2.53 ± 0.20
Vd (L/Kg)	0.84	0.48	0.54	0.60	0.47	0.78	0.62 ± 0.06
V_{dB} (L/Kg)	3.25	2.65	2.67	3.23	2.29	3.008	2.68 ± 0.16
$V_{d_{area}}$(L/Kg)	2.40	1.92	1.90	2.36	1.65	2.34	2.30 ± 0.13
$V_{d_{ss}}$(L/Kg)	1.85	1.42	1.42	1.77	1.24	2.29	1.67 ± 0.16
Cl_B (L/Kg h)	1.43	0.56	0.63	0.71	0.50	1.07	0.82 ± 0.15

Fig.-4

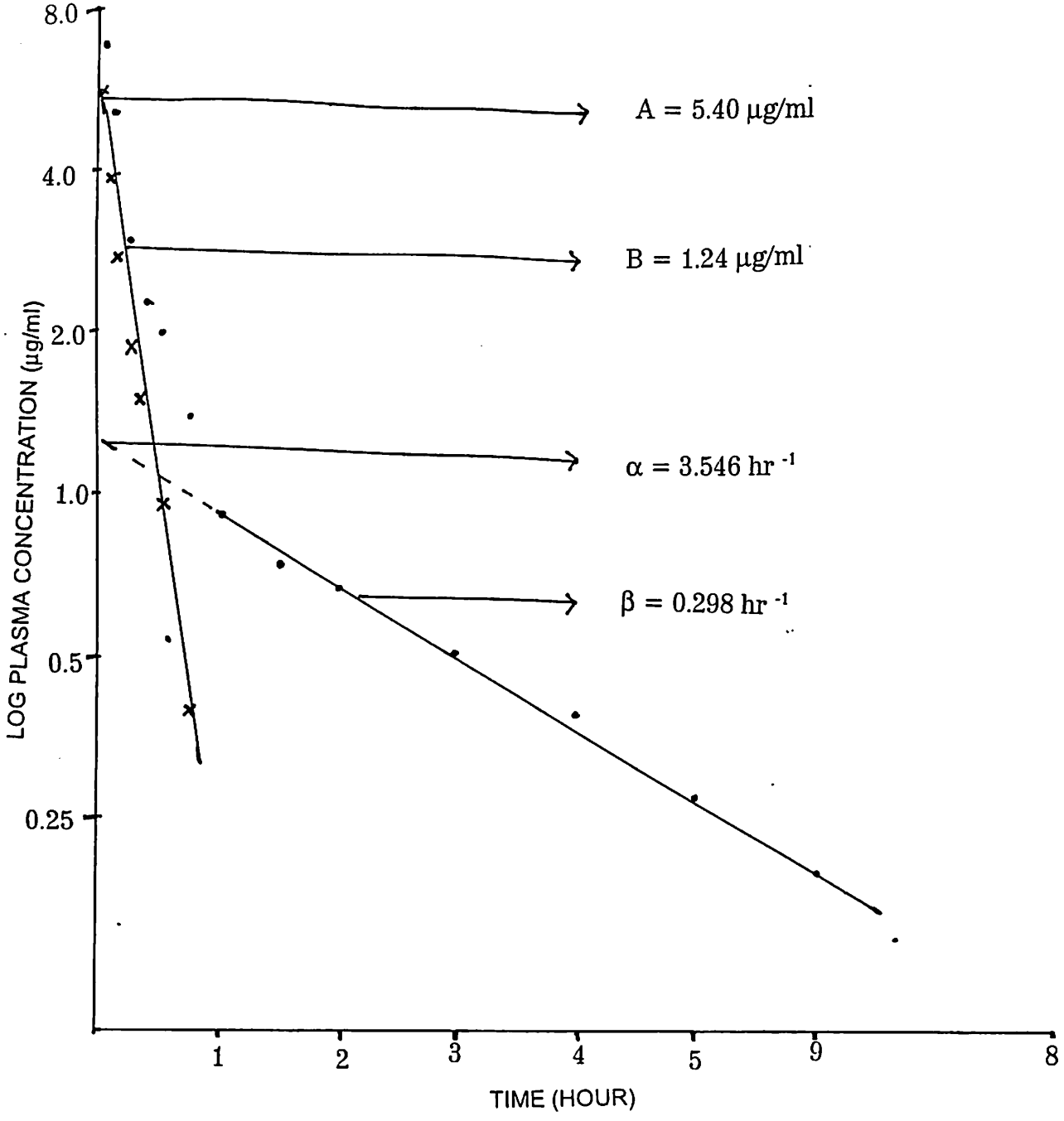

8.0
4.0
2.0
1.0
0.5
0.25
LOG PLASMA CONCENTRATION (µg/ml)
A = 5.40 µg/ml
B = 1.24 µg/ml
α = 3.546 hr -1
β = 0.298 hr -1
1
2
3
4
5
9
8
TIME (HOUR)

Table – 5 Calculated dosage regimen of ofloxacin after a single i.v. dose. (4mg/kg).

$C^{\infty}_{p\,min}$ = 0.12 µg/ml ↓		Animal Number						Mean ± S.E. ↓
		1	2	3	4	5	6	
γ = 8 h	D^*	33.62	2.36	3.25	3.07	2.20	10.96	9.24 ± 5.06
	D_0	33.33	2.13	3.02	2.79	2.00	10.68	8.99 ± 5.05
γ = 12 h	D^*	363.29	7.57	12.25	10.12	7.33	68.44	78.17 ± 57.84
	D_0	363.00	7.34	12.02	9.84	7.14	68.15	77.92 ± 57.83

$C^{\infty}_{p\,min}$ = Minimum inhibitory concentration

D^* = Loading dose in mg/Kg

D_0 = Maintenance dose in mg/Kg

γ = Dose interval

II. PHARMACOKINETIC STUDY OF OFLOXACIN IN FEBRILE GOATS AFTER A SINGLE I.V. ADMINISTRATION

1. Plasma levels

The plasma drug concentration versus time profile after i.v. administration (4mg/Kg) of ofloxacin in febrile goat has been depicted in Table – 6 and Fig –1. The mean plasma concentration of the drug at 2.5 min (0.042 h) was noted to be 6.29 ± 0.77 μg/ml. The drug was detectable up to 5 h in all goats and the mean plasma drug concentration was observed to be 0.18 ±0.04 μg/ml. The mean therapeutic concentration (≥0.12 μg/ml) of the drug was maintained from 2.5 min to 6 h.

2. Milk levels

The concentrations of ofloxacin in milk following i.v. administration (4 mg/Kg) in febrile goats are shown in Table – 7 and Fig – 2. The drug appeared in milk samples of all goats at 0.75 h with a mean of 0.49 ± 0.10 μg/ml. The drug reached its mean peak concentration (2.33 ± 0.28 μg/ml) at 3 h. The drug was detectable up to 10 h in all goats with a mean of 0.21 ± 0.03 μg/ml. In three out of six goats, it was detectable even at 12 h. The therapeutic concentration (≥ 0.12 μg/ml) was maintained from 0.75 to 10 h.

Table – 6 Plasma concentrations of ofloxacin (μg/ml) in febrile goats after a single i.v. administration.

Time (h) ↓	Animal Number						Mean ± S.E. ↓
	1	2	3	4	5	6	
0.042	3.83	5.85	9.12	6.80	4.78	7.35	6.29 ± 0.77
0.083	1.52	3.80	6.75	6.15	4.00	5.90	4.68 ± 0.79
0.167	1.28	3.00	4.48	5.78	3.55	4.15	3.71 ± 0.62
0.25	1.08	2.20	2.98	5.00	3.07	3.43	2.96 ± 0.53
0.333	0.91	1.70	1.98	4.15	2.68	2.84	2.38 ± 0.46
0.50	0.64	1.70	1.31	2.96	2.05	1.95	1.76 ± 0.31
0.75	0.58	1.45	0.87	1.98	1.40	1.20	1.25 ± 0.20
1	0.54	1.05	0.58	1.35	0.95	0.88	0.89 ± 0.12
1.5	0.33	0.90	8.39	0.54	0.42	0.76	0.56 ± 0.09
2	0.27	0.82	0.14	0.45	0.33	0.60	0.43 ± 0.10
3	0.23	0.60	0.11	0.35	0.27	0.45	0.34 ± 0.07
4	0.19	0.50	0.08	0.24	0.22	0.32	0.26 ± 0.06
5	0.12	0.36	0.05	0.19	0.15	0.20	0.18 ± 0.04
6	0.08	0.28	N.D.	0.13	0.12	0.16	0.13 ± 0.04
8	N.D.	0.15	--	0.07	0.08	0.08	0.06 ± 0.02
10	--	0.09	--	N.D.	N.D.	N.D.	0.02 ± 0.02

N.D. = Non-Detectable

Table – 7 Milk concentrations of ofloxacin (μg/ml) in febrile goats after a single i.v. administration. (4mg/kg).

Time (h) ↓	Animal Number						Mean ± S.E. ↓
	1	2	3	4	5	6	
0.042							
0.083							
0.167							
0.25							
0.333	--	N.D.	N.D.	--	N.D.	--	
0.50	N.D.	0.18	0.12	N.D.	0.14	N.D.	0.07 ± 0.03
0.75	0.28	0.83	0.58	0.25	0.68	0.30	0.49 ± 0.10
1	0.64	1.13	0.95	0.50	0.98	0.68	0.81 ± 0.10
1.5	1.39	1.89	1.48	1.25	1.76	1.45	1.54 ± 0.10
2	1.53	2.45	1.82	1.46	2.25	1.60	1.85 ± 0.17
3	1.69	2.78	2.15	1.75	3.52	1.88	2.33 ± 0.28
4	1.39	3.60	2.94	1.58	2.10	1.42	2.17 ± 0.37
5	1.15	2.15	1.88	1.10	1.58	1.18	1.51 ± 0.18
6	0.78	1.46	0.95	0.82	1.05	0.80	0.98 ± 0.11
8	0.60	1.05	0.82	0.65	0.88	0.62	0.77 ± 0.07
10	0.12	0.25	0.20	0.15	0.35	0.16	0.21 ± 0.03
12	N.D.	0.14	0.10	N.D.	0.12	N.D.	0.06 ± 0.03

N.D. = Non-Detectable

3. *Urine levels*

The concentrations of the drug in urine post i.v. administration (4mg/kg) in febrile goats are shown in Table – 8 and Fig – 3. The drug appeared in urine samples of all the goats at 2.5 min with a mean concentration of 18.15 ± 9.80 μg/ml. The drug reached its mean peak concentration 499.9 ± 75.39 μg/ml at 1 h and the drug was detectable up to 30 h in all goats with its mean concentration of 0.21 ± 0.02 μg/ml. The therapeutic concentration was maintained from 2.5 min to even beyond 30 h.

4. *Kinetic parameters*

Kinetic parameters were calculated by 2 compartment open model since plasma drug concentration versus time profile had shown biphasic curve. Table – 9 shows the value of different kinetic parameters. The mean extrapolated zero time concentration during distribution phase (A), elimination phase (B) and theoretical zerotime concentration (C_o^p) were observed to be 4.75 ± 0.90, 0.82 ± 0.18 and 5.58 ± 0.94 μg/ml, respectively. The distribution rate constant (α) ranged from 2.127 to 4.371 h^{-1} with a mean of 2.945 ± 0.352 h^{-1}. The range of elimination rate constant (β) varied from 0.253 to 0.345 h^{-1} with an average of 0.304 ± 0.015 h^{-1}. The mean distribution ($t_{1/2}\alpha$) and elimination ($t_{1/2}\beta$) half-life were noted to be 0.25 ± 0.03 and 2.31 ± 0.12h, respectively. The average rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from

Table – 8 Urine concentrations of ofloxacin (μg/ml) in febrile goats after a single i.v. administration (4mg/kg).

Time (h) ↓	Animal Number						Mean ± S.E. ↓
	1	2	3	4	5	6	
0.042	65.5	8.50	6.00	4.00	4.12	20.80	18.15 ±9.80
0.083	108.0	28.9	20.5	16.25	17.55	82.45	45.61 ± 16.13
0.167	135.0	42.2	24.2	19.10	22.80	118.8	60.35 ± 21.40
0.25	192.2	115.5	95.8	88.50	88.95	180.8	127. ± 19.31
0.50	390.0	138.2	172.2	135.6	152.6	222.2	201.8 ± 39.81
0.75	540.0	165.2	228.8	200.8	225.5	418.6	296.5 ± 60.61
1	790.8	228.8	408.0	540.2	485.6	545.8	499.9 ± 75.39
1.5	910.8	190.6	230.5	315.6	282.2	644.6	429 ± 116.8
2	552.0	159.8	192.8	205.6	188.5	378.7	179.6 ± 63.16
3	380.0	126.5	85.9	108.8	117.5	190.8	168.25 ± 44.71
4	214.8	105.0	62.8	80.90	91.0	125.2	113.25 ± 22.06
5	150.5	74.8	36.0	53.6	53.2	66.6	72.45 ± 16.52
6	78.95	27.90	16.2	30.85	24.4	42.45	36.78 ± 9.13
8	26.50	18.40	9.85	18.80	16.85	15.95	17.72 ± 2.20
10	15.50	12.58	7.10	14.00	10.05	12.00	11.87 ± 1.22
12	9.26	9.10	4.50	9.55	6.85	7.95	7.87 ± 0.79
24	2.05	4.20	3.20	4.58	4.25	2.15	3.41 ± 0.45
30	0.20	0.22	0.14	0.22	0.28	0.18	0.21 ± 0.02

central (Kel) compartment were calculated to be 1.222 ± 0.269, 0.716 ± 0.096, and 1.160 ± 0.099 h^{-1} , respectively. The fraction of drug available for elimination from central compartment (Fc) and approximate tissue to plasma concentration ratio (T≈P) were noted to be 0.25 ± 0.03 and 3.28 ± 0.42, respectively. The average value of area under curve in plasma (AUC), area under first moment curve (AUMC) and mean residential time (MRT) were 4.47 ± 0.61 mg/L.h., 9.88 ± 2.11 mg/L.h^2 and 2.17 ± 0.29 h, respectively various values of volume distribution obtained by different methods are presented in Table-9. The mean value of Vd_{area} was calculated to be 3.29 ± 0.54 L/kg. The total body clearance (Cl_B) ranged from 0.67 to 1.74 with a mean of 1.00 ± 0.17 ml/Kg/min.

5. ***Calculated dosage regimen***

Table – 10 shows the calculated dosage regimen of ofloxacin for i.v. route in febrile goats. For treating systemic infections at the dosage interval (γ) of 8 h the D* and D_{0} were calculated to be 4.61 ± 0.88 and 4.21 ± 4.21 ± 0.82 mg/kg, respectively, while at γ of 12 h D* and D_{0} were calculated to be 16.10 ± 3.46 and 15.71 ± 3.41 mg/kg, respectively.

Table – 9 Kinetic parameters of ofloxacin in febrile goats after a single i.v. administration (4mg/Kg).

Parameter Units ↓	Animal Number						Mean ± S.E. ↓
	1	2	3	4	5	6	
A (μg/ml)	1.44	3.08	5.81	6.83	4.35	6.98	4.75 ± 0.90
B (μg/ml)	0.54	1.40	0.29	0.87	0.58	1.24	0.82 ± 0.18
(C) μg/ml)	1.98	4.48	6.18	7.70	4.93	8.22	558 ± 0.94
α (h^{-1})	2.869	3.528	2.543	2.229	2.127	4.371	2.945 ± 0.352
β (h^{-1})	0.300	0.274	0.341	0.313	0.253	0.345	0.304 ± 0.015
$t_{1/2}\alpha$ (h)	0.24	0.20	0.27	0.31	0.33	0.16	0.25 ± 0.03
$t_{1/2}\beta$ (h)	2.31	2.53	2.03	2.21	2.74	2.01	2.31 ± 0.12
AUC (mg/L.h.)	2.30	5.98	3.13	5.84	4.34	5.20	4.47 ± 0.61
AUMC (mg/L.h^2)	6.18	18.65	3.39	10.25	10.02	10.79	9.88 ± 2.11
MRT (h)	2.68	3.12	1.08	1.76	2.31	2.07	2.17 ± 0.29
K_{12} (h^{-1})	1.309	1.780	0.470	0.825	0.770	2.180	1222 ± 0.269
K_{21} (h^{-1})	0.999	0.775	0.439	0.659	0.473	0.952	0.716 ± 0.096
Kel (h^{-1})	0.861	1.247	1.075	1.058	1.137	1.584	1.160 ± 0.099
Fc	0.35	0.22	0.17	0.30	0.22	0.22	0.25 ± 0.03
T≈P	1.87	3.55	4.79	2.38	3.50	3.59	3.28 ± 0.42
Vd (L/Kg)	2.02	0.89	0.65	0.52	0.81	0.49	0.90 ± 0.23
Vd_B (L/Kg)	7.40	2.86	13.79	4.60	6.90	3.23	6.45 ± 1.64
Vd_{area}(L/Kg)	5.80	2.44	3.75	2.19	3.33	2.23	3.29 ± 0.54
Vd_{ss}(L/Kg)	4.67	2.93	1.35	1.17	2.13	1.61	2.31 ± 0.54
Cl_B (L/Kg h)	1.74	0.67	1.28	0.69	0.84	0.77	1.00 ± 0.17

Table – 10 Calculated dosage regimen of ofloxacin in febrile goats after a single i.v. dose (4mg/kg).

$C^{\infty}_{p\,min}$ = 0.12 µg/ml ↓		Animal Number						Mean ± S.E. ↓
		1	2	3	4	5	6	
8 h	D^*	7.67	2.62	6.89	3.21	3.02	4.23	4.61 ± 0.88
	D_o	6.98	2.33	6.44	2.95	2.62	3.96	4.21 ± 0.82
12 h	D^*	25.47	7.84	26.94	11.24	8.32	16.81	16.10 ± 3.46
	D_o	24.78	7.55	26.49	10.98	7.92	16.54	15.71 ± 3.41

$C^{\infty}_{p\,min}$ = Minimum inhibitory concentration

D^* = Loading dose in mg/Kg.

D_o = Maintenance dose in mg/Kg.

γ = Dose interval.

III. COMPARISON OF PHARMACOKINETICS OF OFLOXACIN BETWEEN HEALTHY AND FEBRILE GOATS AFTER A SINGLE I.V. ADMINISTRATION

1. *Plasma Levels*

Comparative plasma concentrations of ofloxacin between healthy and febrile goat after its i.v. administration (4mg/kg) are presented in Table - 11 and Fig. - 1. The mean therapeutic concentration ($\geq 0.12\ \mu g/ml$) was maintained from 2.5 min to 6 h both in healthy and febrile goats. Though the drug concentrations in plasma were found to be slightly lower at all time intervals in febrile goats but they were statistically insignificant as compared to healthy goats.

2. Milk levels

The present study shows that significantly higher concentrations of the drug in milk were found at 0.5 to 8 h in febrile goat as compared to healthy goat. (Table –11). The mean peak milk concentration of 1.45 ± 0.08 and $2.33 \pm 0.28\ \mu g/ml$ in healthy and febrile goats, respectively, were attained at similar time of 3 h. The mean therapeutic concentration ($\geq 0.12\ \mu g/ml$) was maintained from 0.5 to 10 h in both healthy and febrile goats.

Table – 11 Comparison of concentrations (μg/ml) of ofloxacin in various biological fluids between healthy and febrile goats after single i.v. administration (4 mg/kg).

	HEATHY (n= 6)			FEBRILE (n= 6)		
	PLASMA	MILK	URINE	PLASMA	MILK	URINE
0.042	7.64 ± 0.34		20.05 ± 11.40	6.29 ± 0.77		18.15 ± 9.80
0.083	5.73 ± 0.50		46.70 ± 17.03	4.68 ± 0.79		45.61 ± 16.13
0.167	4.23 ± 0.66		59.69 ± 21.72	3.71 ± 0.62		60.35 ± 21.40
0.25	3.24 ± 0.48		126.88 ± 19.83	2.96 ± 0.53		126.95 ± 19.31
0.333	2.62 ± 0.37		201.17 ± 39.43	2.38 ± 0.46	N.D.	201.80 ± 39.81
0.50	2.01 ± 0.24	N.D.	298.2 ± 62.26	1.76 ± 0.31	0.07 ± 0.03*	296.5 ± 60.60
0.75	1.53 ± 0.17	0.12 ± 0.07	499.5 ± 74.87	1.25 ± 0.20	0.49 ± 0.10*	499.9 ± 75.39
1	1.10 ± 0.16	0.30 ± 0.08	431.8 ± 118.61	0.89 ± 0.12	0.81 ± 0.10**	429.15 ± 116.8
1.5	0.82 ± 0.09	0.62 ± 0.14	280.81 ± 62.69	0.56 ± 0.09	1.54 ± 0.10***	279.61 ± 63.16
2	0.70 ± 0.09	0.88 ± 0.16	171.11 ± 45.57	0.43 ± 0.10	1.85 ± 0.17**	168.31 ± 44.71
3	0.50 ± 0.7	1.45 ± 0.08	115.3 ± 22.04	0.43 ± 0.07	2.33 ± 0.28*	113.3 ± 22.06
4	0.34 ± 0.07	1.02 ± 0.27	73.28 ± 16.73	0.26 ± 0.06	2.17 ± 0.37*	72.45 ± 16.52
5	0.25 ± 0.05	0.68 ± 0.21	37.30 ± 29.80	0.18 ± 0.04	1.51 ± 0.18*	36.78 ± 9.13
6	0.19 ± 0.04	0.42 ± 0.16	19.97 ± 2.23	0.13 ± 0.04	0.98 ± 0.11*	17.72 ± 2.20
8	0.07 ± 0.03	0.29 ± 0.13	11.72 ± 1.31	0.06 ± 0.02	0.77 ± 0.07*	11.87 ± 1.22
10	N.D.	0.17 ± 0.11	7.61 ± 0.81	0.02 ± 0.02	0.21 ± 0.03	7.87 ± 0.79
12		0.04 ± 0.04	3.51 ± 0.37	N.D	0.06 ± 0.03	3.41 ± 0.45
24		N.D.	0.19 ± 0.02		N.D.	0.21 ± 0.02

N.D. = Non-Detectable

*$p < 0.05$ **$p < 0.01$ ***$p < 0.001$

Rest data are non-significant

3. Urine levels

Table – 11 and Fig – 3 depict the urine concentrations of ofloxacin at various time intervals in healthy and febrile goats after its i.v. administration. There was no significant difference of drug concentrations in febrile goats as compared to healthy ones. More or less similar mean peak urine concentration of 499.5 ± 74.87 μg/ml in healthy and 499.9 ± 75.39 μg/ml in febrile goats were attained at 0.75 h. The therapeutic concentration (≥ 0.12 μg/ml) was maintained from 2.5 min to 24 h in both the groups.

4. Kinetic parameters

Table – 12 shows the comparison of kinetic parameters of ofloxacin in healthy and febrile goats after its i.v. administration (4 mg/kg). There was no significant difference observed in all kinetic parameters except zero time concentration during elimination (B) phase which was significantly ($p<0.05$) lower as compared to healthy goats.

5. Calculated Dosage Regimen

Table 13 shows the comparison of calculated dosage regimen of ofloxacin between healthy and febrile goats after i.v. administration. The present study calculated the loading (D*) and maintenance (D_{γ}) doses to maintain C_p^{ss} min of 0.12 μg/ml in plasma at desired dosage interval (γ) of 8 h and 12 h to be lower in febrile goats as compared to healthy goats for i.v. route, though the data

Table – 12 Comparison of pharmacokinetic parameters of ofloxacin between healthy and febrile goats after single i.v. administration (4 mg/Kg).

Parameters	Unit	Healthy (No= 6)	Febrile (No. 6)
A	μg/ml	5.37 ± 0.58	4.75 ± 0.90
B	μg/ml	1.42 ± 0.08	0.82 ± 0.18*
C_o^p	μg/ml	6.79 ± 0.65	5.58 ± 0.94
α	h^{-1}	3.718 ± 0.27	2.944 ± 0.35
β	h^{-1}	0.379 ± 0.05	0.304 ± 0.01
$t_{1/2}$ α	h	0.23 ± 0.05	0.25 ± 0.03
$t_{1/2}$ β	h	1.96 ± 0.20	2.31 ± 0.12
K_{12}	h^{-1}	1.856 ± 0.02	1.222 ± 0.27
K_{21}	h^{-1}	1.097 ± 0.15	0.716 ± 0.10
Kel	h^{-1}	1.287 ± 0.09	1.160 ± 0.10
Fc	--	0.29 ± 0.02	0.25 ± 0.03
T≈P	--	2.59 ± 0.23	3.28 ± 0.42
AUC	mg/L.h	5.63 ± 0.82	4.47 ± 0.61
AUMC	$mg/L.h^{-1}$	13.07 ± 2.51	9.88 ± 2.11
MRT	h	2.14 ± 0.21	2.17 ± 0.29
Vd	L/Kg	0.62 ± 0.06	0.90 ± 0.23
Vd_h	L/Kg	2.86 ± 0.16	6.45 ± 1.64
Vd_{area}	L/Kg	2.30 ± 0.13	3.29 ± 0.54
$Vd_{s.s}$	L/Kg	1.67 ± 0.16	2.31 ± 0.54
Cl_B	ml/Kg/min	0.91 ± 0.16	1.00 ± 0.17

* $p < 0.05$ Rest data are non-significant

Table – 13 Comparison of calculated dosage regimen of ofloxacin between healthy and febrile goats after a single i.v. administration.

C_p^{∞} min=0.12 μg/ml		Healthy (No – 6)	Febrile (No –6)
γ = 8 h	D*	9.24 ± 5.06	4.61 ± 0.88
	D_o	8.99 ± 5.05	4.21 ± 0.52
γ = 12 h	D*	78.17 ± 57.84	16.10 ± 3.46
	D_o	77.92 ± 57.83	15.71 ± 3.41

$C_{p\,min}^{\infty}$ - Minimum inhibitory concentration

D^* - Loading dose in mg/Kg.

D_o - Maintenance dose in mg/Kg.

γ - Dose time interval.

were non-significant. The calculated D* and D_o at γ of 8 h were found to be 9.24 ± 5.06 and 4.61 ± 0.88 mg/Kg in healthy goats and 4.21 ± 0.82 mg/Kg in febrile goats, respectively. Similarly at γ of 12 h, D* and D_{∞} of 78.17 ± 57.84 and 77.92 ± 57.83 mg/Kg in healthy goats and 16.10 ± 3.46 and 15.71 ± 3.41 mg/Kg in febrile goats, respectively, were calculated. No significant difference was noted between healthy and febrile goats.

IV. PHARMACOKINETICE STUDY OF OFLOXACIN IN HEALTHY GOAT AFTER A SINGLE I.M. ADMINISTRATION

1. *Plasma levels*

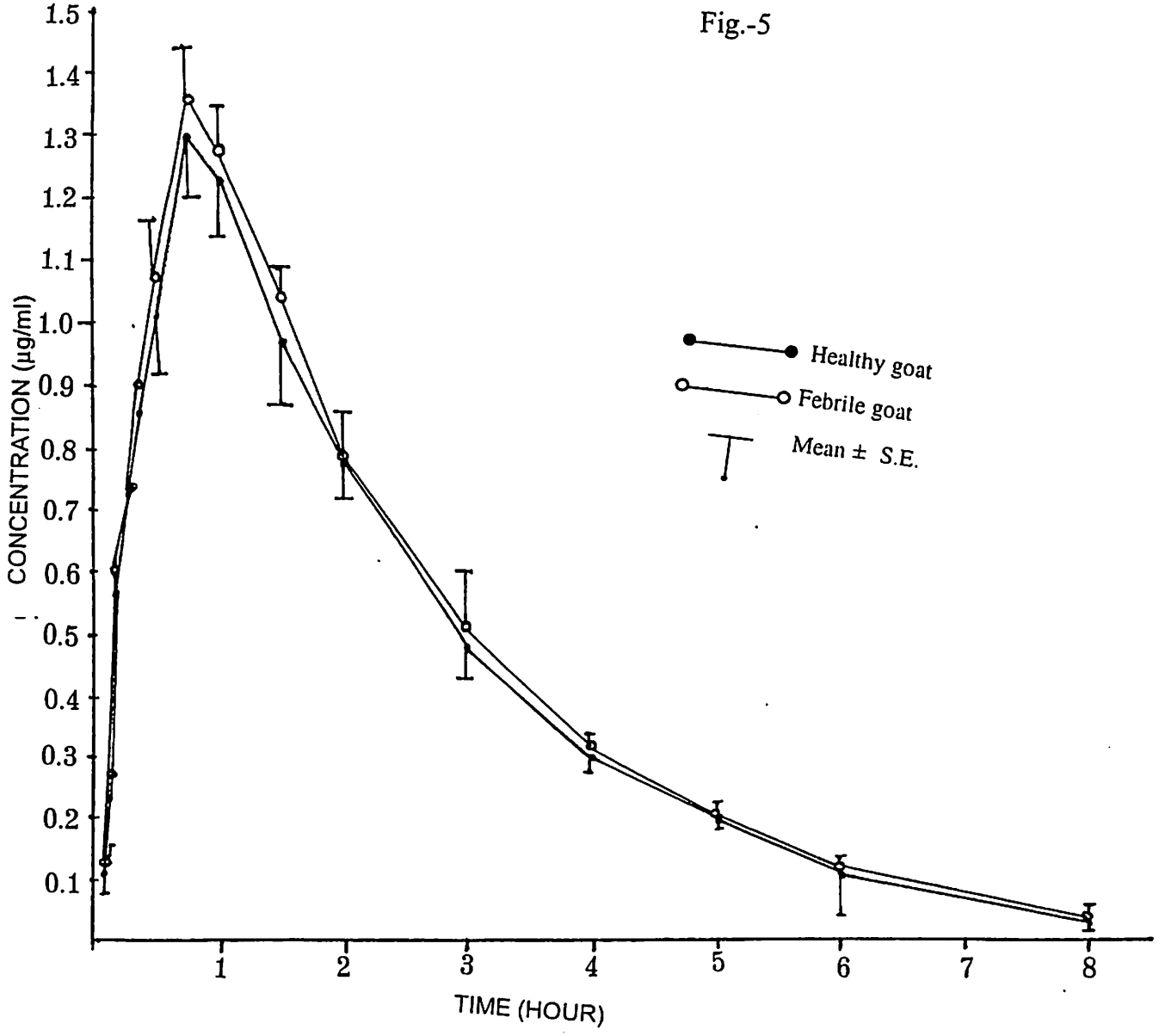
Table – 14 and Fig – 5 present the plasma drug concentration profile at different time intervals after a single i.m. administration (4mg/Kg). The drug appeared at 2.5 min (0.11 ± 0.03 μg/ml) in five out of six goats. Mean peak plasma concentration of 1.30 ± 0.09 μg/ml was attained at 0.75 h. The drug was persisted in all animals up to 6 h with its mean concentration of 0.11 ± 0.07 μg/ml. The drug was present in three animals at 8 h and none at 10 h. The therapeutic concentration (≥0.12 μg/ml) of the drug was maintained from 5 min to 5 h.

Table – 14 Plasma concentrations of ofloxacin (μg/ml) in healthy goats after a single i.m. administration (4mg/Kg).

Time (h) ↓	Animal Number						Mean ± S.E. ↓
	1	2	3	4	5	6	
0.042	0.08	0.12	N.D.	0.20	0.10	0.15	0.11 ± 0.03
0.083	0.15	0.25	0.12	0.38	0.22	0.28	0.23 ± 0.04
0.167	0.44	0.68	0.30	0.72	0.48	0.75	0.56 ± 0.07
0.25	0.54	0.85	0.48	0.95	0.58	0.90	0.72 ± 0.08
0.333	0.67	0.92	0.60	1.15	0.72	1.05	0.85 ± 0.09
0.50	0.83	1.00	0.75	1.30	0.94	1.22	1.01 ± 0.09
0.75	1.28	1.15	1.00	1.60	1.42	1.35	1.30 ± 0.09
1	1.03	1.40	0.92	1.32	1.20	1.52	1.23 ± 0.09
1.5	0.67	1.10	0.70	1.20	0.95	1.20	0.97 ± 0.10
2	0.58	0.92	0.60	0.88	0.82	0.88	0.78 ± 0.06
3	0.29	0.50	0.38	0.60	0.46	0.62	0.48 ± 0.05
4	0.18	0.32	0.26	0.34	0.32	0.35	0.30 ± 0.03
5	0.12	0.22	0.16	0.25	0.20	0.258	0.20 ± 0.02
6	0.08	0.12	0.10	0.13	0.11	0.12	0.11 ± 0.07
8	N.D.	0.05	N.D.	0.06	N.D.	0.06	0.03 ± 0.01
10	--	N.D.	--	N.D.	--	N.D.	--

N.D. = Non-Detectable

Fig.-5


CONCENTRATION (µg/ml)
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
1
2
3
4
5
6
7
8
TIME (HOUR)
Healthy goat
Febrile goat
Mean ± S.E.

2. ***Milk levels***

Concentrations of ofloxacin at different time intervals following i.m. administration are presented in Table-15 and Fig-6. The drug appeared in milk in four out of six at 1.5 h (0.23 ± 0.08 μg/ml) and in all six goats at 2 h (0.47 ± 0.06 μg/ml). The drug attained its means peak concentration (1.05 ± 0.12 μg/ml) at 5 h. The drug was detectable up to 6 h in all goats with its mean concentration of 0.60 ± 0.12 μg/ml. In five, four, three and one out of six goats the drug was detectable at 8, 10, 12 and 24 hr, respectively. The mean therapeutic concentration (≥0.12 μg/ml) was maintained from 1.5 to 10 h.

3. ***Urine levels***

Table – 16 and Fig –7 depict the concentrations of ofloxacin in urine at various time intervals post i.m. infection. The drug appeared in effective therapeutic concentration at 2.5 min (3.59 ± 0.95 μg/ml) and persisted up to 24 h in all goats (0.48 ± 0.06 μg/ml). The peak urine concentration of 455.5 μg/ml was achieved at 2 h.

4. ***Kinetic parameters***

The plasma drug concentration versus time profile had shown monophasic curve (fig-8), and hence, the kinetic parameters were calculated using 1-compartment open model.

Table-17 presents the values of different kinetic parameters of the drug after its i.m. administration (4mg/Kg). The

Table – 15 Milk concentrations of ofloxacinin healthy goats after a single i.m. administration. (4mg/Kg).

Time (hr) ↓	Animal Number						Mean ± S.E. ↓
	1	2	3	4	5	6	
0.042							
0.083							
0.167							
0.25							
0.333							
0.50							
0.75							
1.0	--	N.D.	N.D.	N.D.	N.D.	--	--
1.5	N.D.	0.22	0.45	0.36	0.32	N.D.	0.23 ± 0.08
2	0.28	0.54	0.68	0.55	0.46	0.32	0.47 ± 0.06
3	0.56	0.65	0.88	0.68	0.58	0.72	0.68 0_ 0.05
4	0.98	0.82	0.96	0.88	0.70	1.10	0.91 ± 0.07
5	0.66	1.15	1.38	1.25	1.12	0.74	1.05 ± 0.12
6	0.20	0.58	0.95	0.90	0.62	0.32	0.60 ± 0.12
8	N.D.	0.26	0.52	0.42	0.28	0.15	0.27 ± 0.08
10	--	0.15	0.32	0.25	0.20	N.D.	0.15 ± 0.05
12	--	N.D.	0.20	0.16	0.10	--	0.08 ± 0.04
24	--	--	0.15	N.D.	N.D.	--	0.03 ± 0.03

N.D. = Non-Detectable

Fig.-6

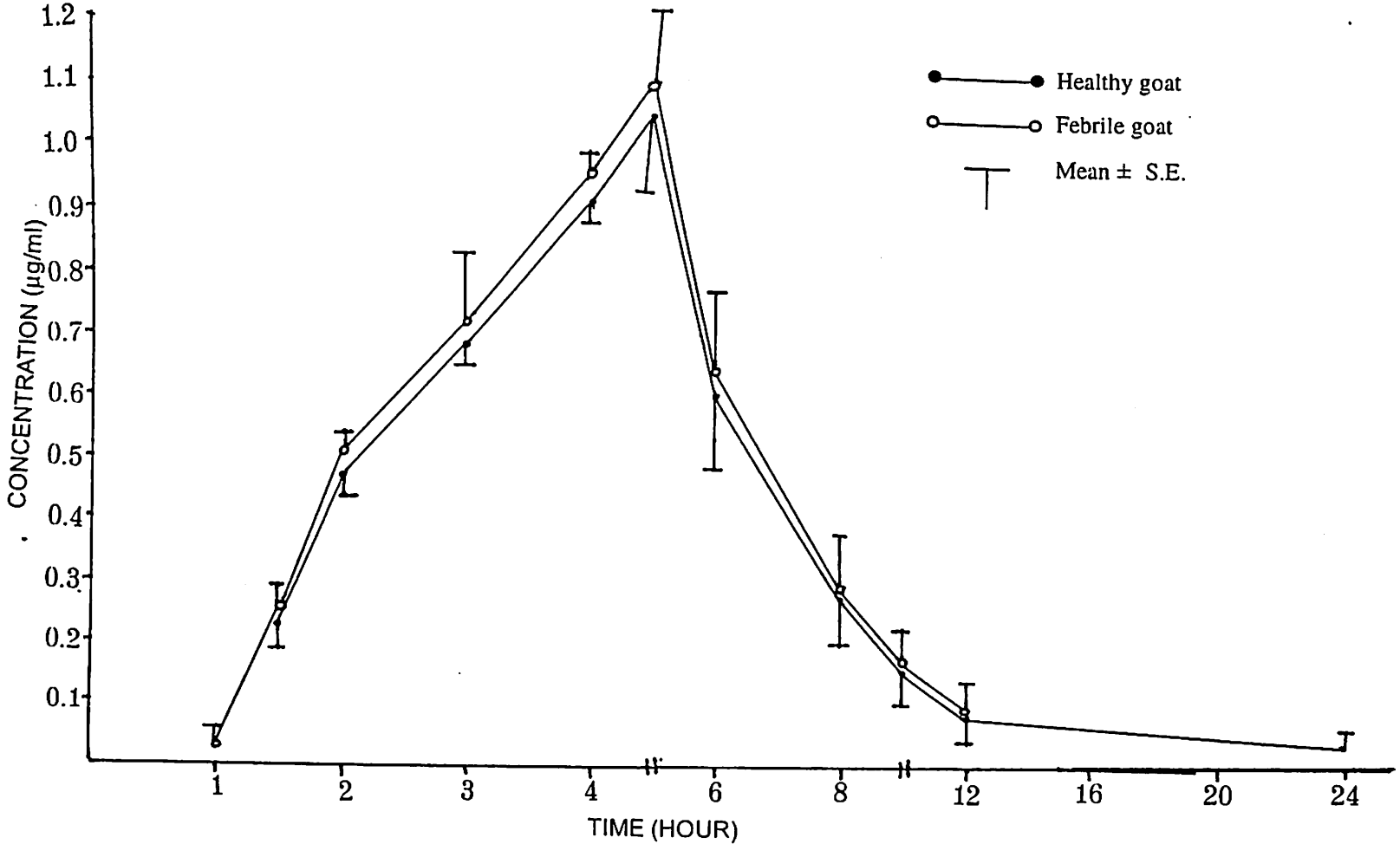

1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
CONCENTRATION (µg/ml)
Healthy goat
Febrile goat
Mean ± S.E.
1
2
3
4
6
8
12
16
20
24
TIME (HOUR)

Table – 16 Urine concentrations of ofloxacin in healthy goats after a single i.m. administration (4 mg/Kg).

Time (h) ↓	Animal Number						Mean ± S.E. ↓
	1	2	3	4	5	6	
0.042	7.75	2.84	2.00	2.25	1.85	4.85	3.59 ± 0.95
0.083	24.6	15.52	14.54	16.82	14.20	20.26	17.66 ± 1.65
0.167	78.3	27.78	22.25	32.28	25.50	70.28	42.73 ± 10.12
0.25	98.8	80.8	75.80	88.45	78.85	92.25	85.83 ± 36.10
0.333	120.9	102.7	98.88	112.6	101.2	115.8	108.7 ± 3.67
0.50	235.5	153.1	145.5	225.8	152.6	216.8	188.5 ± 17.03
0.75	362.7	182.7	175.6	350.2	185.8	352.2	268.2 ± 38.89
1	431.2	262.8	250.8	410.8	275.5	398.2	338.2 ± 34.05
1.5	513.8	338.2	310.6	490.2	358.6	455.6	411.2 ± 35.10
2	636.5	252.0	425.5	580.5	285.8	552.8	455.5 ± 65.57
3	291.00	144.8	206.6	270.2	168.6	275.2	226.7 ± 25.08
4	153.00	103.2	148.2	150.0	108.2	150.2	135.5 ± 9.46
5	1.00	80.55	88.65	86.26	82.45	88.26	86.20 ± 1.63
6	49.00	40.40	45.6	46.45	42.26	48.45	45.36 ± 1.39
8	33.34	28.65	30.22	32.25	34.18	35.20	32.31 ± 1.01
10	18.67	14.55	17.50	17.80	15.65	19.25	17.24 ± 0.74
12	8.67	6.88	7.85	8.15	7.12	8.86	7.92 ± 0.32
24	0.67	0.28	0.45	0.52	0.36	0.62	0.48 ± 0.06

Fig.-7

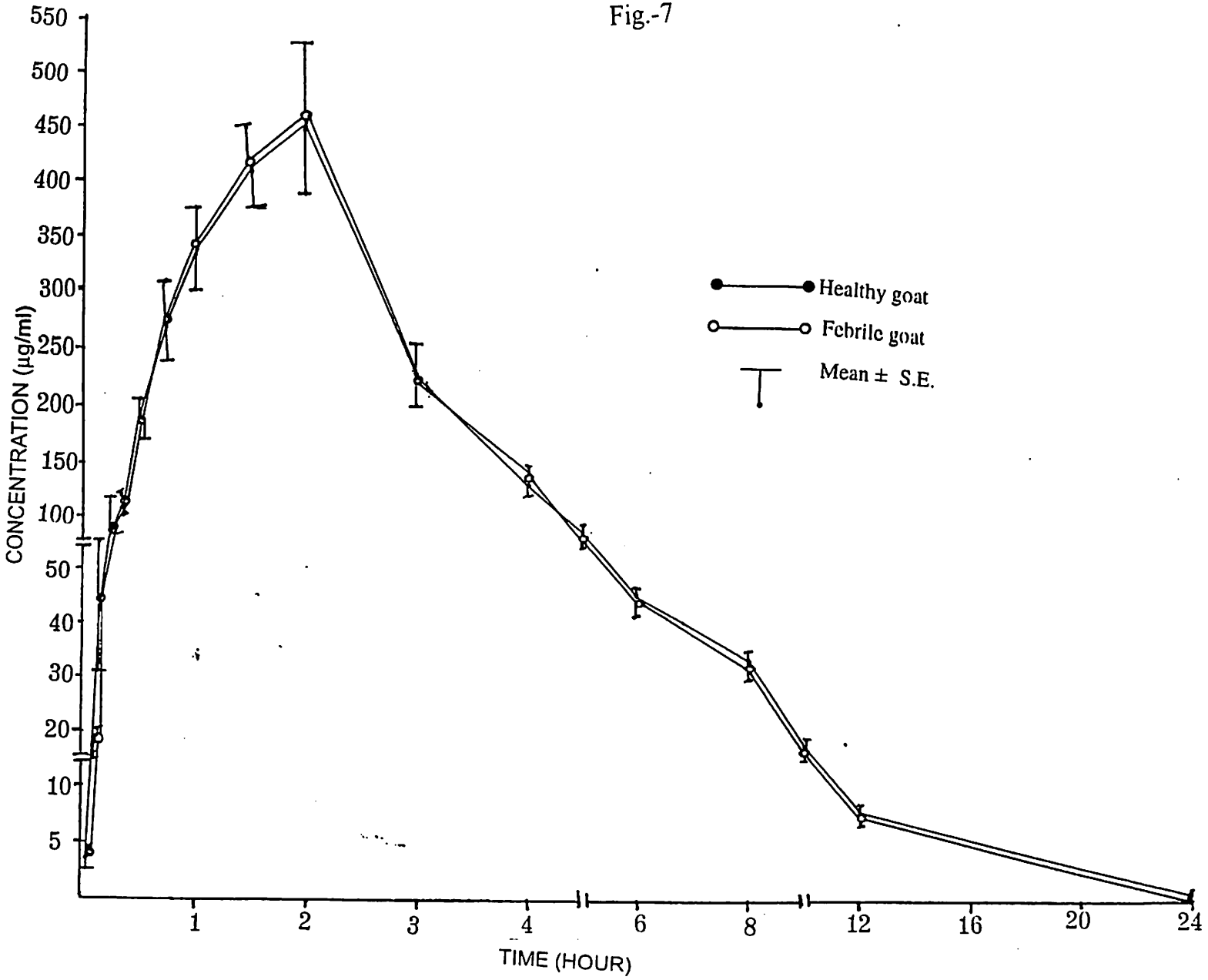
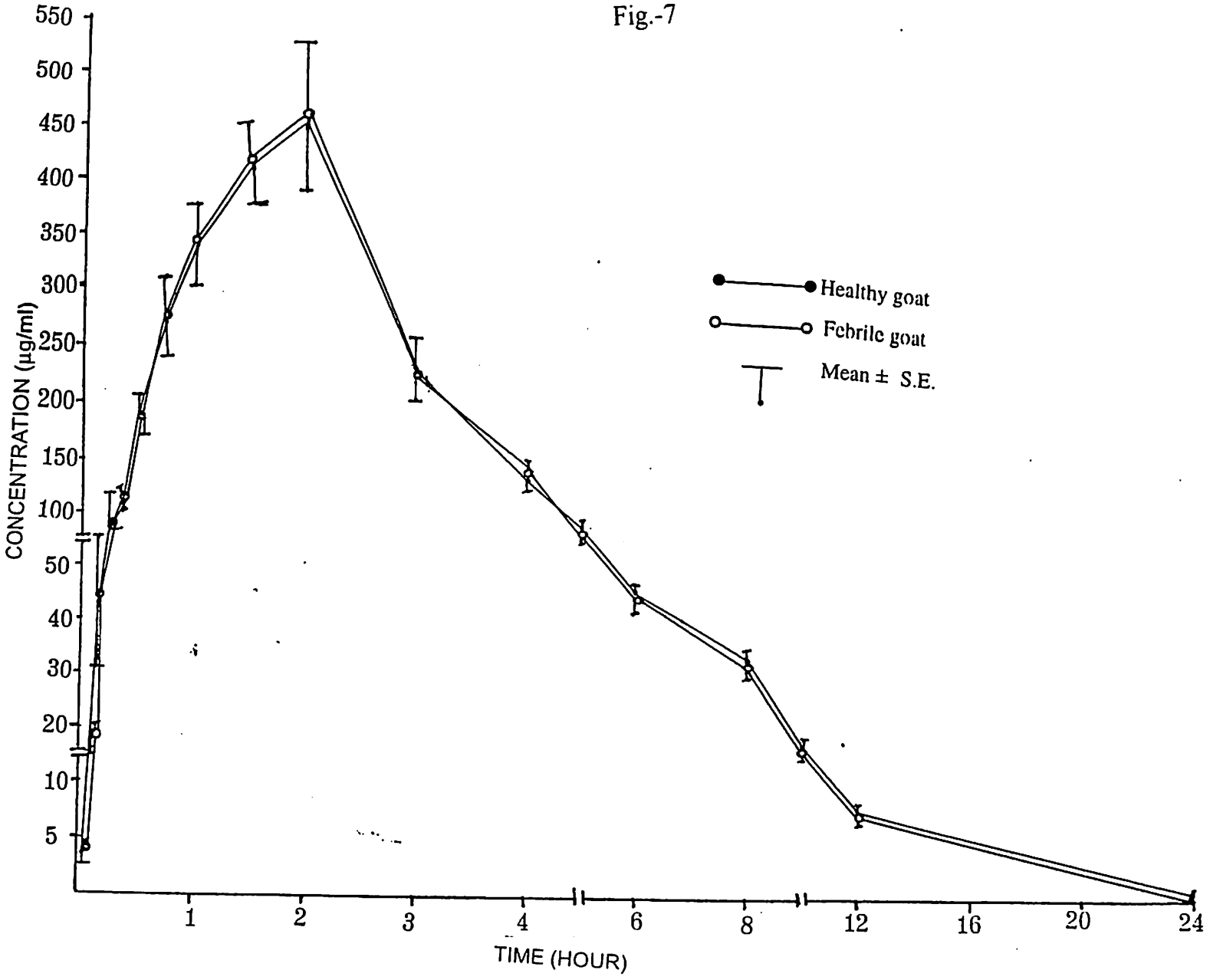

550
500
450
400
350
300
250
200
150
100
50
40
30
20
10
5
CONCENTRATION (µg/ml)
1
2
3
4
6
8
12
16
20
24
TIME (HOUR)
Healthy goat
Febrile goat
Mean ± S.E.

Fig.-7


CONCENTRATION (µg/ml)
550
500
450
400
350
300
250
200
150
100
50
40
30
20
10
5
1
2
3
4
6
8
12
16
20
24
TIME (HOUR)
Healthy goat
Febrile goat
Mean ± S.E.

mean extrapolated zero time concentration in plasma during absorption phase (A) and elimination phase (B) were estimated to be 2.02 ± 0.13 and 1.97 ± 0.16 μg/ml, respectively. The absorption rate constant (Ka) ranged from 2.143 to 3.149 h^{-1} with a mean value of 2.617 ± 0.164. The elimination rate constant (β) ranged from 0.359 to 0.525 h^{-1} with a mean value of 0.454 ± 0.023 h. The mean absorption half-life ($t_{1/2}Ka$) and elimination half-life $t_{1/2}\beta$ were noted to be 0.27 ± 0.02 and 1.55 ± 0.09 h, respectively. The total area under the curve (AUC) was noted to be 5.18 ± 0.46 mg/L.h. The values of volume distribution i.e. Vd_β and Vd_{area} were calculated to be 2.10 ± 0.19 and 1.78 ± 0.16 L/kg, respectively. The total body clearance (C_{lB}) varied from 0.64 to 1.07 with a mean value of 0.81 ± 0.08 ml/Kg. min.

5. ***Calculated dosage regimen***

Table – 18 shows the calculated dosage regimen of ofloxacin for i.m. route in healthy goats. For treating systemic infections (C_o^∞ min = 0.12μg / ml) at the dosage interval (γ) of 8 h, the mean D* and D_0 were calculated to be 8.68 ± 1.69 and 8.47 ± 1.69 mg/Kg, respectively, while at γ of 12 h mean D* and D_0 were calculated to be 58.39 ± 15.98 and 58.22 ± 16.01 mg/Kg, respectively.

Table – 17 Kinetic parameters of ofloxacin in healthy goats after a single i.m. administration (4mg/Kg).

Parameters (Unit) ↓	Goat Number						Mean ± S.E. ↓
	1	2	3	4	5	6	
A (μg/ml)	1.74	2.17	1.58	2.28	1.96	2.40	2.02 ± 0.13
B (μg/ml)	1.64	2.27	1.40	2.23	1.92	2.38	1.97 ± 0.16
K_a (h^{-1})	2.771	2.146	2.847	3.149	2.143	2.643	2.617 ± 0.164
β (h^{-1})	0.525	0.480	0.435	0.456	0.359	0.470	0.454 ± 0.023
$t_{1/2}$ K_a (h)	0.25	0.32	0.24	0.22	0.32	0.26	0.27 ± 0.02
$t_{1/2}$ β (h)	1.32	1.44	1.59	1.52	1.93	1.47	1.55 ± 0.09
AUC (mg/L.h)	3.75	5.74	3.77	5.61	6.26	5.97	5.18 ± 0.46
Vd_b (L/Kg)	2.44	1.76	2.86	1.79	2.08	1.68	2.10 ± 0.19
Vd_{area} (L/Kg)	2.03	1.45	2.44	1.56	1.77	1.42	1.78 ± 0.16
Cl_B (ml/Kg/min)	1.07	0.69	1.06	0.71	0.64	0.67	0.81 ± 0.08

Fig - 8

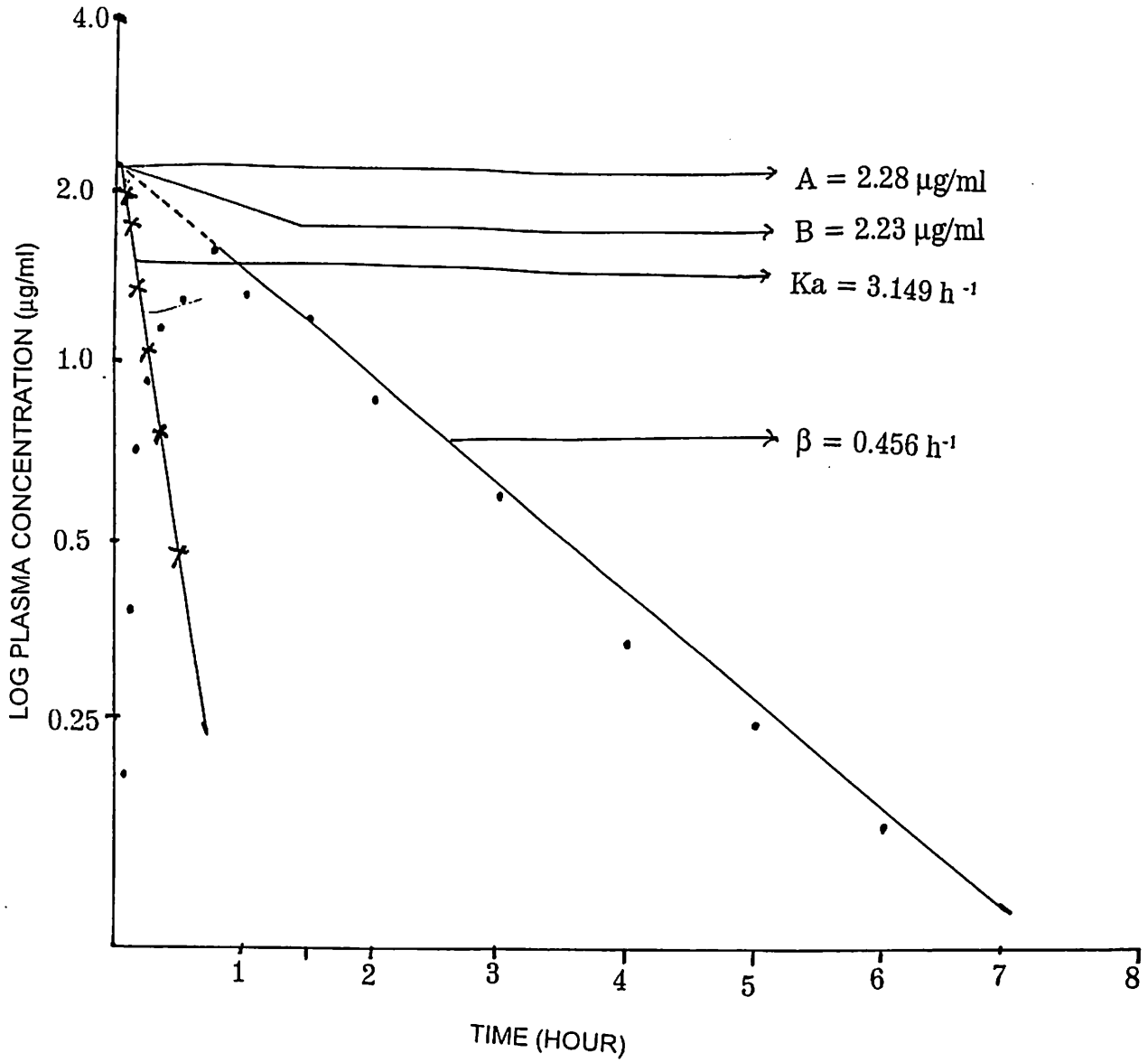

4.0
2.0
1.0
0.5
0.25
LOG PLASMA CONCENTRATION (µg/ml)
A = 2.28 µg/ml
B = 2.23 µg/ml
Ka = 3.149 h -1
β = 0.456 h-1
1
2
3
4
5
6
7
8
TIME (HOUR)

Table – 18 Calculated dosage regimen of ofloxacin in healthy goats after a single i.m. dose.

$C^{\infty}_{p\ min}$ = 0.12 µg/ml ↓		Animal Number						Mean ± S.E. ↓
		1	2	3	4	5	6	
γ = 8 h	D^*	16.24	8.09	9.50	7.19	3.75	7.32	8.68 ± 1.69
	D_o	16.00	7.92	9.21	7.00	3.54	7.15	8.47 ± 1.69
γ = 12 h	D^*	132.66	55.22	54.15	44.54	15.78	47.96	58.39 ± 15.98
	D_o	132.65	55.09	53.86	44.35	15.59	47.79	58.22 ± 16.01

$C^{\infty}_{p\ min}$ - Minimum inhibitory concentration

D^* - Loading dose in mg/Kg.

D_o - Maintenance dose in mg/Kg.

γ - Dose time interval.

V. PHARMACOKINETIC STUDY OF OFLOXACIN IN FEBRILE GOATS AFTER I.M. ADMINISTRATION

1. *Plasma levels*

Table – 19 and Fig. – 5 depict the plasma drug concentrations at various time intervals in febrile goats after i.m. administration of ofloxacin. The drug appeared at 2.5 min (0.13 ± 0.02 μg/ml). Mean peak concentration (1.36 ± 0.09 μg/ml) was achieved at 0.75 h and the drug persisted up to 6 h (0.12 ± 0.01 μg/ml) in all animals. In four out of six goats, it was detectable at 8 h and none at 10 h. The mean therapeutic concentration (≥ 0.12 μg/ml) was maintained from 2.5 min to 6 h.

2. *Milk levels*

Table – 20 and Fig – 6 show the concentrations of ofloxacin in milk after i.m. administration (4 mg/Kg) in febrile goats. The drug appeared at 1.5 h in all goats with the mean value of 0.26 ± 0.05 μg/ml. The drug reached its mean peak concentration (1.10 ± 0.11 μg/ml) at 5 h. The drug was detectable up to 6 h in all goats with its mean concentration of 0.64 ± 0.13 μg/ml. In five and four goats it was detectable even at 10 h and 12 h, respectively. The therapeutic concentration was maintained from 1.5 to 10 h.

Table - 19 Plasma concentrations of ofloxacin (μg/ml) in febrile goats after a single i.m. administration (4mg/Kg).

Time (h) ↓	Animal Number						Mean ± S.E. ↓
	1	2	3	4	5	6	
0.042	0.10	0.15	0.06	0.22	0.12	0.15	0.13 ± 0.02
0.083	0.22	0.28	0.15	0.40	0.25	0.32	0.27 ± 0.04
0.167	0.48	0.78	0.28	0.75	0.50	0.78	0.60 ± 0.08
0.25	0.60	0.92	0.45	0.90	0.60	0.92	0.73 ± 0.08
0.333	0.72	1.05	0.58	1.12	0.75	1.15	0.90 ± 0.10
0.50	0.95	1.20	0.68	1.28	0.98	1.30	1.07 ± 0.10
0.75	1.35	1.50	0.90	1.45	1.45	1.50	1.36 ± 0.09
1	1.15	1.32	1.05	1.58	1.25	1.30	1.28 ± 0.07
1.5	0.96	1.10	0.88	1.20	0.98	1.10	1.04 ± 0.05
2	0.60	0.82	0.62	1.10	0.72	0.82	0.78 ± 0.07
3	0.45	0.52	0.50	0.62	0.44	0.54	0.51 ± 0.03
4	0.26	0.35	0.32	0.40	0.26	0.35	0.32 ± 0.02
5	0.18	0.20	0.24	0.25	0.15	0.20	0.20 ± 0.02
6	0.08	0.14	0.14	0.15	0.09	0.14	0.12 ± 0.01
8	ND	0.06	0.06	0.07	N.D	0.05	0.04 ± 0.01
10	-	N.D	N.D	N.D	-	N.D	-

N.D. = Non-Detectable

Table – 20 Milk concentrations of ofloxacin (μg/ml) in febrile goats after a single i.m. administration (4mg/Kg).

Time (h) ↓	Animal Number						Mean ± S.E. ↓
	1	2	3	4	5	6	
0.042							
0.083							
0.167							
0.25							
0.333							
0.50							
0.75	--	--	N.D.	--	--	--	--
1	N.D.	N.D.	0.15	N.D.	N.D.	N.D.	0.03 ± 0.03
1.5	0.12	0.25	0.40	0.32	0.34	0.12	0± 0.05
2	0.30	0.60	0.72	0.56	0.50	0.35	0.51 ± 0.06
3	0.58	0.72	0.92	0.72	0.62	0.78	0.72 ± 0.45
4	1.05	0.85	1.05	0.92	0.75	1.15	0.96 ± 0.06
5	0.72	1.20	1.40	1.24	1.18	0.84	1.10 ± 0.11
6	0.25	0.56	1.10	0.88	0.60	0.42	0.64 ± 0.13
8	0.00	0.24	0.64	0.40	0.25	0.22	0.29 ± 0.09
10	0.00	0.14	0.38	0.22	0.18	0.10	0.17 ± 0.05
12	0.00	0.00	0.28	0.15	0.08	0.00	0.09 ± 0.05

3. ***Urine levels***

Table - 21 and Fig – 7 depict the concentrations of ofloxacin in urine following single i.m. dose of 4 mg/kg. The drug appeared in effective therapeutic concentration even at 2.5 min and it was maintained up to 24 h. Mean peak concentration of 457.8 ± 66.81 μg/ml was attained at 2 h.

4. ***Kinetic parameters***

Table – 22 presents the various kinetic parameters obtained for ofloxacin following i.m. administration (4 mg/kg). The mean extrapolated zero time concentration of the drug in plasma during absorption phase (A) and elimination phase (B) were calculated to be 2.15 ± 0.09 and 2.00 ± 0.14 μg/ml. respectively. The mean value of absorption rate constant (Ka) and elimination rate constant (β) were noted to be 2.811 ±0.177 and 0.468 ± 0.012 h^{-1}, respectively. The absorption half life ($t_{1/2}Ka$) varied from 0.20 to 0.30 h with mean of 0.25 ± 0.02 h while elimination half life ($t_{1/2}\beta$) ranged from 1.31 to 1.73 h with a mean of 1.50 ± 0.06 h. The mean value of total area under curve (AUC) was found to be 5.22 ± 0.27 mg/L.h. The values of volume distribution i.e. Vd_B and Vd_{area} were calculated to be 1.97 ± 0.13 and 1.67 ± 0.11 L/Kg, respectively. The total body clearance (Cl_B) ranged from 0.63 to 0.86 ml/Kg/min with a mean value of 0.78 ± 0.04 ml/Kg/min.

Table – 21 Urine concentrations of ofloxacin in febrile goats after a single i.m. administration (4mg/Kg).

Time (h) ↓	Animal Number						Mean ± S.E. ↓
	1	2	3	4	5	6	
0.042	8.05	2.80	2.50	3.00	1.65	4.05	3.68 ± 0.93
0.083	25.28	14.82	15.15	17.15	14.00	19.85	17.70 ± 1.74
0.167	80.35	28.10	23.05	33.58	24.20	68.28	42.93 ± 10.16
0.25	100.2	81.20	76.45	89.25	76.95	91.05	85.85 ± 3.80
0.333	118.8	103.50	99.15	115.80	100.20	116.20	108.95 ± 3.65
0.50	230.6	150.50	146.20	228.40	151.80	218.20	187.62 ± 17.15
0.75	358.8	184.6	177.8	355.00	186.20	355.50	269.65 ± 38.83
1	435.4	265.80	252.2	414.80	278.50	400.0	341.13 ± 34.30
1.5	515.4	340.40	315.8	495.40	359.80	458.6	414.23 ± 35.07
2	642.4	250.00	428.6	585.60	284.80	555.5	457.8 ± 66.81
3	288.8	145.8	208.8	272.80	165.50	270.8	225.42 ± 24.85
4	155.2	101.8	150.2	151.60	105.60	151.6	136.00 ± 10.25
5	92.2	78.22	87.85	85.44	79.85	87.56	85.19 ± 1.70
6	50.00	38.60	44.68	45.25	41.16	47.65	44.56 ± 0.96
8	32.54	29.05	29.68	31.85	35.00	34.10	3204 ± 0.96
10	17.88	15.10	17.00	18.25	15.05	18.85	17.02 ± 0.66
12	8.52	7.12	7.05	8.05	7.02	8.06	7.64 ± 0.27
24	0.58	0.30	0.42	0.45	0.32	0.58	0.44 ± 0.05

Table – 22 Kinetic parameters of ofloxacin in febrile goats after a single i.m. administration (4mg/Kg).

Parameter (Unit) ↓	Animal Number						Mean ± S.E. ↓
	1	2	3	4	5	6	
μg/ml)	2.03	2.08	1.82	2.50	2.19	2.26	2.15 ± 0.09
μg/ml)	1.95	2.06	1.57	2.47	2.13	2.14	2.00 ± 0.14
h^{-1})	2.510	3.166	2.641	2.721	2.338	3.490	2.811 ± 0.177
$^{-1}$)	0.509	0.448	0.400	0.454	0.529	0.465	0.468 ± 0.012
K_a (h)	0.28	0.22	0.26	0.25	0.30	0.20	0.25 ± 0.02
β (h)	1.36	1.55	1.73	1.53	1.31	1.49	1.50 ± 0.06
C (mg/L.h)	4.64	5.26	4.61	6.36	4.96	5.46	5.22 ± 0.27
$_b$ (L/Kg)	2.05	1.94	2.55	1.62	1.88	1.79	1.97 ± 0.13
$_{area}$ (L/Kg)	1.69	1.70	2.16	1.38	1.53	1.57	1.67 ± 0.11
(ml/Kg/min)	0.86	0.76	0.86	0.63	0.81	0.73	0.78 ± 0.04

Table – 23 Calculated dosage regimen of ofloxacin in febrile goats after a single i.m. dose. (4mg/Kg).

$C_{p\,min}^{\infty}$ =0.12 μg/ml ↓		Animal Number						Mean ± S.E. ↓
		1	2	3	4	5	6	
γ = 8 h	D*	11.89	7.35	6.36	6.26	12.64	7.77	8.71 ± 1.15
	D_0	11.69	7.35	6.09	6.09	12.46	7.59	8.51 ± 1.16
γ = 12 h	D*	91.15	44.09	31.49	38.47	104.89	49.94	60.01 ± 12.10
	D_0	90.94	43.89	31.24	38.30	104.72	44.75	58.97 ± 12.57

$C_{p\,min}^{\infty}$ = Minimum inhibitory concentration

D* - Loading dose in mg/Kg

D_o - Maintenance dose in mg/Kg

γ - Dose interval

5. *Calculated dosage regimen*

Table – 23 shows the calculated dosage regimen of ofloxacin for i.m. route in febrile goat for treating systemic infections (C_o^{∞} min = 0.12 μg/ml). The calculated D* and D_o at the desired dose interval (γ) of 8 h were noted to be 8.71 ± 1.15 and 8.51 ± 1.16 mg/Kg respectively, while at γ of 12 h D* and D_o were calculated to be 60.01 ± 12.10 and 58.97 ± 12.57 mg/Kg., respectively.

VI. COMPARISON OF PHARMACOKINETICS OF OFLOXACIN BETWEEN HEALTHY AND FEBRILE GOAT AFTER A SINGLE I.M. ADMINISTRATION

1. *Plasma levels*

Table – 24 and Fig – 5 depict the plasma concentrations of ofloxacin between healthy and febrile goats following i.m. administration (4 mg/Kg). The drug maintained its therapeutic concentration up to 5 and 6 h in healthy and febrile goats, respectively. The mean peak concentrations of the drug were found to be 1.30 ± 0.09 and 1.36 ± 0.09 μg/ml at 0.75 h in both healthy and febrile goats. The drug maintained its mean therapeutic concentration (≥ 0.12 μg/ml) from 5 min to 5 h and 2.5 min to 6 h in healthy and febrile goats, respectively. No significant difference in plasma levels at various time intervals was observed between healthy and febrile goats.

2. *Milk levels*

Table – 24 Fig. – 6 present the milk concentration of ofloxacin in healthy and febrile goats post i.m. administration peak concentration of 1.05 ± 0.12 and 1.10 ± 0.11 μg/ml was attained at similar time of 5 h in healthy and febrile goats, respectively. The therapeutic concentration was maintained from 1.5 h to 10 h in both the group of goats.

3. *Urine levels*

Table 24 and Fig. – 7 depict the urine concentrations of ofloxacin in healthy and febrile goats post i.m. administration. There was no significant difference in urine concentration between healthy and febrile goats. Peak concentrations of 455.5 ± 65.57 μg/ml and 457.8 ± 6.1 μg/ml were attained in 2 h in healthy and febrile goats, respectively. The therapeutic concentration was maintained from 2.5 min to even beyond 24 h in both healthy and febrile goats.

4. *Kinetic parameters*

Table 25 shows the comparison of kinetic parameters of ofloxacin in healthy and febrile goats after a single i.m. administration (4mg/kg). None of the kinetic parameters differed significantly between healthy and febrile goats.

Table – 24 Comparison of concentrations of ofloxacin in various biological fluids between healthy and febrile goats after a single i.m administration (4 mg/kg).

Time	HEALTHY (n= 6)			FEBRILE (n= 6)		
(h)	PLASMA	MILK	URINE	PLASMA	MILK	URINE
0.042	0.11 ± 0.03		3.59 ± 0.95	0.13 ± 0.02		3.68 ± 0.093
0.083	0.23 ± 0.04		17.66 ± 1.65	0.27 ± 0.04		17.70 ± 1.74
0.167	0.56 ± 0.07		42.73 ± 10.12	0.60 ± 0.08		42.93 ± 10.16
0.25	0.72 ± 0.08		85.83 ± 36.10	0.73 ± 0.08		85.85 ± 3.80
0.333	0.85 ± 0.09		108.68 ± 3.67	0.90 ± 0.10		108.95 ± 3.65
0.50	1.01 ± 0.09		188.05 ± 17.03	1.07 ± 0.10		187.62 0± 17.15
0.75	1.30 ± 0.09		268.20 ± 38.89	1.36 ± 0.09	N.D.	269.65 ± 38.83
1	1.23 ± 0.09	N.D.	338.22 ± 34.05	1.28 ± 0.07	0.03 ± 0.03	341.13 ± 34.30
1.5	0.97 ± 0.10	0.23 ± 0.08	411.17 ± 35.10	1.04 ± 0.05	0.26 ± 0.05	414.23 ± 35.07
2	0.78 ± 0.06	0.47 ± 0.06	455.52 ± 65.57	0.78 ± 0.07	0.51 ± 0.06	457.82 ± 66.381
3	0.48 ± 0.05	0.68 ± 0.05	226.07 ± 25.08	0.51 ± 0.03	0.72 ± 0.45	225.42 ± 24.85
4	0.30 ± 0.03	0.91 ± 0.07	135.47 ± 9.46	0.32 ± 0.02	0.96 ± 0.06	136.00 ± 10.25
5	0.20 ± 0.02	1.05 ± 0.12	86.20 ± 1.63	0.20 ± 0.02	1.10 ± 0.11	85.19 ± 2.15
6	0.11 ± 0.07	0.60± 0.12	45.36 ± 1.39	0.12 ± 0.01	0.64 ± 0.13	44.56 ± 1.70
8	0.03 ± 0.01	0.27 ± 0.08	32.31 ± 1.01	0.04 ± 0.01	0.29 ± 0.09	32.04 ± 0.96
10	N.D.	0.15 ± 0.05	17.24 ± 0.74	N.D.	0.17 ± 0.05	17.02 ± 0.66
12		0.08 ± 0.04	7.92 ± 0.33		0.09 ± 0.05	7.64 ± 0.27
24		0.03 ± 0.03	0.48 ± 0.06		N.D.	0.44 ± 0.05

N.D. = Non-Detectable

All data are non-significant.

Table – 25 Comparison of kinetic parameters of ofloxacin between healthy and febrile goats after a single i.m. administration (4mg/Kg).

Parameter (Unit)	Healthy	Febrile
A (μg/ml)	2.02 ± 0.13	2.15 ± 0.09
B (μg/ml)	1.97 ± 0.16	2.00 ± 0.14
K_a (h^{-1})	2.617 ± 0.16	2.811 ± 0.18
β (h^{-1})	0.454 ± 0.02	0.468 ± 0.02
$t_{1/2}$ K_a (h)	0.27 ± 0.02	0.25 ± 0.02
$t_{1/2}$ β (h)	1.55 ± 0.09	1.50 ± 0.06
AUC (mg/L.h)	5.18 ± 0.46	5.22 ± 0.27
Vd_b (L/Kg)	2.10 ± 0.19	1.97 ± 0.13
Vd_{area} (L/Kg)	1.78 ± 0.16	1.67 ± 0.11
Cl_B (ml/Kg/min)	0.81 ± 0.08	0.78 ± 0.04

Table – 26 Comparison of calculated dosage regimen between healthy and febrile goats after a single i.m. dose (4mg/kg).

$C^{\infty}_{p\ min}$ =0.12 µg/ml		Healthy (No = 60)	Febrile (No=6)
γ = 8 h	D*	8.68 ± 1.69	8.71 ± 1.15
	D_0	8.47 ± 1.69	8.51 ± 1.16
γ = 12 h	D*	58.39 ± 15.98	60.01 ± 12.40
	D_0	58.22 ± 16.01	58.97 ± 12.57

$C^{\infty}_{p\ min}$ - Minimum inhibitory concentration

D^* - Loading dose in mg/Kg

D_0 - Maintenance dose in mg/Kg

γ - Dose interval

5. *Dosage regimen*

Table 26 presents the comparison of calculated dosage regimen, post i.m. administration of ofloxacin (4 mg/Kg). More or less similar D* and D_o of 8.68 ± 1.69 and 8.47 ± 1.69 mg/Kg were calculated for non-febrile goats and 8.71 ± 1.15 and 8.51 ± 1.16 mg/Kg for febrile goats, respectively, at γ of 8 h for maintaining C_p' min of 0.12 μg/ml. Similarly, the D* and D_o of 58.39 ± 15.98 and 58.22 ± 16.01 mg/Kg in non-febrile goats and 60.01 ± 12.40 and 58.97 ± 12.57 mg/Kg in febrile goats were calculated at γ of 12 h for maintaining C_p^{∞} min of 0.12 μg/ml.


Discussion

DISCUSSION

The fluoroquinolone class of antimicrobiol agents are frequently used in veterinary practice to treat a variety of microbial infections (Greene and Budsberge, 1993). They possess broad spectrum with bactericidal activity (Wolfson and Hooper, 1985; Vancutsem *et al.*, 1990; Chu, 1996). Ofloxacin, one of the latest flouroquinolones is currently used in clinical practice in human due to its wide spectrum of antimicrobial activity, excellent distribution in different tissues and lower toxicity. The available literature reveals that no kinetic study has been conducted in goats, particularly in febrile state. Commonly, febrile state is produced by most of the infectious diseases that alters the metabolism and excretion of the drugs (Song *et al.*, 1972), and thus variations in disposition kinetic behaviour of drugs as well as change in dosage regimen have been reported by many workers (Jaychandran 1994; Ansari 1997; Singh 1998). Keeping this fact in view, pharmacokinetic study of ofloxacin in healthy and febrile goats was carried out in order to assess the effects of fever on various kinetic parameters, so as to decide the dosage regimen for effective therapy of microbial infections in animals.

KINETIC STUDY OF OFLOXACIN IN GOATS

A. *Distribution in body fluids*

Concentrations of ofloxacin in plasma after single i.v. administration (4 mg/Kg) were noted to be lower at all time intervals in febrile goats as compared to healthy goats but they were non significant (Table - 11). Therapeutic concentration of 0.12 μg/ml was maintained up to six hour both in health and febrile goats. Significantly higher concentration of ofloxacin in milk were noted from 0.5 to 8 h in febrile goats as compared to healthy goats while non-significant difference was observed at 10 and 12 h between both the groups. The therapeutic concentration of ofloxacin (0.12 μg/ml) was maintained from 0.75 to 10 h in both healthy and febrile goats (Table - 11). Similar to plasma, no significant difference was observed in urinary concentrations of ofloxacin collected at different time intervals between healthy and febrile goats. The therapeutic concentration of the drug (0.12 μ/ml) was maintained from 2.5 min to > 24 h in both the groups of goats.

After i.m. administration of ofloxacin, the drug concentrations at all time intervals were noted to be higher in febrile goats but the data were non-significant which is similar to the pattern after i.v. administration. The therapeutic concentration in plasma was maintained around 2.5 min to 6 h in both the conditions. Unlike i.v. dose, the milk drug concentrations were noted to be non

significantly higher at various time intervals in febrile goats as compared to healthy goats. The therapeutic concentration in milk was maintained from 0.5 to 10 h in both healthy and febrile goats. In urine, the drug concentrations were found to differ only non-significantly in healthy and febrile goats and the therapeutic concentration was maintained from 2.5 min to > 24 h in both the groups of goats. Peak concentrations in plasma, milk and urine were attained at similar time of 0.75 h, 5 h and 2h, respectively, in both healthy and febrile goats.

Febrile condition may possibly increase the permeability of cell membrane including that of blood capillary. Significant increase in drug concentrations in milk were noted at most of the time intervals in febrile goats after i.v. administration while non significant increase was noted after i.m. administration. The better distribution of the drug in milk of febrile goats after i.v. administration may be due to higher maintenance of blood concentration of the drug for a longer period and the possible increase in the permeabily of capillary membrane caused by fever. An increase in concentration of oxytetracycline in lungs of pneumonia calves was explained by increased in its permeability (Ames *et al.*, 1983).

Pharmacokinetic study of perfloxacin (Ansari, 1997) and ciprofloxacin (Singh, 1997) showed lower plasma drug concentrations

at most of the time intervals accompanied by corresponding increase in urine drug concentration after i.v. and i.m. administration in febrile goats as compared to afebrile goats. In contrast, enrofloxacin, a fluorinated quinolone showed no significant change in plasma and urine drug concentrations post i.v. and i.m. administration in febrile goats as compared to afebrile goats. (Uday Kumar, 2000). The present findings of ofloxacin is similar to that of enrolfoxacin.

B. Kinetic Parameters

On comparing the disposition kinetics of ofloxacin in febrile goats with that of healthy ones, it was observed that almost none of the kinetic parameters of healthy goats differed significantly as compared to febrile goats. Significantly ($p < 0.05$) lower zero time concentration during elimination phase (B) was noted in febrile goats, while zero time concentration during distribution phase (A) and theoretical zero time concentration (C_0^p) were non significantly differed in febrile goats as compared to healthy goats after its i.v. administration when given @ 4 mg/Kg (Table-12). After i.m. administration (4 mg/Kg), the zero time concentration during absorption those (A) and elimination (B) phase were noted to differ only non significantly (Table-25).

These was no significant difference in absorption rate constant (Ka) between healthy (2.617 ± 0.160 h^{-1}) and febrile goats (2.811 ± 0.177 h^{-1}). Similar values of absorption half life ($t_{1/2}Ka$) of

0.27 ± 0.02 and 0.25 ± 0.02 h were noted in healthy and febrile goats, respectively, after i.m. administration of ofloxacin (Table - 25). The above finding indicates that the rate of absorption of ofloxacin was similar in both the groups after i.m. administration. This finding is supported by similar appearance of drug in plasma (2.5 min) and similar time to reach peak plasma concentration (0.75 h) in both healthy and febrile goats after i.m. administration (Table - 24). Distribution rate constant (α) of 3.718 ± 0.27 h^{-1} and distribution half life of 0.23 ± 0.05 h were noted for healthy goats in the present study (Table - 12). More or less similar value of α and $t_{1/2}\alpha$ and of 2.944 ± 0.35 h^{-1} and 0.25 ± 0.03 h, respectively, were noted in febrile goats. The above findings reveal that the rate of distribution ofloxacin is similar in body tissues under both healthy and febrile condition and not at all influenced by febrile state. This is similar to the findings noted for enrofloxacin (Uday Kumar, 2000), pefloxacin (Ansari, 1997) and ciprofloxacin (Singh, 1997) in goats. More or less similar $t_{1/2}\alpha$ of 0.22 ± 0.05 h was noted in bucks (Takawale *et al.*, 2000 a) and little lower value of 0.14 ± 0.01 h in rams (Takawale *et al.*, 2000 b) were noted which show the rate of distribution was similar in bucks and goats but little faster in rams.

The mean elimination rate constant (β) of 0.39 ± 0.05 and 0.454 ±7 0.09 h and elimination half life ($t_{1/2}\beta$) of 1.96 ± 0.20 and 1.55 ± 0.99 h were obtained in healthy goats after i.v. and i.m.

administration, respectively (table - 12 and 25). Higher $t_{1/2}\beta$ values of 3.852 to 4.540 h (Loade *et al.*,1987) and 5.62 to 6.40 h (Verho, 1985) in man, slightly higher $t_{1/2}\beta$ values of 2.39 h in rabbit (Perkins *et al.*, 1994) and 4.5 ± 0.3 h in matured dogs (Yoshida *et al.*, 1998) were reported. This shows that ofloxcacin is removed comparatively at a faster rate in goats as compared to man and other species . In the present study, no significant difference was observed in $t_{1/2}\beta$ values between healthy and febrile condition which denotes that the drug is removed at a similar rate under febrile condition. This is supported by non significant difference in the values of elimination rate constant of drug from central compartment (Kel) and total body clearance (Cl_B). This is further supported by non significant difference in urinary excretion of ofloxacin after i.v. and i.m. administration in healthy and febrile goats (Table 11 and 24).

There was no significant difference for the rate constant of drug transfer from central to peripheral compartment (K_{12}) between healthy (1.856 ± 0.02 h^{-1}) and febrile (1.222 ± 0.27 h^{-1}) goats. Similarly, non-significant difference was observed for the rate constant of drug transfer from peripheral to central compartment (K_{21}) between healthy (1.110 ± 0.145) and febrile (0.716 ± 0.100 h^{-1}) goats. This non significant difference in the above values led to in significant difference in the values of tissue to plasma concentration ratio ($T \approx P$).

The various other kinetic parameters viz., area under curve (AUC), total area under the first moment of plasma drug concentration curve (AUMC), mean residential time (MRT) and fraction of drug available for elimination from central compartment (Fc) were observed to be non significantly different in febrile goats as compared to healthy goats after i.v. administration (Table - 12) Similarly non significant difference was noted for the value of AUC between healthy and febrile goats after i.m. administration (Table- 25).

The various values of volume distribution did not differ significantly between healthy and febrile condition after i.v. and i.m administration of ofloxcin (Table - 12 & 25). Notari (1980) demonstrated that for a 2 - compartment open model, the value of $Vd_B > Vd_{area} > Vd_{ss}$ and Vd. He further mentioned that among these values of volume distribution, only Vd_{area} correctly predicts the amount of drug in the body during elimination phase where as Vd_B over estimates and Vd_{ss} and Vd under estimates the amount of drug in the body. The values of Vd_{area} of 2.30 ± 0.13 and 1.78 ± 0.16 L/kg were obtained for ofloxacin in healthy goats after its i.v. and i.m. administration, respectively. No significant difference was observed between healthy and febrile goats among the different Vd values. Higher Vd_{area} obtained in goats denotes that the drug is well distributed in different tissues and body fluid. This is supported by

higher drug concentrations obtained in milk post i.v. and i.m. administration. A little lower volume distribution of 1.22 to 1.4 L/Kg in man (Warlich *et al.*, 1990). 1.49 ± 0.20 L/Kg in bucks (Takawale *et al.*, 2000 a) and 1.61 ± 0.24 Lkg^{-1} in rams (Takawale *et al* ., 2000 b) were noted.

In the present study, the total body clearance (Cl_B) did not differ significantly between healthy and febrile goats after i.v. and i.m. administration (Table - 12 and 25). The values of Cl_B were observed to be 0.82 ± 0.15 and 0.81 ± 0.08 ml/Kg/min in healthy goats after i.v. and i.m. administration, respectively. Higher Cl_B values of 380.41 ± 53.10 ml/Kg/h (6.34 ml/Kg/min) in bucks (Takawale *et al.*, 2000a) and 480.40 ± 13.20 ml/Kg/h (8.01 ml/Kg/min) in rams (Takawala *et al.*, 2000b) were noted. The above findings denote that ofloxacin is comparatively slowly removed from the body of goats as compared to the above noted species.

C. Dosage Regimen

The minimum inhibitory concentration (MIC) values of enrofloxacin (close congener of oflaxacin) for different species of microorganisms ranged between 0.01 to 1 µg/ml in veterinary practice (Mevius *et al.*, 1990; Prescott and Yielding., 1990). Sudha Kumari (1998) and Uday Kumar (2000) have taken 0.12 µg/ml as MIC for calculating dosage regimen of enrofloxacin. Similarly, various workers (Singh, 1998; Srivastava, 1987) have taken 0.12

μg/ml as MIC for calculating dosage regimen of ciprofloxacin, another closely related congener of ofloxacin. Edlstein *et al.* (1996) noted that 90% strains of clinical *Legionella* isolates were inhibited by a concentration of 0.032 μg/ml of ofloxacin. Hence, in the present investigation 0.12 μg/ml has been taken as therapeutic concentration for calculating dosage regimen.

In the present study, loading (D*) and maintenance (D_o) doses were calculated for maintaining therapeutic concentration (MIC=C_p^{∞} min) of 0.12 μg/ml at dosage interval (γ) of 8 and 12 h in afebrile and febrile goats for i.v. and i.m. route (Table 13 and 26). Though lower D* and D_o were observed in febrile goats at γ of 8 and 12 h for maintaining C_p^{∞} min of 0.12 μg/ml, but the data were non significant as compared to afebrile goats for i.v. route. In case of i.m. route, more or less similar D* and D_o were obtained at the dosage interval of 8 and 12 h for maintaining C_p^{∞} min of 0.12 μg/ml in order to treat systemic infections. For i.m administration, the required D* and D_o at 8 h for maintaining C_p^{∞} min of 0.12 μg/ml are 8.68 ± 1.69 and 8.47 ± 1.69 ,g/Kg in afebrile state while these are calculated to be 8.71 ± 1.15 and 8.51 ± 1.16 mg/Kg in febrile state.

The drug maintained its therapeutic concentration (0.12 μg/ml) in milk for a period > 10 h in both afebrile and febrile goats

after i.v. and i.m. administration of ofloxacin (4 mg/kg). Hence, the drug may be given at the dose rate of 4 mg/Kg twice daily for treating mastitis caused by ofloxacin sensitive bacteria. The drug can also be used at the dose rate of 4 mg/kg by i.v. and i.m. route in treating drug susceptible urinary tract infections once daily, since the urinary drug concentrations were maintained above the therapeutic concentration for a period of 24 h in both healthy and febrile goats.


Summary

SUMMARY

A detailed pharmacokinetic study of ofloxacin was undertaken in healthy (afebrile) and febrile goats of non descript breed weighing between 20 to 25 Kg. Pharmacokinetic parameters were estimated in healthy and febrile goats for i.v. and i.m. routes using appropriate compartment models. Attempts were made to calculate the rational dosage regimen of the drug on the basis of kinetic data and maintenance of therapeutic concentration (MIC) in plasma. The findings are as follows.

1. Following i.v. administration of ofloxacin (4 mg/Kg), the mean therapeutic concentration (≥ 0.12 μg/ml) of ofloxacin was maintained from 2.5 min to 6 h in both healthy and febrile goats. Though the drug concentrations in plasma were slightly lower at all time intervals but they were non significant as compared to healthy goats. (Table - 11). Significant increase in concentrations of the drug in milk were found between 30 min to 8 h in febrile goats as compared to healthy ones (Table- 11). The therapeutic concentration (≥ 0.12 μg/ml) was maintained from 0.5 to 10 h in both healthy and febrile goats. The mean peak concentration of drug in milk was attained at 3 h in both goats. In urine, there was no significant difference of drug concentrations at all time intervals in febrile goats as compared to healthy goats. More or

less similar mean peak urine concentrations were attained at 45 min in both the group of goats. The therapeutic concentration in urine ($0 \geq 0.12$ µg/ ml) was maintained from 2.5 min to 24 h in both the groups.

2. Following i.m. administration of ofloxacin (4 mg/Kg), the drug maintained its therapeutic concentration (0.12 µg/ml) from 5 min to 5 h and 2.5 min to 6 h in healthy and febrile goats, respectively. The mean peak plasma concentration of the drug was attained at 45 min in both the group of goats. No significant difference in plasma level at various time intervals was observed between healthy and febrile goats (Table - 20). In milk, though the higher concentrations were observed at most of the time intervals in febrile goats as compared to healthy goats but the data were non significant. Peak milk concentration of ofloxacin was attained at 5 h in both the groups of goats. The therapeutic concentration was maintained from 1.5 to 10 h in both healthy and febrile goats (Table - 20). There was no significant difference in urine concentration between healthy and febrile goats. Peak concentration was attained at 2 h in both healthy and febrile goats. The therapeutic concentration in urine was maintained from 2.5 min to even beyond 24 h in both healthy and febrile goats.

3. It was observed that almost all of the kinetic parameters of healthy goats differed non significantly as compared to febrile goats except significantly ($p<0.05$) lower zero time concentration during elimination phase (B) after i.v. administration.

Similar values of absorption half life ($t_{1/2}$ Ka) of 0.27 ± 0.01 and 0.25 ± 0.02 h were noted in healthy and febrile goats, respectively, after i.m. administration of ofloxacin. The above findings indicate that the rate of absorption of ofloxacin was similar in both the groups after i.m. administration. This findings is supported by similar appearance of drug in plasma (2.5min) and similar time to reach peak concentration (0.75 h) in both healthy and febrile goats (Table- 24). More or less similar values of α and $t_{1/2}\alpha$ were obtained in healthy and febrile goats. This denotes that the rate of distribution of ofloxacin in body tissues may not be influenced by febrile state. The mean elimination rate constant (β) of 0.379 ± 0.05 and 0.45 ± 0.02 h^{-1} and elimination half life ($t_{1/2}\beta$) of 1.96 ± 0.20 and 1.55 ± 0.09 h were obtained in healthy goats after i.v. and i.m. administration, respectively (Table - 12 and 25). In the present study, no significant difference was observed in $t_{1/2}\beta$ values between healthy and febrile condition which denote that the drug is removed at a similar rate under febrile condition. This is supported by non significant difference in the values of elimination rate constant from central compartment

(Kel) and total body clearance (Cl_B). This is further supported by non significant difference in urinary excretion of ofloxacin after i.v. and i.m. administration in healthy and febrile goats (Table-11 and 24).

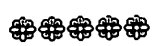
4. There was no significant difference for the rate constant of drug transfer from central to peripheral compartment (K_{12}) between healthy (1.861 ± 0.200 h^{-1}) and febrile (1.222 ± 0.270 h^{-1}) goats. Similarly non significant difference was observed for the rate constant of drug transfer from peripheral to central compartment (K_{21}) between healthy (1.110 ± 0.145 h^{-1}) and febrile (0.716 ± 0.100 h^{-1}) goats. This non-significant difference in the above values led to insignificant difference in the values of tissue to plasma concentration ratio (T≈P). The various other kinetic parameters viz., area under curve (AUC), total area under the first moment curve (AUMC), mean residential time (MRT) and fraction of drug available for elimination from central compartment (Fc) were observed to be non significantly different in febrile goats as compared to healthy goats after i.v. administration. Similar non significant difference was noted for the value of AUC between healthy and febrile goats after i.m. administration (Table - 25).
5. The various values of volume distribution did not differ significantly between healthy and febrile condition after i.v. and i.m. administration of ofloxacin (Table- 12 & 25) . The values of

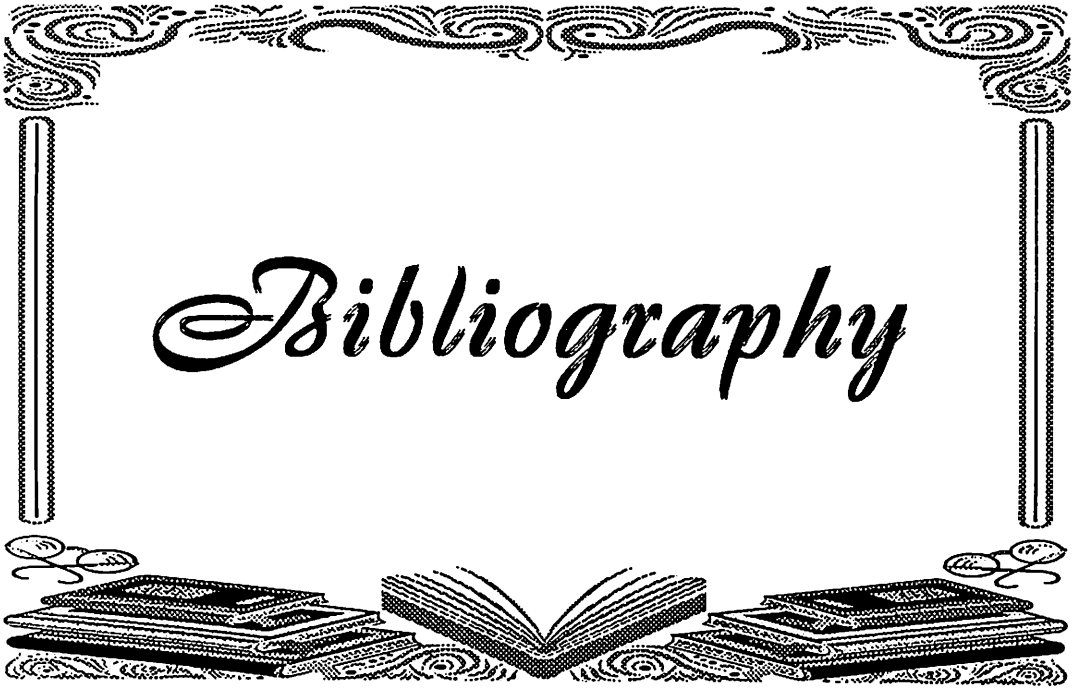
Vd_{area} of 2.30 ± 0.13 and 1.78 ± 0.16 L/kg were obtained for ofloxacin in healthy goats after its i.v. and i.m. administration, respectively. No significant difference was observed between healthy and febrile goats among the different Vd values. Higher Vd_{area} obtained in goats denotes that the drug is well distributed in different tissues and body fluids. This is supported by higher drug concentrations obtained at various time intervals in milk post i.v. and i.m. administration.

6. In the present study, the total body clearance (Cl_B) did not differ significantly between healthy and febrile goats after i.v. and i.m. administration (Table - 12 and 25). The values of Cl_B were observed to be 0.82 ± 0.15 and 0.81 ± 0.08 ml/Kg/min in healthy goats after i.v. and i.m administration, respectively.
7. In the present study, calculated loading (D*) and maintenance (D_o) doses to maintain C_p^{∞} min (MIC) of 0.12 µg/ml at desired dosage interval (γ) of 8 and 12 h were observed to be lower in febrile goats after i.v. administration though the data were non-significantly different from that of healthy goats. On the basis of the present study, ofloxacin may be administered at γ of 8 h at D* and D_o of 9.24 ± 5.06 and 8.9 ± 5.05 mg/Kg in afebrile goats and 4.61 ± 0.88 and 4.21 ± 0.82 mg/Kg in febrile goats, respectively for treating system infections by i.v.route. In case of i.m. route more

or less similar D* and D_o of 8.68 ± 1.69 and 8.47 ± 1.69 mg/Kg for non febrile goats and 8.71 ± 1.15 and 8.51 ± 1.16 mg/Kg for febrile goats, respectively, were required at γ of 8 h for treating system infections.

The drug maintained its therapeutic concentration (0.12 μg/ml) in milk for a period > 10 h in both afebrile and febrile goats after i.v. and i.m. administration of ofloxacin (4 mg/kg), and hence,the drug may be given at the dose rate of 4 mg/kg twice daily for treating mastitis caused by ofloxacin sensitive bacteria. The drug can also be used at the dose rate of 4 mg/Kg by i.v. and i.m. route in treating drug susceptible urinary tract infections once daily since the urinary drug concentrations were maintained above the therapeutic concentration for a period of 24 h in both afebrile and febrile goats.

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Appendix

APPENDIX - I

Calculation of kinetic parameters after i.m. administration of ofloxacin :

Kinetic parameters were calculated from the plasma log drug concentration versus time profile. An example is noted below from the data of goat no - 4 obtained after i.m. injection of ofloxacin (4mg/kg) in healthy goat. The data showed a monophasic curve and hence, well fitted into an one-compartment open model. Elimination phase starts from the time of peak concentration of the drug onwards

Time in h (X)	X^2	Plasma concentration (X) ($\mu g\ ml^{-1}$)	Log (Y x 10)	XY
0.75	0.5625	1.60	1.2041	0.9031
1	1.0	1.32	1.1206	1.1206
1.5	2.25	1.20	1.0792	1.6188
2	4.0	0.88	0.9445	1.8890
3	9.0	0.60	0.7782	2.3346
4	16.0	0.34	0.5315	2.1260
5	25.0	0.25	0.3979	1.9895
6	36.0	0.13	0.1139	0.6839
8	64.0	0.06	0.2218	1.7744
$\Sigma X = 31.25$	$\Sigma X^2 = 157.8125$		$\Sigma Y = 5.9481$	$\Sigma XY = 10.8906$

$\overline{X} = 3.4722$ $\overline{Y} = 0.6609$

$(\Sigma X)^2 = 976.5625$

b, slope of line $= \dfrac{n.\Sigma xy - \Sigma x.\Sigma y}{n.\Sigma x^2 - (\Sigma x)^2}$

$= \dfrac{9 \times 10.8906 - 31.25 \times 5.9481}{9 \times 157.8125 - 976.5625}$

$= \dfrac{98.0154 - 185.8781}{1420.3125 - 976.5625}$

$= \dfrac{-87\ .\ 8627}{443.75}$

$= -0.198 h^{-1}$

β, elimination rate constant = b × - 2.303

$\beta = -0.198 \times 2.303$

$= 0.456\ h^{-1}$

B, Zero time concentration during elimination phase can be obtained from the formula $\bar{y} = a + b\ \bar{x}$

= log 0.6609 - (0.198 x 3.4722)

= log 0.6609 + 0.6875

= log 1.3484

antilog of 1.3484 = 22.305 µg/ml

Since plasma concentration is multiplied earlier by 10 in the above mentioned calculation, the value of 22.305 µg/ml should be divided by 10 to get actual zero time concentration, Hence, zero time concentration (B) = 2.23 µg/ml.

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

Substracting the theoretical values from observed values, a series of residual concentrations were obtained and slope of line in natural log (absorption constant, Ka) and the zero time intercept [zero time concentration during absorption phase (A)] can be calculated as per the method adopted for calculation of B and β. The values of $Ka = 3.149\ h^{-1}$; $A = 2.28\ \mu g/ml$.

$t_{1/2}$ Ka, absorption half life

$$t_{1/2}\ Ka = \frac{0.693}{Ka} = \frac{0.693}{3.149} = 0.22\ h$$

$t_{1/2}\ \beta$, elimination half life

$$t_{1/2}\ \beta = \frac{0.693}{\beta} = \frac{0.693}{0.456} = 1.52\ h$$

AUC, Area under curve

$$AUC = \frac{B}{\beta} + \frac{A}{Ka} = \frac{2.23}{0.456} + \frac{2.28}{3.149} = 5.61\ mg/L.h.$$

V_{dB}, the volume of distribution based on elimination

$$V_{dB} = \frac{D}{B} = \frac{4}{2.23} = 1.79\ L/kg$$

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

$$Vd_{area} = \frac{D}{(AUC).\beta} = \frac{4}{5.61 \times 0.456} = 1.56$$

Cl_B, total body clearance

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

$$= 1.56 \times 0.456 = 0.71 \text{ ml/kg/min.}$$

APPENDIX - II

CALCULATION OF KINETIC PARAMETERS AFTER I.V. ADMINISTRATION OF OFLOXACIN :

Kinetic parameters were calculated from the plasma log drug concentration versus time profile. An example is noted below from the data of goat no-4 obtained after i.v. injection of ofloxacin (4mg/Kg) in healthy goats. The data showed a biphasic curve and hence well filled into a 2 compartment open model. Elimination phase starts from 1 hr.

me in h (X)	X^2	Plasma concentration (X) ($\mu g\ ml^{-1}$)	Log (Y x 10)	XY
1	1	0.94	0.9731	0.9731
1.5	2.25	0.75	0.8751	1.3126
2	4.0	0.68	0.8325	1.6650
3	9.0	0.52	0.7360	2.1480
4	16.0	0.40	0.6021	2.4084
5	25.0	0.28	0.4472	2.2360
6	36.0	0.20	0.3010	1.8060
= 22.5	ΣX^2= 93.25		ΣY = 4.471	ΣXY =12.5491

$\overline{X}$ = 3.214 $\overline{Y}$ = 0.6781

$$\text{b, slope of line} = \frac{n.\Sigma xy - \Sigma x.\Sigma x}{n.\Sigma x^2 - (\Sigma x)^2}$$

where, X = time (h) y = drug concentration, n = no. of observation

$$= \frac{7 \times 12.5491 - 22.5 \times 4.747}{7 \times 93.25 - 506.25}$$

$$= \frac{87.8437 - 106.8075}{652.75 - 506.25}$$

$$= \frac{-18.9638}{146.5} = -0.1294 \ h^{-1}$$

β, elimination rate constant = b x -2.303

= - 0.1294 x - 2.303

= 0.298 h^{-1}

B, zero time concentration during elimination phase can be obtained from the formula $\bar{y} = a + b\bar{x}$

where

$\bar{y}$ = mean drug concentration

$\bar{x}$ = mean time

b = slope of line

a = zero time concentration

Therefore

$a = \bar{y} - b.\ \bar{x}$

= log 0.6781 - (-01294 x 3.214)

= log 0.6781 + 0.4159

= log 1.0940

zero time concentration = antilog of 1.094 = 12.42μg/ml

Since plasma concentration is multiplied earlier by 10 in the above mentioned calculation, the value of 12.42 μg/ml should be divided by 10 to get the actual zero time concentration. Hence, zero time concentration (B) = 1.242μg/ml or 1.24 μg/ml.

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)

Substracting 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 absorption phase, A) can be calculated as per method adopted for calculation of B and β. The value of A is 5.40 μg/ml.

C_p^o, theoretical plasma concentration at time zero

$C_p^o = A + B$

= 6.64 μg/ml

$t_{1/2}$ α, distribution half life

$$t_{1/2}\,\alpha = \frac{0.693}{\alpha} = \frac{0.693}{3.546} = 0.20 \text{ h.}$$

$t_{1/2}\beta$, elimination half life

$$t_{1/2}\,\beta = \frac{0.693}{0.298} = 2.23 \text{ h}$$

AUC, Area under curve

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta} = \frac{5.46}{3.546} + \frac{1.24}{0.298}$$

$$= 5.68 \text{ mg/L.h.}$$

AUMC, Area under first moment curve

$$AUMC = \frac{A}{\alpha^2} + \frac{B}{\beta^2} = \frac{5.40}{(3.546)^2} + \frac{1.24}{(0.298)^2}$$

$$= \frac{5.40}{12.57} + \frac{1.24}{0.09} = 0.43 + 13.78$$

$$= 14.39 \text{ mg/K.h}^2$$

$$MRT = \frac{AUMC}{AUC}\frac{14.39}{5.68} = 2.53$$

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

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

$$= 3.546 + 0.298 - 0.905 - 1.168$$

$$= 3.844 - 2.073 = 1.77\ L^{-1}$$

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

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

$$= \frac{5.40 \times 0.298 + 1.24 \times 3.546}{6.64}$$

$$= \frac{1.609 + 4.397}{6.64} = \frac{6.006}{6.64} = 0.905\ h^{-1}$$

K_{el}, the elimination rate constant of the drug from central compartment.

$$K_{el} = \frac{\alpha.\beta}{K_{21}} = \frac{3.546 \times 0.298}{0.905} = \frac{1.057}{0.905} \quad = 1.168\ h^{-1}$$

Fc, the fraction of drug available for elimination from compartment.

$$F_c = \frac{\beta}{K_{el}} = \frac{0.298}{1.168} = 0.18$$

T≈P, approximate tissue to plasma concentration ratio

$$T \approx P = \frac{K_{12}}{K_{21} - \beta} = \frac{1.77}{0.905 - 0.298} = \frac{1.771}{0.607} = 2.92$$

V_{dc}, the volume of distribution based on distribution and elimination.

$$V_{dc} = \frac{D}{C_p^o} = \frac{4}{6.64} = 0.60 \text{ L / kg}$$

where D = Dose (4mg/kg)

V_{dB}, the volume distribution based on elimination

$$V_{dB} = \frac{D}{B} = \frac{4}{1.24} = 3.23 \text{ L.kg}^{-1}$$

V_{darea} the volume of distribution based on total under curve

$$V_{darea} = \frac{D}{(AUC).\beta} = \frac{4}{5.68 \times 0.298} = 2.366$$

$$= 2.37 \text{ L/kg.}$$

Vd_{SS}, the volume of distribution of steady state

$$V_{ds.s} = \frac{K_{12} + K_{21}}{K_{21}} \times V_d$$

$$= \frac{1.77 + 0.905}{0.905} \times 0.60 = \frac{2.676}{0.905} \times 0.60$$

$$= 2.957 \text{ x } 0.60$$

$$= 1.77 \text{ L/kg}$$

$Cl_B = V_{darea} \text{ x } \beta$

$$= 2.37 \text{ x } 0.298 = 0.71$$

APPENDIX - III

CALCULATION OF DOSAGE REGIMEN :

Dosage regimen of antimicrobial agents are generally calculated to maintain minimum inhibitory concentration (MIC) in plasma at desired dosage interval (γ) using the formulae noted by Baggot (1977) and described by Saini and Srivastava 1997. The data of animal No. 4 obtained after i.v. injection of ofloxacin in healthy goat has been used as an example for calculation of dosage regimen for maintaining C_p^{∞} min (MIC) of 0.12μg/ml at the dosage interval (γ) of 8 and 12 h.

Calculation of loading (D*) and maintenance (D_o) dose

The loading dose (D*) is the initial dose that may be given at the onset of therapy with the aim of achieving target concentration rapidly. The maintenance (D_0) dose is the dose given at a particular dosage interval (γ) for maintaining the C_p^{∞} min (MIC) during the course of treatment. The loading (D*) and maintenance (D_o) doses of ofloxacin can be calculated by the formulae given below.

$$D^* = C_p^o \text{ (min)}. V_{darea}. e^{\beta.\gamma}$$

$D_o = C_p^o$ (min). V_{darea}. $(e^{\beta.\gamma-1})$

Where, D* = Loading dose, D_o = Maintenance dose

C_p^o (min) = Minimum therapeutic plasma drug concentration

Vd_{area} = The volume of distribution based on total area under the plasma drug concentration versus time curve.

β = Elimination rate constant

γ = Dosage interval.

The loading and maintenance dosage of ofloxacin repeated at different time interval (8 and 12 h), to maintain the minimum plasma concentrations of 0.12µg/ml. Hence, by considering 0.12 µg/ml as minimum therapeutic concentration C_p^o (min) at dosage interval of 8hr in animal no-4 after i.v. injection of drug (afebrile goats), D* and Do are calculated to be

$$D^* = C_p^o(\text{min}).V_{darea}.e^{\beta.\gamma}$$

$$= 0.12 \times 2.37\ (e^{0.298 \times 8})$$

$$= 3.07 \text{ mg/kg}$$

$$D_o = C_p^o \text{ (min)}. V_{darea}.(e^{\beta.\gamma-1})$$

$$= 0.12 \times 2.37 \cdot e^{(0.298 \times 8)-1}$$

$$= 2.79 \text{ mg/kg}.$$