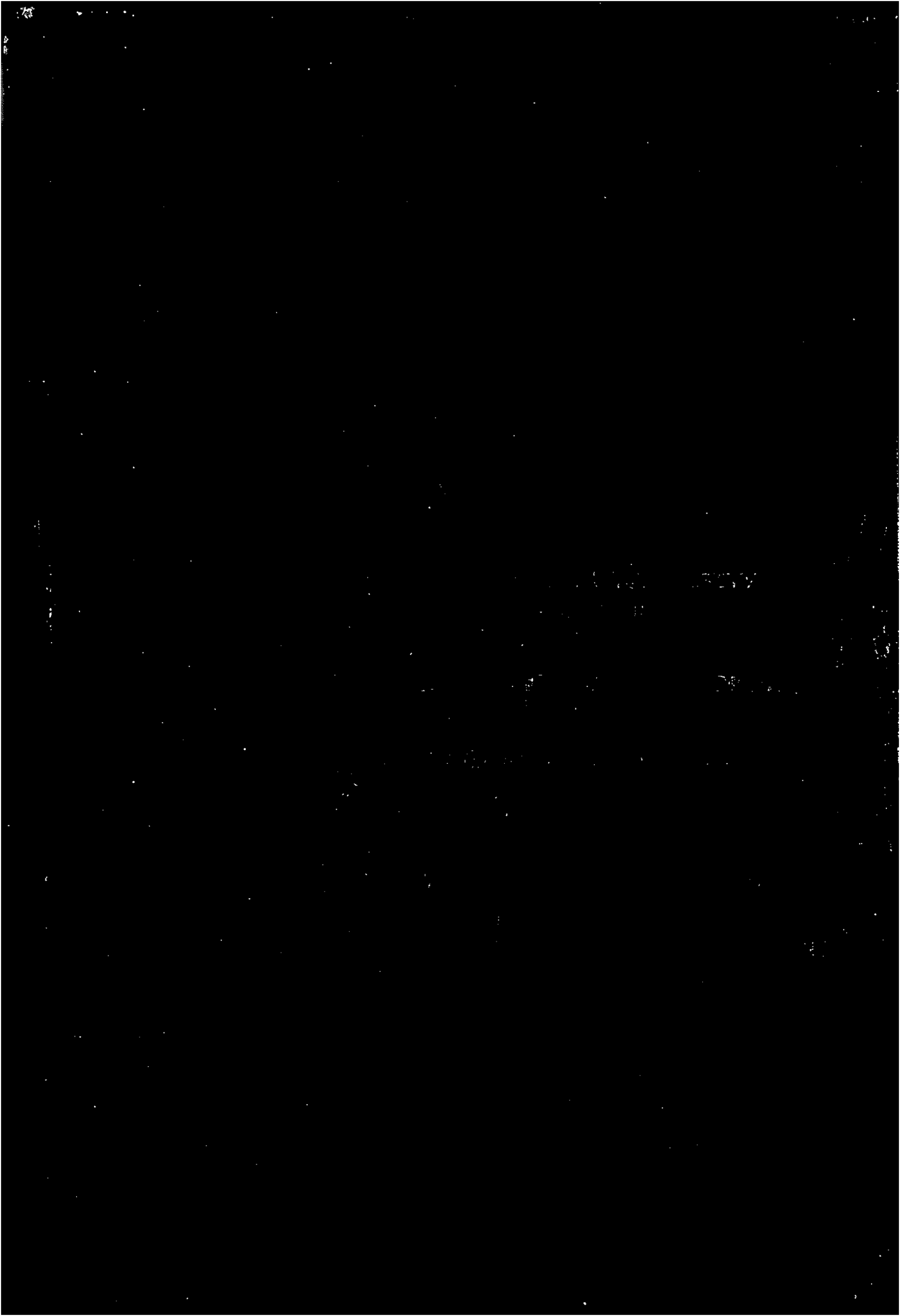

PHARMACOKINETIC AND IMMUNOLOGICAL STUDIES OF SPARFLOXACIN IN GOATS

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY
PUSA (SAMASTIPUR), BIHAR

(FACULTY OF POST-GRADUATE STUDIES)

In the partial fulfilment of the requirement

FOR THE DEGREE OF

MASTER OF VETERINARY SCIENCE
IN
VETERINARY PHARMACOLOGY AND TOXICOLOGY

By
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DEPARTMENT OF VETERINARY PHARMACOLOGY AND TOXICOLOGY
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Dedicated to

my benevolent
and
adorable
PARENTS
&
TEACHERS

DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY

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CERTIFICATE – I

This is to certify that the thesis entitled **"PHARMACOKINETIC AND IMMUNOLOGICAL STUDIES OF SPARFLOXACIN IN GOATS"** submitted in partial fulfillment of the requirement for the degree of **"Master of Veterinary Science (Veterinary Pharmacology & Toxicology)"** of the faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar, is the record of bonafide research carried out by **DR. RAMESH KUMAR NIRALA**, 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.

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We, the undersigned, members of the Advisory Committee of **DR. RAMESH KUMAR NIRALA**, 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 AND IMMUNOLOGICAL STUDIES OF SPARFLOXACIN IN GOATS**" may be submitted by **DR. RAMESH KUMAR NIRALA** in partial fulfillment of the requirements for the degree.

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Date : 31/12/2007

Place : Patna

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CONTENTS

❖❖❖

LIST OF TABLES

LIST OF FIGURES

❖❖❖

Introduction

INTRODUCTION

In the modern scientific era both human and animals are equally important for ecological balance. If one of them facing infectious diseases caused by several microbes like, bacteria, viruses, fungal, and parasite etc., our ecosystem or ecological balance may be disturbed. For maintaining ecological balance, we need antimicrobial agents because antimicrobials are frequently used to suppress or kill microorganisms in man and animals and thereby relieve them from various diseased conditions.

Antimicrobial agents play a vital role in medical and veterinary practices in combating various systemic microbial infections. The fluoroquinolones represent one of the major antimicrobial agents in chemotherapy against the microbial infections. The history of the newer quinolones began with the discovery of nalidixic acid in 1902 as an accidental by product during the synthesis of the anti-malarial compound, chloroquine (Lesher *et al.*, 1962).

In 1970, another group of quinolones (e.g., oxolinic acid, pipemidic acid and cinoxacin) were launched, but these compounds were only marginally better than nalidixic acid. A break through was achieved in early 1980s with the development of fluorinated 4 – quinolones (Wolfson and Hooper, 1985) such as norfloxacin and ciprofloxacin. Theses agents have broad spectrum antimicrobial

activity and are effectively used against wide varieties of infectious diseases with fewer side effects and non-development of microbial resistance.

Sparfloxacin is one of the latest fluoroquinolones with broad spectrum antimicrobial activity which has enhanced activity against gram-positive, gram-negative, mycoplasma etc. The major indication of this drug is in respiratory tract infections, which include pneumonia, excerbation, chronic bronchitis and sinusitis. It has good efficacy against tuberculosis, *Mycobacterium avium* infection in AIDS patients. Sparfloxacin has no active metabolite and the mechanism of action involves the inhibition of DNA gyrase like other fluoroquinolones.

Goat is a versatile animal and mainly reared in tropical countries like India. It is mainly reared by poor sections of society and serve as an easy alternative for uplifting the economic conditions of poor farmers because of its versatile traits like greater adaptability, maintenance on low grade ration, least rearing expenses and early economic return. Hence, it is very essential that proper health coverage may be achieved by sparfloxacin therapy.

For judicious use of an antimicrobial, a rational dosage regimen is a pre-requisite for which detailed pharmacokinetic study is needed. In order to use drug effectively, it is important to investigate the detailed pharmacokinetics of the drug in the same species and also

in similar climate in which the drug is to be used clinically (Nawaz *et al.*, 1980). Pharmacokinetic studies of antimicrobial agents are carried out in healthy animals to obtain detailed pharmacokinetic data. From these data, appropriate dosage regimen can be derived for effective treatment of the disease.

It is well known that many drugs including antimicrobial agents are known to either stimulate or depress the immune system and thereby alter the course of the diseases. Therefore, it is highly desirable to carry out immunological studies of antimicrobial agents.

Pharmacokinetics studies of sparfloxacin were carried out in different species of animal but so far, little work has been done on pharmacokinetics and immunological studies of sparfloxacin in goat.

Keeping these in view, the present study has been carried out with the following aims and objectives: -

1. Estimation of concentrations of sparfloxacin at different time intervals in biological fluids (plasma and urine) following i.v. administration.
2. Determination of kinetic parameters of sparfloxacin.
3. Calculation of dosage regimen of sparfloxacin.
4. To study the immunological properties of sparfloxacin following i.m. administration.

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Review of Literature

REVIEW OF LITERATURE

In general, the fluoroquinolones developed over the past few years have greater potency, a broader spectrum of antimicrobial activity, greater *in vitro* efficiency against resistance organisms and better safety profile than other antimicrobial agents including the oldest quinolones. Minimum inhibitory concentrations of these drugs against gram-positive, gram-negative, anaerobic and atypical organisms demonstrate their increased potency.

Sparfloxacin is a new fluorinated quinolone antimicrobial agent with a broad spectrum of activity against gram-positive microorganisms such as *Staphylococci* and *Streptococci* including *S. pneumoniae*. It is also effective against gram-negative organisms such as *Enterobactereaceae*, *H. influenzae*, *Legionella*, *Chlamydia* and *Mycobacteria*. *In vitro* activity of sparfloxacin is higher against many gram-positive, and against anaerobic bacteria than ciprofloxacin, ofloxacin and norfloxacin. Its *in vivo* activity in experimental model of infections against gram-positive, and gram-negative organisms is higher than that of ciprofloxacin.

FLUOROQUINOLONES :

Fluoroquinolones are synthetic antimicrobial agents that contain a carboxylic acid moiety in the 3 position of the basic ring structure responsible for anti microbial activity.

The chemical structure of 4-quinolone ring is given in Fig. 1.

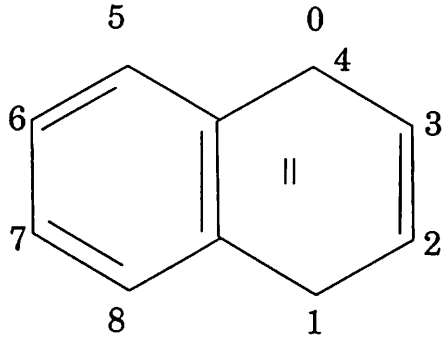

Fig. 1:- Chemical structure of 4-quinolones ring

The fluorine substituent at 6 shows the increased DNA gyrase inhibitory activity and extends the activity against both gram-negative and gram-positive pathogens. The absorption and half life of this compound is increased by addition of fluorine at 8 position and, also better absorption and longer half life by substitution of methyl group for piperazine. This compound contains a piperazine moiety at position F. Addition of cyclopropyl group at N_1, amino group at C_5 and fluorine at position 6 extend the spectrum of activity of these compounds. Modifications at position 2, 5, 7 result in the newer fluoroquinolone compounds with better antimicrobial activity.

PHYSICO-CHEMICAL PROPERTIES OF SPARFLOXACIN :

Sparfloxacin is an amino fluoroquinolone It is chemically designated as 5-amino-1-cyclopropyl-7-(Cis-3, 5-dimethyl-1-pierazinyl) –6, -8 difluoro-1, 4-0 × 0-3 quinolone carboxylic acid. It is a yellow

crystalline powder. The chemical structure of sparfloxacin is given below:

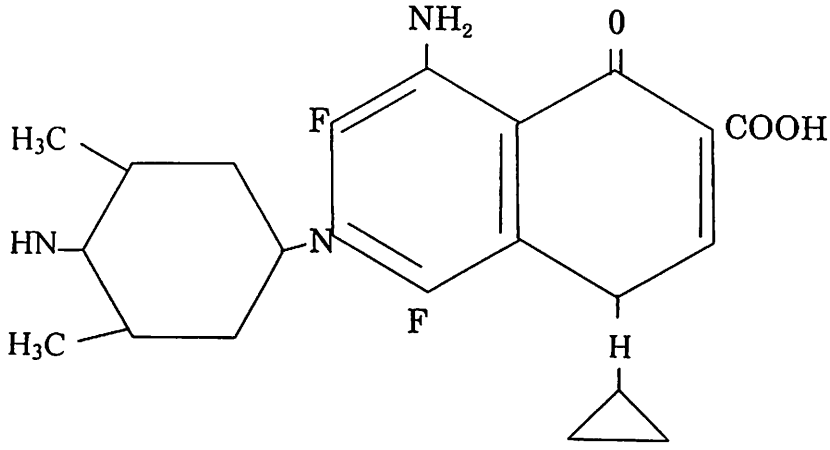

Fig. 2 : Chemical structure of sparfloxacin

Chemical name	:	5 - amino – 1 – cyclopropyl – 7 – (Cis – 3, 5–dimethyl – 1 – piperazinyl) – 6-8. difluoro – 1, 4 –dihydro – 4 – oxo – 3 quinolone carboxylic acid.
Empirical Formula	:	$C_{10}H_{22}F_2N_4O_3$
Mol. wt	:	392.41
Melting Point	:	266 – 269°C
pK Value	:	pK_1 – 6.25 pK_2 – 9.30
Solubility	:	It is a hydrophobic compound and its aqueous solubility is < 0.04 g/L at pH1 and <0.02 g/L at pH 7.4.

Antibacterial Spectrum :

Sparfloxacin also has good activity against *Staphylococci* including methicillin resistant strains (MIC_{90} = 0.1 to 1 $\mu g.ml^{-1}$). Several intracellular bacteria are inhibited by fluoroquinolones at

concentrations that can be achieved in plasma. These include species of *Chlamydia, Mycoplasma, Brucella* and *Mycobacterium.* Sparfloxacin has MIC_{90} values from 0.5 to 3 $\mu g.ml^{-1}$ for *M. Fortunetum* and *M. tuberculosis.* Most anaerobic microorganisms are resistant to the fluoroquinolones except sparfloxacin. The MIC values of susceptible microorganisms to sparfloxacin are given below :

MICRO ORGANISM	**MIC ($\mu g.ml^{-1}$)**
Staphylococcus	0.1 – 1
M. Fortunetum	0.5 – 3
Mycoplasma pneumoniae	0.063
Mycobacterium tuberculosis	0.5 – 3
S. aureus	0.125 – 0.06
Mycobacterium Kansasi	0.5 – 3
Vibrio Venificus	0.03 – 0.06
Mycobacterium maximum	2
Spirochete Borodia burgdorferi	1
Pneumococci	0.25 – 1
S. Pneumoniae	0.5

(Trautmann *et al.*, 1996; Cremieux *et al.,* 1996; Goa *et al.*, 1997; Aubry *et al.*, 2000; Kraiczy *et al.*, 2001; Gillespil *et al.*, 2001; Kaku *et al.*, 1994)

Mechanism of Action :

The quinolone antibiotics target bacterial DNA gyrase and topoisomerase IV (Drlica and Zhao, 1997). For many gram- positive

bacteria such as *S. aureus*, topoisomerase IV is the primary activity inhibited by the quinolones (Ng *et al.*, 1996). In contrast, for many gram-negative bacteria such as *E. coli*, DNA gyrase is the primary quinolone target (Hooper, 2000; Alovero *et al.*, 2000). The two strands of double helical DNA must be separated to permit DNA replication or transcription. However, anything that separates the strands result in over winding or excessive positive super coiling of the DNA in front of the point of separation. Sparfloxacin acts by inhibiting the super coiling activity of DNA gyrase, which is an enzyme essential for DNA replication.

Fate of Target Cell After Effect of Fluoroquinolone :

The morphological alterations caused by the fluoroquinolones include decreased cell division, filamentation and lysis (Foerster, 1987). Besides altered cell division, numerous bacterial cell ghost, (*i.e.* bacterial cell with only one cell wall and no internal cell components) were formed. This was thought to be due to the inhibition of DNA gyrase that eventually lead to generalized bacterial cellular dysfunction, dysfunction of normal cellular replication, repair and eventfully the death of cell.

PHARMACOKINETICS :

Though many studies were carried out in man but only few reports are available with regards to animals about the pharmacokinetics of sparfloxacin. The details are given below.

Man :

Jhonson *et al.* (1992) studied the pharmacokinetics of sparfloxacin following oral administration of a single dose of 400 mg in healthy male volunteers. They observed a mean peak plasma concentration (1.6 $\mu g.ml^{-1}$) at mean time of 2.7 h. The mean elimination half life in plasma and inflammatory fluid were 17.6 h and 19.7 h, respectively. Urinary recovery of sparfloxacin was 8.8% of the total administered dose that occurred within the first 52 h.

Naora *et al.* (1992) showed the effect of fenbuten on pharmacokinetics of sparfloxacin after i.v. administration. They collected arterial blood, CSF and whole brain simultaneously and detected their concentrations at various time intervals.

Shimada *et al.* (1993) reported pharmacokinetics of sparfloxacin in healthy volunteers. They studied the effect of change of pH of urine on the kinetic behaviour of sparfloxacin. The difference between treatment for Cp_{max}, AUC and Cl_B were found to be significant. The bioavailability of sparfloxacin was found to be 84.4% and 122.3% after acidification and alkalization of urine, respectively.

Montay *et al.* (1994) studied the pharmacokinetics of sparfloxacin in human after single oral administration at dose of 200, 400, 600, 800 mg. They determined the mean C_{max} value ranged from 705 ± 158 to 1966 ± 620 $\mu g.ml^{-1}$ and t_{max} ranging from 4 – 5 h and AUC

was 87 to 88%. The elimination half life values were constant i.e. ranging from 18 to 21 h. The metabolic ratio conjugated/free drug was not modified by increasing dose.

Davey *et al.* (1994) studied the ciprofloxacin and sparfloxacin penetration into human brain tissue and their activity as antagonist of GABA receptor of rat vagus nerve after a single dose of sparfloxacin (400 mg) or ciprofloxacin (750 mg). Drug concentrations in brain tissues exceeded those in CSF by 1.8 to 19.4 fold. The peak sparfloxacin concentration in brain tissue may have occurred later than 3 to 5 h.

Bron *et al.* (1994) studied about penetration of sparfloxacin into the aqueous humour after oral administration (400 mg) in 28 patients undergoing cataract surgery. The aqueous levels were 0.12 ± 0.036 $\mu g.ml^{-1}$ to 0.404 ± 0.159 $\mu g.ml^{-1}$ from 2 – 24 h after ingestion. The therapeutic level of sparfloxacin may be achieved in non inflamed, non infected eyes under going cataract or vitreous surgery.

Goa *et al.* (1997) studied about antibacterial activity, pharmacokinetic properties, clinical efficacy and tolerability of sparfloxacin in lower respiratory tract infection. Sparfloxacin showed similar efficacy to that of amoxycillin, erythromycin roxithromycin, amoxicillin/clavulanic acid and amoxicillin plus ofloxacin, producing clinical cure rates of 80 to 84%. They observed the good activity of

sparfloxacin against *S. pseudominae* and other respiratory pathogens. It showed good efficacy after once daily dosing and good gastro intestinal tolerability. It was noted that lack of interaction with theophylline was advantageous.

Rat :

Matsunaga *et al.* (1991) studied the disposition and metabolism of sparfloxacin in rats during and after 14 consecutive daily oral administration at 10 $mg.kg^{-1}$. Plasma levels after the 1st and 14th administration were similar in terms of t_{max} (1 h), C_{max} (1.35 $\mu g.ml^{-1}$), $t_{1/2}$ (3-4 h) and AUC (7.3 $\mu g.ml^{-1}$ h). Tissue distribution after the repeated administration was similar to that after single administration level in kidney, liver, pancreas, submaxillary glands, lungs and many others were higher than or similar to those in plasma and in brain and in some others were lower.

Furukawa *et al.* (1991) studied the disposition and metabolism of sparfloxacin in rats after oral administration of 10 $mg.kg^{-1}$. They observed the extent of absorption was 70% when estimated by AUC, urinary excretion and biliary excretion. C_{max} estimated was 1.32 $\mu g.ml^{-1}$ within 1 h. after oral administration and decreased with a half life of about 4 h in lactating rats. Milk was found to contain radioactivity several times as high as plasma levels which decreased with a similar half life.

Hamster :

Kaku *et al.* (1994) studied the *in vitro* and *in vivo* activities of sparfloxacin against *Mycoplasma pneumoniae* that were compared with those of erythromycin, levofloxacin, ofloxacin and minofloxacin. The MIC of sparfloxacin was noted to be 0.063 for 90% of the *M. pneumoniae*. Sparfloxacin was effective as erythromycin when orally administered at 15 $mg.kg^{-1}$ twice daily. The peak concentration of sparfloxacin in hamster serum after administration of single dose of 15 $mg.kg^{-1}$ was almost the same as that of human serum after administration of single oral dose of 200 mg and half life of sparfloxacin in hamster serum was shorter than in human serum after administration of single oral dose of 200 mg.

Rabbit :

Satia *et al.* (2005) evaluated the pharmacokinetic parameters in rabbits after measuring the sparfloxacin concentrations in aqueous humour after 15 minutes of instillation of sparfloxacin (0.3%). The aqueous humour level was found to be 1.47 $\mu g.ml^{-1}$ which reached to the peak level of 3.7 $\mu g.ml^{-1}$ at 1.3 h and declined to 0.57 $\mu g.ml^{-1}$ at 6 h. The level of sparfloxacin in aqueous humour in the study was sufficiently higher than the MIC reported for common ocular pathogen such as *Staphylococcus*, *Streptococcus etc.*

Chicken :

The pharmacokinetic properties of this drug in broiler chicken following single oral administration of sparfloxacin at the dose of 20 $mg.kg^{-1}$ were studied. The various pharmacokinetic parameters studied include AUC (45.29 $mg.h.ml^{-1}$), Vd_{area} (2.4 $l.kg^{-1}$), Cl_B (4.55 $ml.min^{-1}.kg^{-1}$) $t_{1/2}$ β (5.94 h) and MRT (10.54 h). The C_{max} of 2.63 $\mu g.ml^{-1}$ was noticed as 6 h (Mathuram *et al.*, 2005).

Some important kinetic parameters of sparfloxacin in different species are given below :

Species	Dose $mg.kg^{-1}$	Route of admini-stration	Distribution half-life $t_{1/2}$ α (h)	Eliminati on half-life ($t_{1/2}$ β) (h)	Volume distribution ($L.kg^{-1}$)	Total body clearance $ml.kg.^{-1}min^{-1}$	Reference
Broiler chicken	20 $mg.kg^{1}$	Oral	--	5.94	2.4	4.55	Mathuram *et al.* (2005)
Goats	20 $mg.kg^{1}$	Oral	0.64 ± 0.17	8.4 ± 0.74	0.97 ± 0.09	1.33 ± 0.07	Kujur, R.S. (2005)
Human	400 $mg.kg^{1}$	Oral	--	17.6	--	--	Johnson (1992)
Rat	10 $mg.kg^{1}$	Oral	--	4	--	--	Furukawa (1991)
Rabbit	40 $mg.kg^{1}$	I.V.	--	2.39±0.29	--	--	Liu et al. (1998)

IMMUNOLOGICAL STUDIES :

The knowledge of possible influence of antimicrobial on the immune response seems to be of great importance for the clinical approach to the process of therapy. This is especially interesting when antimicrobial are given to immune suppressed animals and man.

Literature on immunological studies of fluoroquinolones carried out in some species of animal are stated below :-

Fluoroquinolone do not have any effect on phagocytic cell function, lymphocyte proliferation, cytokine production or bone marrow tissue (Manzella and Clark 1988). In fact production of cytokines producing the immuno stimulatory effect [e.g. Inter leukin -1 (1L-1), inter leukin-2 (1L-2) and the colony stimulating factors] produced by stimulated lymphocyte and splenocytes is enhanced at the therapeutic concentration of the fluoroquinolones. Higher dosages around 50 $\mu g.ml^{-1}$ have been found to inhibit bone marrow cell and lymphocyte proliferation *in vitro* (Shalit, 1991). Hence, administering and fluoroquinolones over 10-20 times the MIC would not be beneficial for treatment. Ciprofloxacin (and hence its congeners) have been reported to stimulate bone marrow cell in experimentally immuno suppressed rats. Soback *et al.* (1994) observed a stimulation and increased in neutrophil function following administration of fluoroquinolones at the therapeutic dose.

Jose *et al.* (1993) studied the effect of pefloxacin on certain natural host defense mechanism and immune response in rabbit. Among the different parameters assessed, the agglutinating antibody titres and the absolute lymphocyte counts were reduced slightly by

pefloxacin. The total immunoglobulin concentration, total serum protein and total leukocyte count were not affected.

GENERAL PHARMACOKINETICS :

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

The compartment model is a hypothetical structure, which can be used to characterize with reproducibility of behaviour and fate of drugs in a biological system, when administered by certain route in a particular dosage form. In pharmacokinetic studies compartment is an entity, which has definite volume and in that concentration a drug exists at any time. The disposition kinetic of a drug is described either by one compartment or multi compartment open models. Body distributes the drug in all tissues widely at 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 semi logarithmic scale, a straight line is obtained (Sams, 1978) and the plasma drug level declines according to the following equation:-

$$Cp = Be^{-\beta t} \qquad \ldots.1$$

Where,

Cp = Concentration of drug in plasma

B = Extrapolated zerotime intercept of mono exponential curve.

β = Over all elimination rate constant.

t = Time elapsed after drug administration

e = Base of natural logarithm

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

Two-Compartment Open Model :

The pharmacokinetics of most of the drugs following i.v. administration are accurately describe by two compartment open model.

Baggot (1974) stated that in two-compartment open model, the drug distribution is instantaneous and homogenous 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, semi logarithmic plot of plasma drug concentration against time show 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 biexpential mathematical expression as a function of time :

$$Cp = Ae^{-\alpha t} + Be^{-\beta t} \qquad \ldots (2)$$

Where,

Cp = 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 value of A, B, α, and β are essential in calculating other kinetics rate constant (K_{12}, K_{21} and K_{el}) 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 drug may also follow three or multiple compartment model. In three compartment open model, the semi logarithmic plot of plasma drug concentration against time show 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 :

$$C_p = Ad^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t} \quad \ldots (3)$$

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

Non-Compartmental Model :

Non compartmental method does not require the assumption of a specific compartmental division of body to study the disposition pattern of drug and/or its metabolites. This method is applied for linear/non-linear pharmacokinetics. In compartmental open model, some of the parameters, such as estimation of bioavailability, total body clearance, apparent volume of distribution are derived from method based on non compartmental analysis. Only the fact is that this term is comparatively new although being used for long time. In case of compartmental open model, the parameters are derived from the best-fitting curve whereas, in non-compartmental model the method are statistical involving derivations and integration the whole analysis is based on statistical moment theory (Bhupinder Singh, 1999).

Pharmacokinetics of Clinical Importance :

Clinically, the pharmacokinetics studies consist of :

(a) Calculation of various kinetics 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 PHARMACOKINETICS 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 (i.m./s.c./oral) administration.

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

These parameters indicate the rate of distribution (faster or slower) of a drug from plasma to body fluids and tissue 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 the following :

(i) Elimination half life ($t_{1/2}$ β)

(ii) Volume of distribution by area method (Vd_{area})

(iii) Total body clearance (Cl_B)

(iv) Drug withdrawal period for drug residues in milk and tissues of food producing animals.

(4) Elimination half life ($t_{1/2}$ β) :

Gibaldi 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 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 distribution is not dependent upon the method used for its calculation if the drug distribute truly according to one compartment open model. The apparent volume of distribution indicates the amount of distribution of drug without providing and clue whether the drug is uniformly distributed or restricted to certain tissues (Baggot, 1977). A large volume of distribution (> 1 $L.kg^{-1}$) 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 compartment 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) that 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 processes of drug distribution, biotransformation and excretion. The parameter, body clearance, on the other hand is independent of these processes and indicate 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 Kel, the total body clearance changes exactly in proportion to Kel (Jusko and Gibaldi, 1972; Rowland *et al.*, 1973).

It is reported that the various constants namely A, α, B, β, $t_{1/2}$ α, $t_{1/2}$ β and Vd_{area} etc. Change disproportionably 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 fluid. 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 effect and toxicity. Thus, the objective of a multiple dosage regimen is to maintain the plasma concentration of the drug within the limit of the maximum safe concentration and the minimum effective levels.

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Material and Methods

MATERIALS AND METHODS

In the present study, clinically healthy female goats of non-descript breed between 20 to 24 months of age and 18-22 kg body weight were used.

EXPERIMENTAL DESIGN :

A. Pharmacokinetic study :

Sparfloxacin was administered in each of six healthy goats by i.v. route. A minimum period of 15 days was allowed to elapse before administration of the next dose of the drug.

B. Immunological study :

For conducting immunological study, clinically healthy goats were divided into three groups consisting of four animals in each group. Details of treatment given to different groups are given below:-

Group I - Saline control

Group II - Antigen control – 2 ml of 7%

Sheep red blood cell (SRBC) suspension i.v. given in each goat on the first day of treatment *i.e.* sensitizing dose, and on the 10^{th} day of experiment *i.e.* challenging dose.

Group III – Sparfloxacin + Antigen – Apart from antigen (SRBC) given as in group II, sparfloxacin was administered @ 5 mg/kg, i.m. daily for 7 days during sensitizing and challenging period.

Sterilization of Glasswares :

For immunological study only, sterilization process is adopted. All glasswares were washed properly with liquid solution in running tap water. These were again rinsed with distilled water and finally air dried. Test tubes, centrifuge tubes and vials were plugged with cotton and pipettes were wrapped with papers. All these materials were sterilized in hot air oven at 160^0C for an hour. For administration of drug and for collection of blood, sterile disposable needles were used.

COLLECTION OF BIOLOGICAL FLUIDS AND THEIR TIMING :

1. PHARMACOKINETIC STUDY :

The samples of various biological fluids were collected after i.v. administration of the drug in healthy goat. The samples of plasma and urine were collected at 2.5, 5, 10, 15, 20, 30, 45 min. and 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h but samples of urine were collected further upto 48 h (at 30, 36 and 48 h).

A. <u>Blood</u> :

Before collection of blood, the sites around the jugular vein on either side of the neck of the animals were aseptically prepared. The site was sterilized prior to each collection with rectified spirit. Blood samples were collected in centrifuge tube containing appropriate amount of heparin by vene-puncture with disposable 18 G needles at various above noted time intervals after drug administration. The

blood samples were centrifuge at 3000 rpm for 10 min. for the separation of plasma. Plasma samples were then kept under refrigeration until estimation was carried out. For the preparation of standards, normal plasma prior to drug administration was also collected.

B. Urine :

Urine samples were collected for analysis by introducing a sterile Foley's balloon catheter (No. 12) lubricated with glycerin through urethra into the urinary bladder of the experimented goats with the aid of a flexible metal probe. Xylocaine 2% was used as local anesthetic for the entry of metal probe. The balloon of the catheter was inflatted by injecting 25-30 ml of water through syringe to keep the catheter in position. The opening of the catheter was blocked with a pressure clip to check dripping of urine. Prior to drug administration, urine sample was also collected in a test tube for the preparation of standards. After administration of the drug, urine samples were collected in test tubes at various above noted time intervals. The samples were kept in a refrigerator and were analyzed in successive days.

2. IMMUNOLOGICAL STUDY :

Blood samples were collected on day 1, 7, 10, 14, 21, 28, 35 and 42 days of the experiment without anticoagulant for serum

separation. The separated serum samples were used for HA (Haemagglutination) test. Blood samples were also collected on various days as noted above for absolute lymphocyte count.

ADMINISTRATION OF DRUGS :

1. PHARMACOKINETIC STUDY :

For this purpose sparfloxacin powder obtained from Wochardt Pharmaceutical, Bombay, India, was dissolved in sterile water containing NaOH (final solution pH_8) was injected at the dose rate of 5 $mg.kg^{-1}$ by i.v. route.

2. IMMUNOLOGICAL STUDY :

Sparfloxacin (5 $mg.kg^{-1}$) was administered i.m. for 7 days in healthy goats of group III during period of pre and post challenge.

ESTIMATION OF SPARFLOXACIN BY HPLC METHOD IN BIOLOGICAL FLUIDS :

Apparatus :

The HPLC equipment used comprised of a HPLC pump (model 515 – Waters®), a dual wavelength absorbance detector (model 2487 – Waters®), a rheodyne manual injector with 25 µl loop size and chromatographic separation were performed using column size of 3.9 × 150 mn (Navapak C_{18} – Water®). Computer software of waters® were used for final estimation of concentrations of test samples.

Chromatographic Condition :

The flow rate was 1.2 ml min^{-1}, the effluent wavelength was monitored at 295 nm, loop size 25 µl, injection volume was 100 µl, the chart speed was 0.25 $mm.min^{-1}$ and detector sensitivity as 2.000 AUFS (area under full scale) were adopted for HPLC analysis for sparfloxacin.

Reagent :

All solvent used were of HPLC grade. All other chemicals and reagents were of analytical grade and freshly prepared triple distilled water was used for HPLC analysis.

Mobile Phase :

The mobile phase comprised of Acetonitrile : 0.1 M phosphate buffer (25 : 75). The pH of mobile phase was 2.5 approximately.

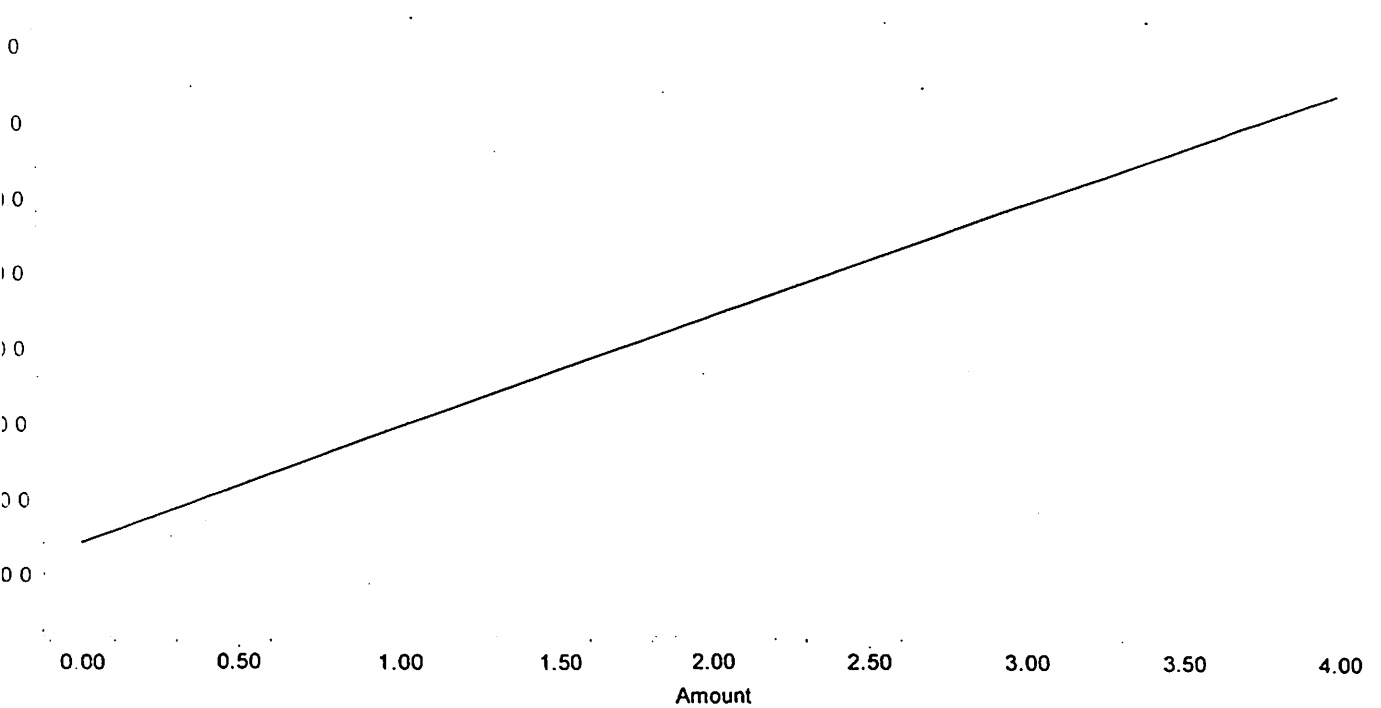
PREPARATION OF STANDARDS OF SPARFLOXACIN IN BIOLOGICAL SAMPLES : (PLASMA AND URINE) :

Sparfloxacin preparation containing 20 mg/ml was used in the present study. Sparfloxacin was diluted in triple distilled water to have different strength viz, 40, 20, 10, 5, 2.5, 1, 0.5, 0.25 $\mu g.ml^{-1}$. From each standard solution, 0.1 ml was added to a centrifuge tube containing 0.9 ml plasma or urine collected prior to drug administration. This yielded sparfloxacin standard of 4, 2, 1, 0.5, 0.25, 0.1, 0.05, 0.025, in the above mentioned biological fluids.

Blank plasma/urine containing no drug was also prepared. These standards were used simultaneously with test samples for determination of the drug concentrations in the test samples.

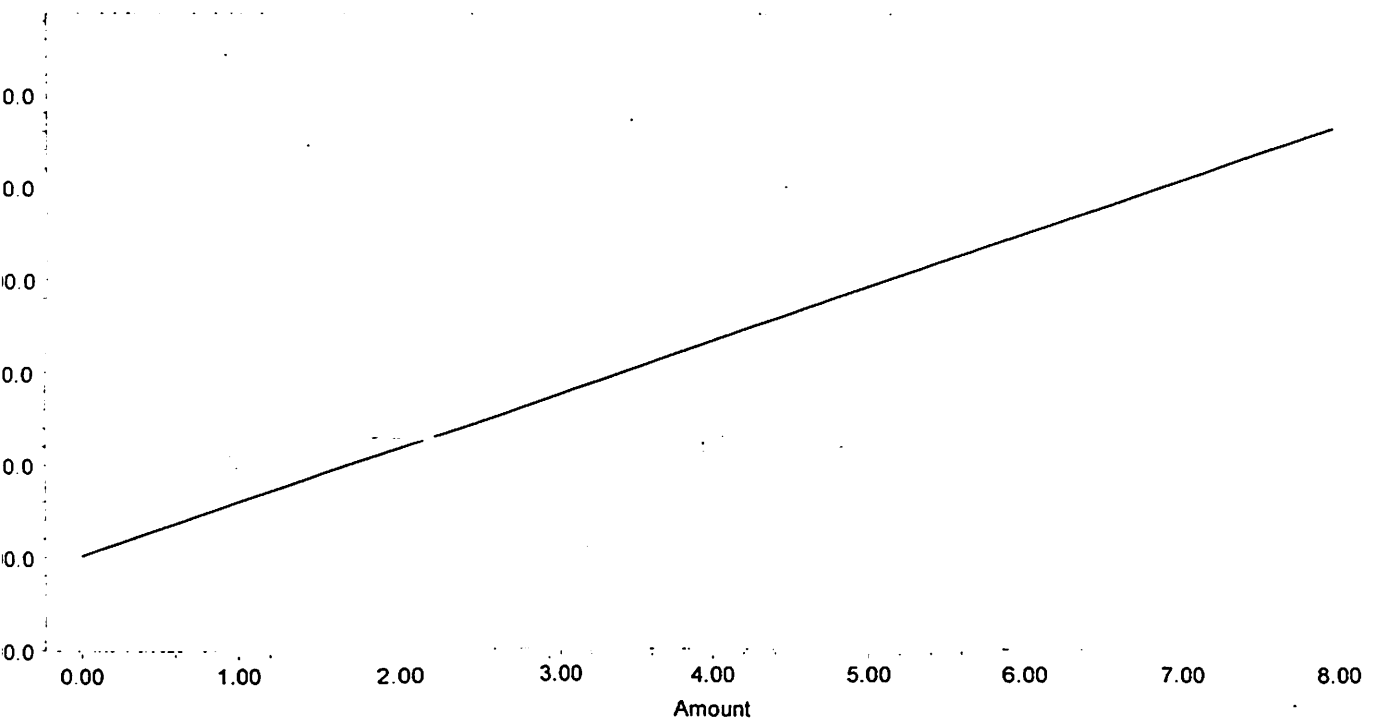
Analytical Method :

1. In a clean and dry centrifuge tube, 400 µl of plasma sample was taken and 600 µl of acetonitrile was added for precipitation of plasma proteins.
2. The mixture was vortexed for 1 min. And then centrifuged for 15 min at 3000 rpm.
3. After that 300 µl of supernatant was transferred to a clean tube and mixed with 600 µl of triple distilled water.
4. An aliquot of this mixture (upto 100 µl) was injected directly into the loop injector and the data was recorded which were stored in computer (retention time and area).
5. From various concentrations of standard *versus* area, standard curve was plotted for sparfloxacin (Fig 1: Standard curve of plasma; Fig 2: Standard curve of urine).
6. By using the standard graph and the area obtained from tests (plasma and urine samples), concentrations were obtained separately for plasma as well as for urine samples.



Peak Name: Sparfloxacin12; RT: 2.880; Fit Type: Linear (1st Order); Cal Curve Id: 1894; R: 0.993101; R^2: 0.986249; Weighting: None; Equation: Y = 1.50e+004 X + 4.12e+003; Normalized Intercept/Slope: 0.129375; RSD(E): 11.392214

Fig. 1: Plasma standard curve of sparfloxacin



Peak Name: Sparfloxacin; RT: 2.623; Fit Type: Linear (1st Order); Cal Curve Id: 2065; R: 0.923259; R^2: 0.852408; Weighting: None; Equation: Y = 5.78e+003 X + 7.20e+005; Normalized Intercept/Slope: 27.696231; RSD(E): 1.228864

Fig. 2: Urine standard curve of sparfloxacin

ANALYSIS OF IMMUNOLOGICAL PARAMETERS :

1. Preparation of Buffer :

(a) Phosphate buffer saline (Aziz, 1985) – The composition of buffers used for reconstitution and preparation of sheep red blood cell (SRBC) is as follows :-

NaCl	-	2.0 gm
KCl	-	0.05 gm
$Na_2HPO_4.2H_2O$	-	0.14 gm
KH_2PO_4	-	0.05 gm
Distilled water	-	250 ml
PH	-	7.2 to 7.4

This solution was autoclaved at 15 lb pressure for 15 min and stored at refrigerated temperature till use. This buffer was used for reconstitution and preparation of RBC suspension.

(b) Alsever's solution – the equal volume of following composition was used ad anticoagulant for collection of sheep blood.

Dextrose	-	5.125 gm
Sodium citrate	-	2.0 gm
Sodium chloride	-	1.05 gm
Citric acid	-	0.137 gm
Distilled water	-	250 ml

This solution was autoclaved in running steam (15 lb pressure) for one hour and kept at 4^0C for 1-2 weeks.

2. Mitogen used for delayed type hypersensitivity :

(a) DNCB (1 chloro – 2, 4 – dinitrobenzene):1% DNCB solution was prepared in acetone (10 mg/ml).

(b) PPD (Tuberculin) : A. commercial preparation of tuberculin (purified protein derivative) available in the diluted form of 10 i.v./0.1 ml manufactured by Beacon diagnostic Pvt. Ltd. 424 New GIDC, Kabilpore, Navsari – 396424 India warp procured.

3. Preparation sheep red blood cell (SRBC) suspension :

10 ml of blood was collected from jugular vein of sheep in a clean sterilized test tube containing equal volume of Alsever's solution as anticoagulant and left it for 24 h. The blood was pooled and mixed with equal amount of phosphate buffer saline (PBS) and centrifuged at 500-800 rpm for 10 min. The supernatant was removed and packed cell volume was washed 3 times with PBS. Finally 7% SRBC suspension and 0.8% SRBC suspension was made in fresh PBS and stored at refrigerated temperature (4^0C) this SRBC suspension was used only for four days from the day of preparation. For further work fresh SRBC suspension was prepared as and when required.

4. Collection of serum sample from goats :

5-10 ml of blood was taken from the regular vein of each goat with the help of 18 G needle by vene-puncture. The blood was

taken into a sterilized test tube and kept in slanting position for 4 to 5h at room temperature. The separated serum was collected in a clean sterilized vial of 2 ml capacity and was preserved by adding sodium azide (1:10,000) and stored at 0^0C until used.

5. Assessment of immune response after administration of drug :

A. Humoral Immune Response (HIR) – For assessing HIR, HA test was done which is stated below :

Haemagglutination (HA) test :

Humoral Immune response (HIR) was assessed by HA test. Antigen used for haemogglutination reaction in sheep red blood cell. HA test was performed in the test and goat sera control of the anti-SRBC antibody titres were measured by using micro-titration technique as described by Beard (1980).

HA test was performed in 'U' shaped micro perspex plate. By taking 0.5 ml of serum, two fold serial dilution was made in PBS except in control well in which only PBS (0.5 ml) was added. In next step, 0.5 ml of 0.8% SRBC suspension was added to all the wells. A known positive and negative control was also included. The plate was stirred gently for mixing and uniform distribution of erythrocyte and left at room temperature for 40 min. the goat's serum produce a diffuse sheet of agglutination RBC covering the bottom of well where as negative control well showed circumscribed compact button at the bottom.

The HA pattern was read with the aid of reading mirror and result of HA titre was recorded as reciprocal of the highest dilution showing 100% HA (Complete agglutination of erythrocyte) and expressed as $\log_2$. HA titre / 0.5 ml of goat's serum.

B. Cell Mediated Immune Response (CMIR) :-

(i) Cell mediated immune response was assessed by Delayed type hypersensitivity (DTH) reaction, mitogen used for cutaneous basophilic hypersensitivity reaction are as follows :

(a) DNCB (1 Chloro – 1, 4 – Dinitro Benezene)

(b) PPD (Purified Protein Derivative)

DNCB Skin Sensitivity Test : The test was done as per the method described by Chauhan and Verma (1983) with minor modification. Three goats from each group were taken. Area of about 10 – 15 cm^2 was shaved with the help of razor on left and right lateral neck for DNCB application. These areas were cleaned with acetone and dried. 0.25 ml of DNCB (10 mg/ml) in acetone vertical was applied on right side. On left side 0.25 ml of acetone was applied which served as control. DNCB was applied on 5^{th} day and challenged on 15^{th} day of experiment by applying 0.25 ml of DNCB (10 mg/ml) in acetone on right side and 0.25 ml acetone on the left side at the same site of first application. The skin thickness was measured with the help of slide caliper at 4, 8, 12, 24, 48, 72 and 96 h. during pre and post challenge.

The CMIR was calculated by subtracting the thickness of right side from left side.

PPD Skin Sensitivity test : The test was done as per the method described by Singh (1987). About 10 – 15 cm^2 area of both side of the neck were shaved, cleaned with acetone and dried. 0.3 ml of PPD (tuberculin) emulsified in 0.7 ml of Freund's complete adjuvant was injected intradermally on right side of neck and similar volume of PBS was inoculated on the left side. On 10^{th} day post-sensitization, 0.1 ml of PPD (tuberculin) was injected on right side of neck skin and left neck skin received 0.1 ml PBS measurement of skin thickness was made by the aid of slide caliper on 4, 8, 12, 24, 48, 72 and 96 h. post injection. The results were expressed as the difference of swelling on PPD injected site and PBS injected site at 4, 8, 12, 24, 48, 72 and 96 h. post injection during pre and post challenge period.

Measurement of skin thickness by slide caliper :-

Main scale × Vernier scale × Limit factor (0.01)

Diameter measured on three different positions.

(ii) Cell-mediated immune response was also assessed by as the method described in Schalm's veterinary haematology.

Absolute lymphocyte count for CMIR

A thin blood smear was made from whole blood. After proper drying of the smear, it was stained with Leishman's stain and air dried. The edge of the smear was examined under oil immersion of

the microscope. A total no. of 100 leukocytes were counted using the blood cell counter. The different type of leukocytes were expressed in percentage. The absolute lymphocyte was calculated after enumerating the total leukocyte count of the sample by using the following formula.

$$\text{Absolute Lymphocyte Count} = \frac{\text{Total No. of Lymphocyte}}{100} \times \text{Total No. of Leukocyte}$$

CALCULATION OF PHARMACOKINETIC PARAMETERS :

The following pharmacokinetic parameters of sparfloxacin was calculated after its single i.v. administration from semilog plot of plasma drug concentration *vs.* time curve. The experimental data was analyzed using two compartment (for i.v. route) open model as described by Gibaldi and Perrier (1982) and Notari (1980).

The concentration of the drug in plasma at any time 't' is obtained by following formula.

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \quad \text{(two-compartment open model)}$$

Where,

C_p = drug concentration in plasma at time 't'

e = base of natural logarithm

The description of different kinetic parameter are as follows :-

(a) A, The zerotime concentration of the drug in plasma during distribution phase ($\mu g.ml^{-1}$).

(b) α, The regression coefficient (distribution rate constant) for distribution phase was calculated by the method of resident yield.

(c) B, The zero time concentration of the drug in plasma during elimination phase ($\mu g.ml^{-1}$).

(d) β, the regression coefficient (elimination rate constant) for the elimination phase was calculated by the method of least squares.

(e) $C_p^o(A+B)$, the theoretical zerotime concentration of the drug in plasma ($\mu g.ml^{-1}$).

(f) $t_{1/2}\,\alpha$ distribution half life ($t_{1/2}\,\alpha$)

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

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

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

(h) AUC, the total area under plasma drug concentration time curve ($mg.L^{-1}.h$).

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

(i) AUMC, the total area under the first moment of plasma drug concentration curve ($mg.L^{-1} h^2$).

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

(j) MRT, mean residential time (h)

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

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

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

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

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

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

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

(n) $T \approx P$, the approximate tissue to plasma concentration ratio

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

(o) Vdc, The volume of distribution, based on distribution and elimination ($L.kg^{-1}$)

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

(p) Vd_B, the volume of distribution based on elimination ($L.kg^{-1}$)

$$Vd_\beta = \frac{D}{\beta}$$

(q) Vd_{area}, the volume of distribution based on total area under curve ($L.kg^{-1}$)

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

(r) Vd_{ss}, the volume of distribution at steady state ($L.kg^{-1}$)

$$Vd_{ss} = \frac{K_{12\ +21}}{K_{21}} - Vdc$$

(s) Cl_B = the total body clearance ($ml.kg^{-1}.min^{-1}$)

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

STATISTICAL ANALYSIS :

Comparison of effect of sparfloxacin in immune response at different time interval and an various days of post treatment in three groups were done by analysis of variance.

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Results

RESULTS

PHAMACOKINETIC STUDY AFTER SINGLE INTRAVENOUS ADMINISTRATION

Pharmacokinetic study of sparfloxacin was conducted in six healthy female goats following its single intravenous dose (i.v.) of 5 $mg.kg^{-1}$ and the results are presented below.

1. Plasma Levels :

Concentrations of sparfloxacin in plasma at various time intervals following its single i.v. administration at the dose rate of 5 $mg.kg^{-1}$ have been shown in Table-1 and Fig. 1. The mean peak plasma concentration of 59.76 ± 1.91 $\mu g.ml^{-1}$ was attained at 0.042 h. The drug was detectable upto 12 h in all the animals with the mean of 0.37 ± 0.01 $\mu g.ml^{-1}$.

2. Urine Levels :

Table 2 and Fig. 2 reveal urine concentrations of sparfloxacin after its single i.v. administration (5 $mg.kg^{-1}$). The drug appeared in urine of all six animals at 0.042 h with a mean of 37.85 ± 2.18 $\mu g.ml^{-1}$. The mean peak urine drug concentration of 63.16 ± 2.13 $\mu g.ml^{-1}$ was achieved at 0.333 h. The drug was detectable upto 48 h in three out of six animals with a mean of 0.17 ± 0.07 $\mu g.ml^{-1}$ but the drug was detectable upto 36 h in all six animals with a mean of 0.41 ± 0.05 $\mu g.ml^{-1}$.

3. Kinetic Parameters :

Plasma drug concentration *versus* time profile has confirmed a two–compartment open model for sparfloxacin as depicted in Fig. 3. Table 3 shows the values of different kinetic parameters calculated by the above noted compartment model.

The mean extrapolated zero time concentration during distribution phase (A), elimination phase (B) and theoretical zero time concentration (C_p^o) were noted to be 12.54 ± 0.08, 1.08 ±0.02 and 13.63 ± 0.09 $\mu g.ml^{-1}$, respectively. The distribution rate constant (α) ranged from 1.42 to 1.68 h^{-1} with a mean of 1.57 ± 0.03 while elimination rate constant (β) ranged from 0.071 to 0.086 with a mean of 0.081 ± 0.002 h^{-1}. The mean distribution half-life ($t_{1/2}\ \alpha$) and elimination half life ($t_{1/2}\ \beta$) were noted to be 0.43 ± 0.01 and 8.56 ± 0.25 h, respectively.

The mean area under curve (AUC) of 21.37 ± 0.37 $mg.L^{-1}$ h, area under first moment curve (AUMC) of 171.16 ± 7.74 $mg.L^{-1}$ h^2 and mean residential time (MRT) of 7.98 ± 0.22 h were noted in the present study. The average rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central (Kel) compartment were calculated to be 0.816 ± 0.019, 0.199 ± 0.005 and 0.639 ± 0.014 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 0.126 ± 0.001 and 6.92 ± 0.085. Various values of volume of distribution obtained by different methods are shown in Table 3. A mean Vd_{area} of 2.89 ± 0.043

LKg^{-1} was noted. The total body clearance (Cl_B) value ranged from 0.218 to 0.245 with a mean of 0.233 ± 0.004 $ml.kg^{-1}.min^{-1}$.

4. Dosage Regimen :

The dosage regimen required to maintain the different level of therapeutic concentration [$C_p^o min$ (MIC) = 0.125, 0.25 and 0.50 $\mu g.ml^{-1}$] in plasma for i.v. route in goats at different dosage interval (γ) of 12 and 24 h are presented in Table 4. For maintaining C_p^∞ min of 0.125 $\mu g.ml^{-1}$, the loading doses (D*s) were calculated to be 0.956 ± 0.015 and 2.540 ± 0.088 $mg.kg^{-1}$ while maintenance doses (Dos) were calculated to be 0.595 ± 0.018 and 2.178 ± 0.091 $mg.kg^{-1}$ at the dosage interval (γ) of 12 and 24 h, respectively. The D*s were calculated to be 1.912 ± 0.030 and 5.080 ± 0.177 $mg.kg^{-1}$ while Dos were noted to be 1.190 ± 0.035 and 4.358 ± 0.183 $mg.kg^{-1}$ at γ of 12 and 24 h respectively, for maintaining $C_p^\infty min$ of 0.25 $\mu g.ml^{-1}$. Like wise, to maintain $C_p^\infty min$ of 0.50 $\mu g.ml^{-1}$, the D*s were calculated to be 3.826 ± 0.061 and 10.162 ± 0.354 while Dos were found to be 2.38 ± 0.072 and 8.716 ± 0.367 $mg.kg^{-1}$ at γ 12 and 24 h, respectively.

IMMUNOLOGICAL STUDIES AFTER MULTIPLE INTRAMUSCULAR ADMINISTRATION

A. Humoral Immune Response (HIR)

Effect of sparfloxacin on humoral immune response in goats was recorded using sheep red blood cell (SRBC) as an indicator of humoral immunity.

Table 5 depicts HIR of sparfloxacin against SRBC antigen (LS mean ± S.E) to Haemoagglutination (HA) antibody titer (log_2 value) in goats. The study reveals that no immunomodulatory effect on humoral immunity by sparfloxacin. Though the HA antibody titer recorded in sparfloxacin treated group was found to be slightly higher (1.70126 ± 0.0770) but it was found to be non significant as compared to antigen treated group (1.57102 ± 0.07569). The antibody forming response against SRBC was recorded to be increased gradually from 7^{th} day onwards and highest HA titer was observed on 21^{st} days and then declining trend was observed.

B. Cell mediated Immune Response (CMIR) :

Effect of sparfloxacin on CMIR in goats after multiple intramuscular administrations was observed by using two different mitogens (DNCB and PPD/Tuberculin) and absolute lymphocyte count as indicator of cell mediated immunity.

Table 6 depicts CMIR of sparfloxacin to DNCB mitogen (LS mean ± S.E) in increase in skin thickness (mm) in goats after multiple intramuscular administrations. The study indicates that immunomodulatory action on cell mediated immunity or host immunity by sparfloxacin. Significant increase in skin thickness was observed in sparfloxacin treated group (1.1955 ± 0.0335 mm) as compared to both the control group (0.9073 ± 0.0335 mm – saline control and 1.1064 ± 0.0335 mm - antigen control). Increases in cutaneous basophilic hypersensitivity (CBH) response to DNCB was

gradually increased significantly from 4 h (0.5898 ± 0.0512 mm) and there after with the highest CBH reaction was found at 12 h (1.9577 ± 0.0512 mm) and there after significantly declining trend was observed upto 96 h (0.7157±0.0512 mm) during pre challenge period i.e. 5th day and post challenge period i.e. 15th day of experiment.

Table 7 presents CMIR of sparfloxacin (LS mean ± S.E) to PPD/tuberculin. An increase in skin thickness (mm) in goats after multiple intramuscular administration of drug as well as increase in CBH response was observed in sparfloxacin treated group (0.8456 ± 0.0153 mm). The increase is not significantly higher as compared to antigen control (0.5792 ± 0.0153 mm). The study did not show immunomodulatory action on cell mediated immunity. Gradual increase in CBH response to PPD/ Tuberculin was noted at initial hours and mean peak skin thickness was noted at 12 h during pre challenge and post challenge period of experiment; later on, significantly declining trend was noted.

The result of absolute lymphocyte count (per cubic millimeter) reveals that simultaneous administration of sparfloxacin caused significantly increased lymphocyte count of sparfloxacin treated group (6499 ± 19.8670 cubic/mm) as compared to both control (5226.6250 ± 19.8670 cu/mm – saline control and 5485 ±19.8670 cu/mm – antigen control). Increase in lymphocyte count was gradually increased from 1st day and thereafter with the highest count was found at 14th day and there after declining trend was observed (Table – 8).

Table – 1

Plasma concentrations ($\mu g.ml^{-1}$) of sparfloxacin in she goats after its i.v. administration @ 5 mg/kg

Time (h)	Animal Number						Mean ± S.E.M.
	1	2	3	4	5	6	
0.042	61.18	52.26	65.18	62.26	56.21	61.50	59.76 ± 1.91
0.083	21.00	19.12	22.00	25.08	21.12	29.50	22.97 ± 1.52
0.167	8.80	8.16	9.00	9.20	9.5	11.20	9.31 ± 0.42
0.25	8.07	7.50	7.86	7.96	8.05	8.50	7.99 ± 0.13
0.333	6.49	6.12	7.12	6.25	7.02	6.52	6.58 ± 0.16
0.50	4.43	4.40	4.68	4.82	4.92	5.05	4.71 ± 0.10
0.75	4.02	3.65	3.60	3.96	3.85	4.20	3.88 ± 0.09
1	2.89	2.65	2.58	2.89	3.05	3.25	2.88 ± 0.10
1.5	1.90	2.00	1.95	2.50	2.15	2.80	2.21 ± 0.14
2	1.46	1.4	1.52	1.32	1.52	2.10	1.56 ± 0.11
3	0.87	1.00	1.10	0.85	0.95	0.98	0.95 ± 0.03
4	0.82	0.75	0.92	0.72	0.75	0.85	0.80 ± 0.03
5	0.68	0.64	0.80	0.65	0.67	0.68	0.68 ± 0.02
6	0.57	0.54	0.68	0.59	0.61	0.60	0.59 ± 0.01
8	0.56	0.50	0.60	0.52	0.52	0.51	0.53 ± 0.01
10	0.55	0.42	0.58	0.45	0.42	0.46	0.48 ± 0.02
12	0.38	0.34	0.42	0.38	0.37	0.35	0.37 ± 0.01
24	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

N.D. = Non-detectable

Fig. 1

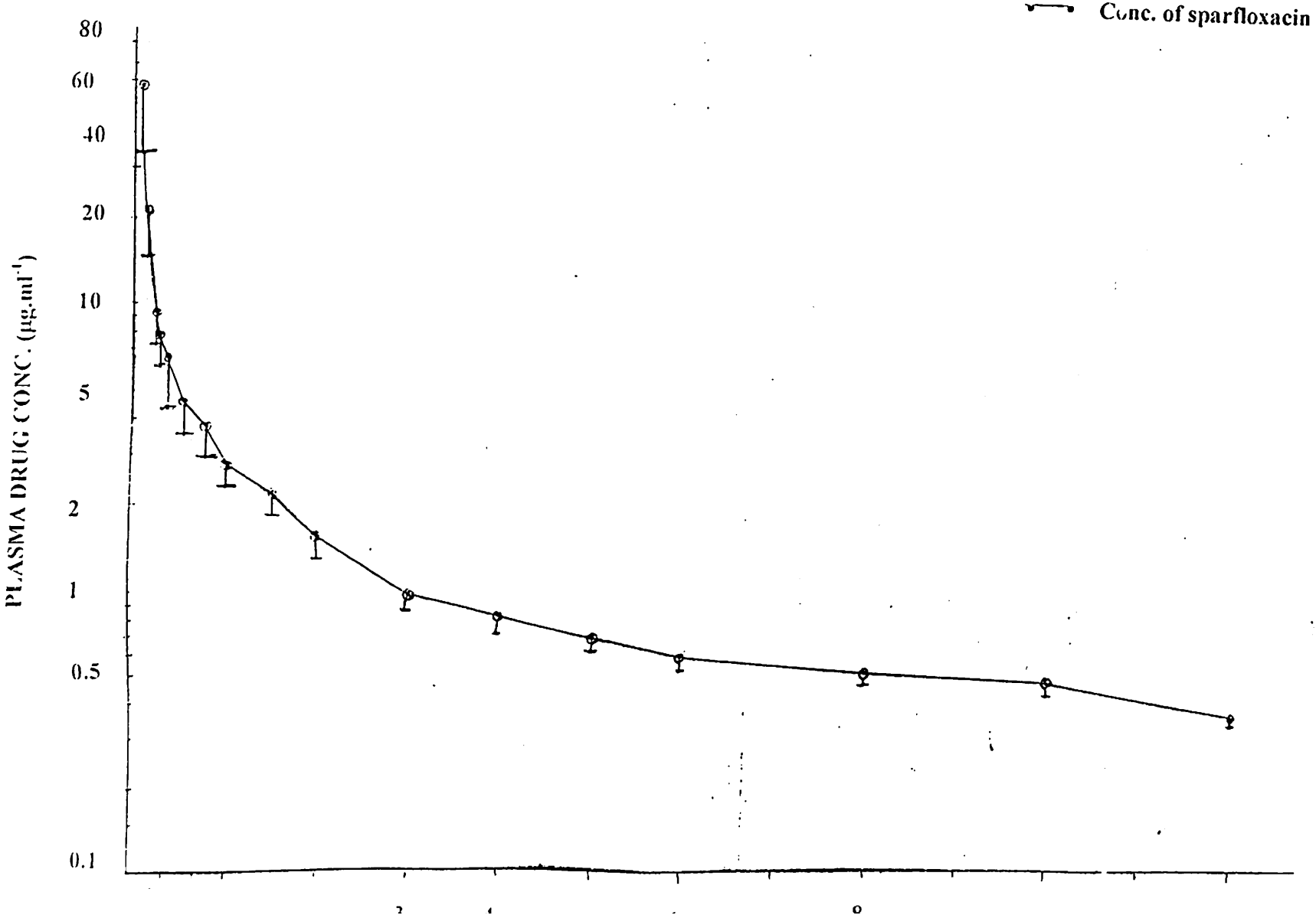
Conc. of sparfloxacin
PLASMA DRUG CONC. (μg.ml⁻¹)
80
60
40
20
10
5
2
1
0.5
0.1

Table – 2

Urine concentrations ($\mu g.ml^{-1}$) of sparfloxacin in she goats after its i.v. administration @ 5 mg/kg

Time (h)	Animal Number						Mean ± S.E.M.
	1	2	3	4	5	6	
0.042	46.70	43.52	35.86	32.22	35.18	33.62	37.85 ± 2.18
0.083	61.60	59.13	54.58	48.56	47.25	39.79	51.81 ± 3.04
0.167	67.60	65.56	62.08	58.00	54.58	49.60	59.57 ± 2.54
0.25	56.20	52.31	75.45	62.20	67.21	53.73	61.18 ± 3.3
0.333	58.80	54.40	64.60	68.80	63.3	69.07	63.16 ± 2.13
0.50	53.70	51.72	58.86	60.25	62.22	61.016	57.98 ± 1.59
0.75	54.90	50.95	54.50	56.40	59.16	58.46	55.72 ± 1.11
1	51.20	48.40	52.26	50.28	53.59	51.39	51.18 ± 0.657
1.5	43.60	41.62	44.82	42.85	49.37	42.89	44.19 ± 1.02
2	42.60	39.65	43.16	38.12	42.05	39.98	40.92 ± 0.735
3	40.00	36.22	41.10	33.50	37.51	32.48	36.80 ± 1.28
4	38.90	30.80	37.60	29.40	31.80	27.89	32.73 ± 1.67
5	26.30	24.24	28.12	25.58	26.27	22.67	25.53 ± 0.701
6	27.50	22.30	26.55	23.30	20.24	18.97	23.14 ± 1.26
8	9.71	8.85	10.15	10.85	12.19	1.58	10.56 ± 0.46
10	6.02	6.12	7.18	7.90	8.16	7.96	7.22 ± 0.36
12	4.76	3.12	5.05	5.54	4.39	4.87	4.62 ± 0.31
24	1.69	1.08	1.72	2.00	1.98	1.79	1.71 ± 0.12
30	0.55	0.42	0.60	0.88	0.76	0.62	0.64 ± 0.06
36	0.41	0.30	0.48	0.62	0.33	0.32	0.41 ± 0.05
48	0.30	N.D.	0.32	0.38	N.D.	N.D.	0.17 ± 0.07

N.D. = Non-detectable

Fig. 2

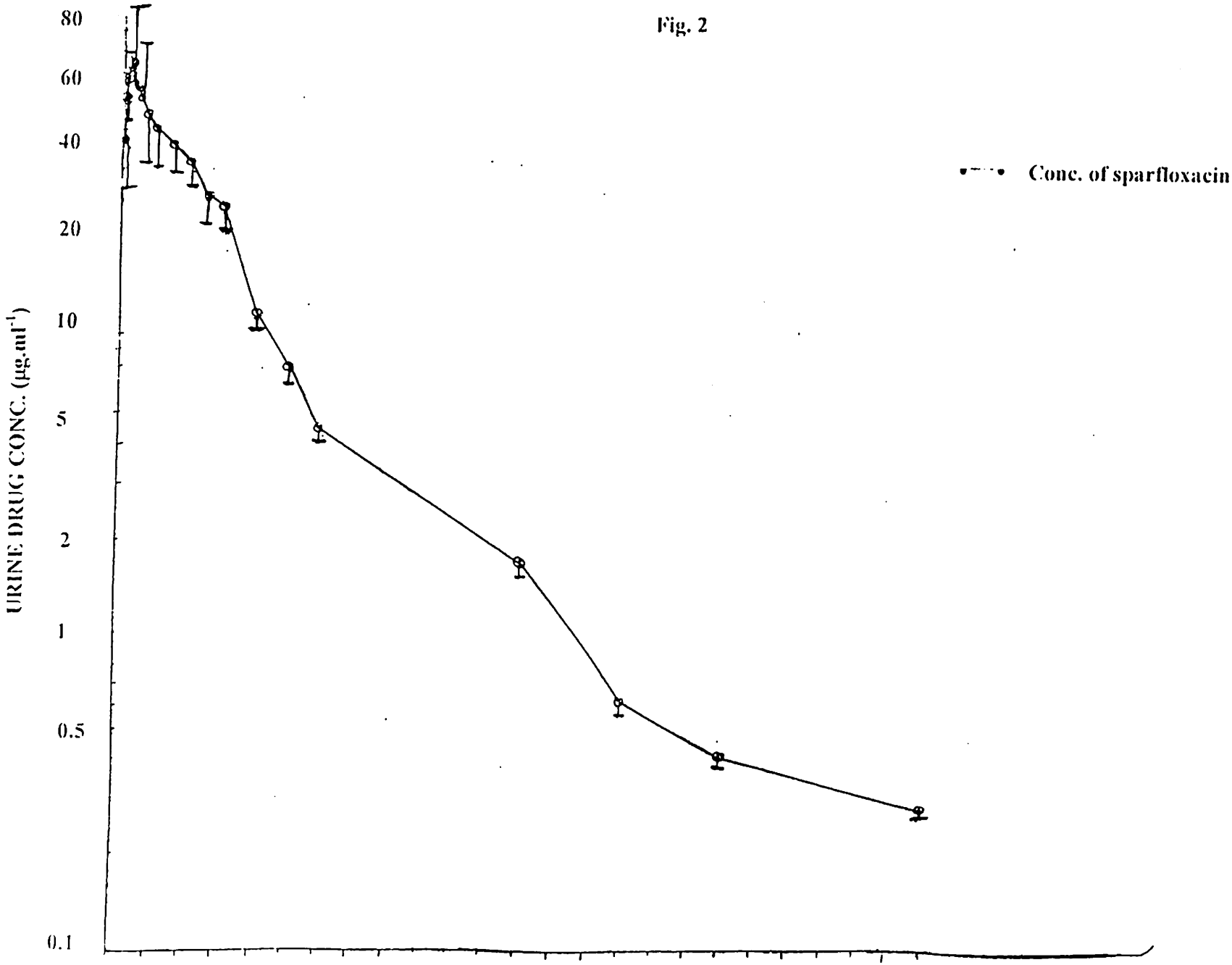
80
60
40
20
10
5
2
1
0.5
0.1
URINE DRUG CONC. (μg.ml⁻¹)
Conc. of sparfloxacin

Table – 3

Kinetic parameters of sparfloxacin in she goats after single i.v. administration @ 5 mg/kg.

Parameter (Unit)	Animal Number						Mean ± SEM
	1	2	3	4	5	6	
A (μg/ml)	12.63	12.24	12.89	12.52	12.43	12.56	12.54 ± 0.08
B (μg/ml)	1.08	1.01	1.10	1.05	1.12	1.15	1.08 ± 0.02
C_p^o (μg/ml)	13.71	13.25	13.99	13.57	13.55	13.71	13.63 ± 0.09
α (h^{-1})	1.60	1.420	1.68	1.56	1.63	1.56	1.57 ± 0.03
$t_{1/2}$ α (h)	0.430	0.488	0.412	0.440	0.425	0.440	0.439 ± 0.017
β (h^{-1})	0.826	0.071	0.086	0.082	0.083	0.080	0.081 ± 0.002
$t_{1/2}$ β (h)	8.38	9.76	8.04	8.44	8.32	8.47	8.56 ± 0.25
AUC ($mg.L^{-1}.h$)	20.96	22.84	20.44	20.81	21.08	22.10	21.37 ± 0.37
AUMC ($mg.L^{-1}.h^2$)	163.22	206.42	152.95	160.92	166.47	177.02	171.16 ± 7.74
MRT (h)	7.78	9.037	7.48	7.73	7.89	8.00	7.98 ± 0.22
K_{12} (h^{-1})	0.826	0.736	0.870	0.793	0.860	0.814	0.816 ± 0.019
K_{21} (h^{-1})	0.202	0.173	0.211	0.196	0.211	0.205	0.199 ± 0.005
Kel (h^{-1})	0.654	0.582	0.685	0.653	0.642	0.622	0.639 ± 0.014
Fc	0.126	0.121	0.125	0.125	0.129	0.131	0.126 ± 0.001
T≈P	6.94	7.21	7.01	7.017	6.77	6.61	6.92 ± 0.09
Vdc ($L.kg^{-1}$)	0.364	0.377	0.357	0.368	0.369	0.364	0.366 ± 0.003
Vd_B ($L.kg^{-1}$)	4.62	4.95	4.54	4.76	4.46	4.34	4.61 ± 0.089
Vd_{area} ($L.kg^{-1}$)	2.88	3.08	2.857	2.92	2.85	2.76	2.89 ± 0.043
Vdss ($L.kg^{-1}$)	5.08	5.25	5.12	5.04	5.07	4.97	5.08 ± 0.038
Cl_B ($ml.kg^{-1}. min^{-1}$)	0.237	0.218	0.245	0.239	0.237	0.225	0.23 ± 0.004

Fig. 3

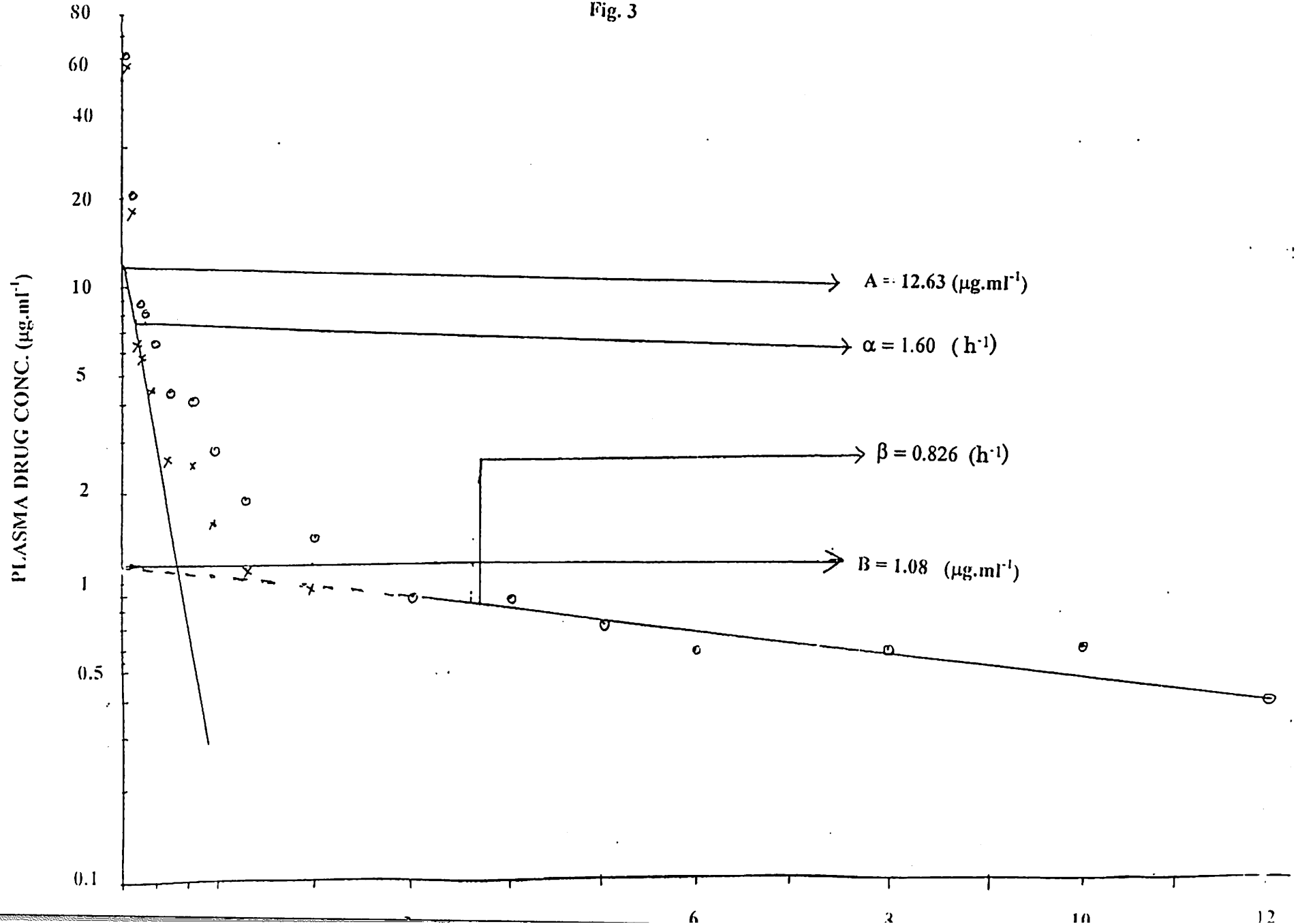
80
60
40
20
10
5
2
1
0.5
0.1
PLASMA DRUG CONC. (μg.ml-1)
A = 12.63 (μg.ml-1)
α = 1.60 (h-1)
β = 0.826 (h-1)
B = 1.08 (μg.ml-1)
6
8
10
12

Table – 4

Dosage regimen of sparfloxacin for i.v. rate in goats

C_p^{∞} min ($\mu g.ml^{-1}$)	γ (h)	Dose ($mg.kg^{-1}$)	Animal Number						Mean ± S.E.M.
			1	2	3	4	5	6	
0.125	12	D*	0.970	0.902	1.003	0.977	0.966	0.920	0.956 ± 0.015
		Do	0.610	0.517	0.646	0.612	0.610	0.515	0.595 ± 0.018
	24	D*	2.612	2.115	2.818	2.618	2.621	2.456	2.540 ± 0.088
		Do	2.252	1.730	2.461	2.253	2.265	2.111	2.178 ± 0.091
0.25	12	D*	1.940	1.805	2.00	1.955	1.932	1.840	1.912 ± 0.030
		Do	1.220	1.035	1.29	1.225	1.220	1.15	1.19 ± 0.035
	24	D*	5.225	4.231	5.637	5.236	5.243	4.913	5.080 ± 0.177
		Do	4.505	3.461	4.923	4.506	4.531	4.223	4.358 0.083
0.50	12	D*	3.880	3.610	4.014	3.910	3.865	3.682	3.826 ± 0.061
		Do	2.440	2.070	2.585	2.450	2.440	2.302	2.381 ± 0.072
	24	D*	10.450	8.463	11.275	10.473	10.487	9.826	10.162 ± 0.354
		Do	9.010	6.923	9.846	9.013	9.062	8.446	8.716 ± 0.367

D* = Priming or loading dose

D_o = Maintenance dose

γ = Dosage interval

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

Table – 5

Humoural Immune Response (HIR) of sparfloxacin against SRBC antigen (LS Mean ± S.E.) to antibody titre (log 2 Value) in Goats.

	LS mean ± S.E
Overall mean (m)	1.3917 ± 0.04395
Factor A : Groups	
1. Saline Control	0.90308^{A} ± 0.07569
2.Antigen Control	1.57102^{B} ± 0.07569
3.Sparfloxacin + Antigen	1.70126^{B} ± 0.07700
Factor B : Days	
1^{st}	0.90308^{a} ± 0.123
7	0.90308^{a} ± 0. 123
10	1.48014^{b} ± 0. 123
14	1.80617^{c} ±0. 123
21	2.25771^{d} ± 0. 123
28	1.73091^{c} ± 0. 123
35	1.20411^{ea} ± 0.123
42	0.84911^{a} ± 0.129

Different superscripts did not differ significantly ($p < 0.01$).

Table – 6

Cell mediated immune Response (CMIR) of sparfloxacin (LS mean ± S.E.) to DNCB mitogen in goats.

	LS mean ± S.E
Overall mean (μ)	**1.0698 ± 0.019**
Factor A : Challenge	
Pre challenge	$0.8732^{a} \pm 0.027$
Post challenge	$1.2663^{b} \pm 0.027$
Factor B : Group	
1. Saline control	$0.9073^{A} \pm 0.034$
2. Antigen control	$1.1064^{B} \pm 0.034$
3. Sparfloxacin + Antigen	$1.1955^{C} \pm 0.034$
Factor C : Hour	
4	$0.5898^{a} \pm 0.051$
8	$0.7494^{b} \pm 0.051$
12	$1.9577^{c} \pm 0.051$
24	$1.4507^{d} \pm 0.051$
48	$1.1069^{e} \pm 0.051$
72	$0.9182^{f} \pm 0.051$
96	$0.7157^{ab} \pm 0.051$

Different superscripts differ significantly ($p < 0.01$).

Table - 8

Cell mediated immune Response (CMIR) of sparfloxacin (LS Mean ± S.E.) to absolute Lymphocyte Count/cubic mm in goats.

	LS mean ± S.E
Overall mean (μ)	5737.125 ±1.4702
Factor A : Group	
1. Saline Control	5226.62^{A} ± 19.867
2.Antigen Control	5485.00^{B} ± 19.867
3.Sparfloxacin + Antigen	6499.75^{C} ± 19.867
Factor B : Days	
1^{st}	5642.33^{a} ± 32.442
7	5677.66^{a} ± 32.442
10	5762.66^{b} ± 32.442
14	5808.33^{c} ± 32.442
21	5805.00^{c} ± 32.442
28	5758.66^{b} ± 32.442
35	$5729.33^{a\,b}$ ± 32.442
42	$5713.00^{a\,b\,c}$ ± 32.442

Different superscripts differ significantly ($p < 0.01$).

❖❖❖

Discussion

DISCUSSION

Sparfloxacin is one of the recent effective members of fluoroquinolones with broad spectrum antimicrobial activity. The major indication of this drug is in respiratory tract infections which include pneumonia, chronic bronchitis and sinusitis *etc*. Sparfloxacin does not have any active metabolite (Tripathi, 2003). It is now well established that many drugs including antimicrobials either stimulate or suppress the immune system and thereby alter the course of the diseases. Therefore it is highly desirable to carry out immunological studies of antimicrobial agents.

Pharmacokinetic study of sparfloxacin has been carried out in healthy volunteer (Zix *et al.*, 1997), Broiler chicken (Mathuram *et al.*, 2005), rat (Matsunga *et al.*, 1991), goat (Kujur, 2005) following oral administration. So far, it seems little work has been done on the pharmacokinetics and immunological studies of sparfloxacin in goats following i.v. administration. With the above mentioned aims and objectives, the present study has been undertaken in goats.

PHARMACOKINETIC STUDY :

(A) Distribution in plasma :-

Sparfloxacin was present with a mean peak plasma concentration of 59.76 ± 1.91 $\mu g.ml^{-1}$ at 0.042 h following its i.v.

administration @ 5 $mg.kg^{-1}$ (Table – 1). The drug declined with time and was traced upto 12 h in all animals with the mean of 0.37 ± 0.01 $\mu g.ml^{-1}$. The mean therapeutic concentration (≥ 0.125 $\mu g.ml^{-1}$) was maintained from 0.042 h to 12 h.

(B) Urinary Excretion :

In case of urine, the drug was present in all animals with a mean concentration of 37.85 ± 2.18 $\mu g.ml^{-1}$ at 0.042 h. Mean peak urine drug concentration of 63.16 ± 2.13 $\mu g.ml^{-1}$ was obtained at 0.333 h. Thereafter, the drug was found to decline with time and it was detectable in all animals up to 36 h post i.v. administration. At 48 h, the drug was detectable in urine of four out of six animals with a mean of 0.17 ± 0.07 $\mu g.ml^{-1}$. The mean therapeutic concentration in urine (≥ 0.125 $\mu g.ml^{-1}$)) was maintained from 0.042 to 48 h.

(C) Kinetic Parameter :

Review of literature reveals that very little kinetic studies of sparflaxacin were conducted after i.v. administration. Majority of studies were conducted after oral administration in different species including man.

Distribution rate constant (α) of 1.57 ± 0.03 h^{-1} and distribution half life ($t_{1/2}\ \alpha$) of 0.439 ± 0.017 h obtained in the present study denotes quicker distribution of sparfloxacin in different body fluids and tissues of goats. Elimination rate constant of (β) of 0.081 ±

0.002 h^{-1} and elimination half life ($t_{1/2}$ β) of 8.56 ± 0.25 h indicates comparatively slow elimination of sparfloxacin as compared to many fluoroquinolones. The values of $t_{1/2}$ β were noted to be 2.956 ± 0.304 h for norfloxacin (Nitesh, 2005), 2.32 ± 0.70 h for ciprofloxacin (Singh *et al.*, 2001), 3.53 ± 2.26 h for pefloxacin (Ansari *et al.*, 2000) and 2.92 ± 0.41 h for enrofloxacin (Nitesh Kumar *et al.*, 2003) in goat after parental administration. More or less similar elimination half life of 8.40 ± 0.74 h in goat was observed by Kujur (2005) after oral administration (20 $mg.kg^{-1}$). Higher $t_{1/2}$ β of 17.6 h, 15 – 20 h and 20 ± 4 in man were noted by Johnson *et al.* (1992), Seth (1999), Zix *et al.* (1997), respectively. However, a lower $t_{1/2}$ β of 5.94 in broiler chicken (Mathuram *et al.*, 2005), 4 h in rat (Furukowa *et al.*, 1991) were noted. Very low $t_{1/2}$ β 2.39 ± 0.29 h was observed after i.v. administration of sparfloxacin @ 40 $mg.kg^{-1}$ in rabbit (Liu *et al.*, 1998). A higher $t_{1/2}$ β of 8.56 ± 0.25 obtained in present study denotes slower elimination of the drug from the body of the goat which is further supported by higher mean residential time (MRT) of 7.98 ± 0.22 h and lower elimination rate constant of drug from central compartment (Kel) of 0.639 ± 0.014 h^{-1} and total body clearance of 0.233 ± 0.004 $ml.kg^{-1}.\ min^{-1}$.

Notari (1980) stated that for a two–compartment open model, the value of $Vd_B > Vd_{area} > Vd_{ss}$ and Vd_c. He further mentioned that among these values of volume of 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_c under estimate the amount of drug in the body. Similarly, the value of Vd_{ss} obtained by non–compartment model correctly predict the amount of drug and / or its metabolite in the body during elimination phase (Singh and Ahuja, 1999). Various values of volume distribution obtained by different methods are shown in Table – 3. Vd_{area} of 2.89 ± 0.043 $L.kg^{-1}$ and Vd_{ss} of 5.08 ± 0.038 $L.kg^{-1}$ were obtained for sparfloxacin in the present study in goat after its i.v. administration. This denotes the drug is expected to be distributed in higher amount in body fluids and tissues of goat which is further supported by tissue to plasma concentration ratio (T≈P) of 6.92 ± 0.09. Kujur (2005) showed a lower Vd_{area} of 0.97 ± 0.09 in goat. However, Mathuram *et al.* (2005) showed higher volume distribution of 2.4 $L.kg^{-1}$ in chicken.

In the present study total body clearance (Cl_B) of 0.233 ± 0.004 $ml.kg^{-1}.min^{-1}$ was obtained which shows that the drug is removed at a slower rate from the body. However, a higher Cl_B of 1.33 ± 0.4 $ml.kg^{-1}.min^{-1}$ in goat (Kujur, 2005) and 4.55 $ml.kg^{-1}.min^{-1}$ in broiler chicken (Mathuram *et al.*, 2005) were obtained.

Dosage Regimen :

The main purpose of conducting kinetic study is to compute rational dosage regimen for treating various disease states. Generally, plasma is the easily accessible body fluid from which drug

enters into the various body fluids and tissues. Therefore, in the present study, dosage regimen for various therapeutic concentrations (C_p^{∞}Min = MIC) of 0.125, 0.25 and 0.50 $\mu g.ml^{-1}$ were taken for calculating dosage regimen. Generally for fluoroquinolones the minimum inhibitory concentration for majority of the organisms ranged from 0.1 to 0.5 $\mu g.ml^{-1}$ (Trautman *et al.*, 1996, Kraiczy *et al.*, 2001, Kaku *et al.*, 1994) for treating of mild infections and highly susceptible bacteria.

In the present study, for treating systemic infections or highly susceptible microbes [$C_{p\,Min}^{\infty}$ (MIC) = 0.125 $\mu g.ml^{-1}$], loading (D*) and maintenance (Do) dose of 2.54 ± 0.08 and 2.17 ± 0.09 $mg.kg^{-1}$ at dosage interval (γ) of 24 h can be effectively used. Similarly, for treating moderate systemic infections or moderately susceptible microbes [$C_{p\,Min}^{\infty}$ (MIC) = 0.25 $\mu g.ml^{-1}$]. D* and Do of 5.08 ± 0.17 and 4.35 ±0.18 $mg.kg^{-1}$ at dosage interval (γ) of 24 h can be successfully used but against severe systemic infections or less susceptible microbes [$C_{p\,Min}^{\infty}$ (MIC) = 0.5 $\mu g.ml^{-1}$] D* and Do of 3.82 ± 0.06 and 2.38 ± 0.07 $mg.kg^{-1}$ at shorten dosage interval (γ) of 12 h can be effectively used.

The drug maintains its therapeutic concentration of ≥ 0.125 $\mu g.ml^{-1}$ for more than 36 h in urine when given @ 5 $mg.kg^{-1}$ i.v. (Table 2). Hence, the drug can be administered daily @ 5 $mg.kg^{-1}$

parentrally or even 36 h against urinary tract infection in goats caused by drug sensitive organisms.

Immunological Study :

All vertebrate animals have two arms of immune response i.e. humoral immune response (HIR) and cell mediated immune response (CMIR). The efficacy of both the arms can be judged. The humoral immune response can be judged by the demonstration of antibody titre against particular antigen (Kolmer *et al*, 1952) where as CMIR can be judged by delayed type hypersensitivity (DTH) and lymphocyte Count (LC). Sheep red blood cell (SRBC) is more often used as an antigen for the evaluation of HIR in different experimental animals by assessing the antibody titre in the sensitized host (Kabat *et al*., 1961).

In the present study, the serum antibody titre to SRBC was evaluated using haemagglutination test for the assessment of HIR as per Beard *et al*., (1980). Like wise, CMIR was determined by DTH (Chauhan *et al*., 1983) reaction *in vivo* and lymphocyte test *in vitro* (Baunous *et al*., 1993).

Humoral Immune Response :

In the present investigation, the haemagglutination antibody titre in sparfloxacin treated group was recorded to be higher (1.70126 ± 0.0756) as compared to antigen treated group (1.57102 ±

0.0756) though it was found to be non-significant. Therefore, it is suggested that no significant immuno modulatory effect on humoral immune response by this drug (Table-5). The above finding is more or less in agreement with the result of Jose *et al.*, (1999) who observed that the simultaneous administration of pefloxacin produced a marginal suppression of humoral immune response, which was not statistically significant in rabbits. Valera *et al.* (1995) reported that humoral immune response by fluoroquinolones is a complex phenomenon which is affected by many factors like dose of the drug, duration of therapy and time of administration of antigen. In the present study, the antibody formation was observed on 7^{th} day of antigen exposure and highest HA titre was observed on 21^{st} day followed declining trend there after upto 42^{nd} days. The above findings are also in agreement with the findings of Jose *et al.* (1999) in rabbit for pefloxacin, Nitesh *et al.* (2005) in goat for pefloxacin and Dhiraj *et al.* (2007) for enrofloxacin respectively, who observed more or less similar observations upto 42^{nd} day.

Cell Mediated Immune Response :

DTH reaction is considered as a valid measure for the *in vivo* assessment of CMIR (Thompson *et al.*, 1975). T-cell provoke development of DTH which is measured by increase in the thickness of skin at the site of antigen inoculation in sensitized goat. Thickening of skin is produced by infiltration of various cells including sensitized T-

lymphocyte at the site of mitogen inoculation (Reuben *et al.*, 1979). In the present study, the sensitized skin in contact with mitogens like DNCB, PPD were used as one of the parameter of CMIR. The inoculated area was reddened, odematous, swollen and subsequently indurated. The reaction was more prominent in DNCB and PPD inoculated group, respectively (Table 6 and 7). The DNCB skin sensitivity showed significant variation in the mean skin thickness between sparfloxacin treated group as compared to antigen treated group. In contrast, PPD skin sensitivity test did not show significant variation in the mean skin thickness between antigen treated group as compared to sparfloxacin treated group. It is well known that PPD possesses weak antigenic properties in comparison to DNCB. PPD did not show any marked mononuclear cell proliferation in goats by this drug.

In the present study DNCB show immunomodulatory action on cell-mediated immunity or T-cell mediated immunity as significant increases in mean skin thickness in sparfloxacin treated group. The above findings are also in agreement with the finding for enrofloxacin (Dhiraj, 2007) and for pefloxacin (Nitesh, 2005) in goats.

The lymphocyte count is used a method for detection of CMIR. The technique is preferred due to its low cost and accuracy as compared to other and sophisticated technique used for the evaluation of CMIR. In the present experiment, the lymphocyte count was

employed as one of the techniques for the evaluation of CMIR. In different experimental group such as SRBC inoculated group (gr. II), sparfloxacin + SRBC treated group (gr. III), normal saline inoculated group (gr. I) by using the lymphocyte from peripheral blood of the experimental goats. The perusal of the Table – 8 showed that the stimulation index (SI) of the lymphocytes stimulated with concanavalin-A, were significantly higher in sparfloxacin + SRBC treated at 14 days post SRBC inoculated group as compared to SRBC alone treated group. The result demonstrates that sparfloxacin potentiates the cell-mediated immunity. The present finding is in agreement with the result of Dhiraj *et al.* (2007) who observed that enrofloxacin potentiate cell- mediated immunity.

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Summary

SUMMARY

Detailed pharmacokinetic and immunological studies of sparfloxacin were carried out in goats weighing between 18 – 22 kg. Concentrations of the drug in plasma and urine as well as various kinetic parameters were calculated by using two compartment open model following intravenous (i.v.) administration. Effects of sparfloxacin on humoral and cell mediated immunity were estimated in goats following multiple intra muscular (i.m.) administration. The salient findings of the present study are as follow:-

Pharmacokinteic study :-

1. Following single i.v. administration (5 $mg.kg^{-1}$), the mean peak plasma concentration of 59.76 ± 1.91 $\mu g.ml^{-1}$ and mean peak urine concentration of 63.16 ± 2.13 $\mu g.ml^{-1}$ were found at 0.042 h and 0.333 h, respectively. The drug was detectable in all animals upto 12 h in plasma and upto 36 h in urine.

2. The mean extrapolated zero time concentration during distribution phase (A), elimination phase (B) and theoretical zero time concentration (C_p^0) were noted to be 12.54 ± 0.08, 1.08 ± 0.02 and 13.63 ± 0.09 $\mu g.ml^{-1}$, respectively.

3. A high value of distribution rate constant (α) of 1.57 ± 0.03 h^{-1} and low value of distribution half life ($t_{1/2}\ \alpha$) of 0.43 ± 0.01 h

denote that the drug is expected to be distributed to peripheral tissues at a faster rate.

4. The elimination rate constant (β) 0.081 ± 0.002 of h^{-1} and the elimination half life ($t_{1/2}$ β) of 8.56 ± 0.25 h obtained in the present study denote that the drug is removed at a slower rate as compared to other fluoroquinolones. This has led to a moderate increase in the value of mean residential time (MRT) of 7.98 ± 0.22 h. The slower elimination of this drug is further supported by lower Kel value of 0.634 h^{-1} and low value of total body clearance (Cl_B) of 0.233 ± 0.04 $ml.kg^{-1}.min^{-1}$.

5. A moderately high value of volume of distribution of 2.89 ± 0.043 $L.kg^{-1}$ and high value of tissue to plasma concentration ratio (T≈P) of 6.92 ± 0.09 denotes better distribution of the drug in different body fluids and tissues of goats.

IMMUNOLOGICAL STUDIES :

(i) Humoral Immune Response :

The agglutinating antibody titre recorded in sparfloxacin treated group produced higher titre value (1.70126 ± 0.0770) as compared to antigen treated group (1.57102 ± 0.0756) but did not differ significantly. Hence, it is assumed that there is no effect of sparfloxacin on humoral immune response. The antibody formation was observed on 10^{th} day of antigen exposure and highest antibody titre was

observed on 21st day followed by declining trend up to 42nd day in all groups except saline control.

Cell Mediated Immune Response :

Cell mediated immune response (CMIR) was evaluated *in vivo* through delayed type hypersensitivity (DTH) reaction and lymphocyte count. In the present study two mitogens such as DNCB and PPD were used for estimating the delayed type hypersensitivity (DTH) response.

The DNCB skin sensitivity test shows significant variation in the mean skin thickness in sparfloxacin treated group as compared to antigen treated group (Table 6). There was significant increase in DNCB mean skin thickness in sparfloxacin treated group (1.1955 ± 0.0335) as compared to antigen treated group (1.1064 ± 0.0335). This result reveals immunopotentiating effect on cell mediated immunity. On the other hand, PPD delayed type hypersensitivity test shows non significant variation in the mean skin thickness in sparfloxacin treated groups as compared to antigen treated groups (Table 7).

In absolute lymphocyte count test, the stimulation index (SI) Value of the lymphocyte counts stimulated with convalin A are significantly higher in sparfloxacin treated group on 14th day as compared to antigen (Ag) treated group, for the respective periods (Table 8). From DNCB skin sensitivity test and absolute lymphocyte count carried out in the present study clearly demonstrate immunopotentiating effect in goats.

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Appendix

APPENDIX – I

CALCULATION OF KINETIC PARAMETERS :

Kinetic parameters were calculated from log plasma drug concentration versus time profile. An example is noted below from the data of animal no. 1 obtained after single i.v. injection of sparfloxacin (5 mg/kg) given in goat. The data showed a biphasic curve and hence, fits well into two compartment open model. Here elimination phase starts from 3 h.

Sl. No.	Time (h) X	X^2	Plasma drug concentration (Y) $\mu g.ml^{-1}$	Log (Y)	XY
1	3	9	0.87	-0.06	-0.18
2	4	16	0.82	-0.09	-0.36
3	5	25	0.68	-0.16	-0.80
4	6	36	0.57	-0.24	-1.44
5	8	68	0.56	-0.25	-2.06
6	10	100	0.55	-0.26	-2.60
7	12	144	0.38	-0.42	-5.04
n= 7	$\Sigma X = 48$ $\ddot{X} = 6.857$	$\Sigma X^2 = 394$		$\Sigma \log Y = -1.48$ $\ddot{Y} = -0.212$	$\Sigma XY = 12.48$

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

$$b = \frac{(7 \times 12.48) - (48 \times 1.48)}{(7 \times 394) - (48 \times 48)}$$

$$= \frac{-87.36 - (-71.04)}{2758 \cdot 2304}$$

$$= \frac{-16.32}{454} = -0.359$$

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

$= -0.0359 \times -2.303$

$= 0.0826\ h^{-1}$.

B, Zero time concentration during elimination phase can be obtained from the formula :

$$\overline{Y} = a + b\overline{x}$$

where,

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

$\overline{x}$ = mean time

b = slope of line

a = Zero time concentration

Therefore,

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

$= -0.212 - (-0.0359 \times 6.857)$

$= \log 0.034$

Zero time concentration (B) = antilog of 0.034 = 1.08 $\mu g.ml^{-1}$.

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

Subtracting the theoretical values from observed values, a series of residual concentrations were obtained and slope of line in natural log (distribution rate constant, α) and the zero time intercept (zero time concentration during distribution phase, A) can be calculated as per method adopted for calculation of B and β. The calculated values are :

$\alpha = 1.60 h^{-1}$

$A = 12.63\ \mu g.ml^{-1}$

C_p^o, the theoretical plasma concentration at time zero

$$C_p^o = A+B = 12.63+1.08 = 13.71\ \mu g.ml^{-1}$$

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

$$\frac{0.693}{1.60} = 0.433\ h$$

Elimination half life ($t_{1/2}\ \beta$) $= \frac{0.693}{\beta} = \frac{0.693}{0.826} = 8.38\ h$

Area under curve, $AUC = \frac{A}{\alpha} + \frac{B}{\beta}$

$$= \frac{12.63}{1.60} + \frac{1.08}{0.0826} = 7.89 + 13.07 = 20.96\ mg.L^{-1}.h$$

Area under the first moment curve, AUMC

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

$$= \frac{12.63}{(1.60)^2} + \frac{1.08}{(0.0826)^2} = 4.93 + 158.29$$

$$= 163.22 \text{ mg.L}^{-1}.\text{h}^2.$$

Mean residential time, MRT

$$MRT = \frac{AUMC}{AUC} = \frac{163.22.}{20.96} = 7.787 \text{ h.}$$

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

$$K_{21} = \frac{A.\beta + B.\alpha}{C_p^o} = \frac{12.63 \times 0.0826 + 1.08 \times 1.60}{13.71} = 0.202 \text{ h}^{-1}$$

Elimination rate constant of the drug from central compartment, Kel

$$Kel = \frac{\alpha\beta}{K_{21}} = \frac{1.60 \times 0.0826}{0.202} = 0.654/\text{h}$$

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

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

$$= 1.60 + 0.0826 - 0.202 - 0.654$$

$$= 0.826/\text{h}.$$

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

$$F_c = \frac{\beta}{Kel} = \frac{0.0826}{0.654} = 0.126.$$

Approximate tissue to plasma concentration ratio, T≈P

$$T \approx P = \frac{K_{12}}{K_{21} - \beta} = \frac{0.826}{0.202 \cdot 0.0826} - \frac{0.826}{0.119} = 6.94$$

Volume of distribution based on both distribution and elimination Vd_c

$$Vd_C = \frac{D}{C_0^P}$$

Where, D = dose rate ($mg.kg^{-1}$)

$$\frac{5}{13.71} = 0.364\ L kg^{-1}$$

The volume of distribution based on elimination, Vd_B

$$Vd_B = \frac{D}{B} = \frac{5}{1.08} = 4.62\ L.kg^{-1}$$

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

$$Vd_{area} = \frac{D}{AUC.\beta} = \frac{5}{20.96 \times 0.0826} = \frac{5}{1.731} = 2.88\ L.kg^{-1}$$

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

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

$$= \frac{0.826 + 0.202}{0.202} = \frac{1.028}{0.202}$$

$$= 5.08 \text{ L.kg}^{-1}$$

The total body clearance, Cl_B,

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

$$= 2.88 \times 0.0826$$

$$= 0.237 \text{ L/kg}^{-1}.\text{h}^{-1}$$

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APPENDIX – II

CALCULARION OF DOSAGE REGIMEN :

Dosage regimen were calculated to maintain the desired levels of therapeutic concentration (MIC) in plasma at desired dosage intervals (γ) using the formulae described by Saini and Srivastava (1997). The data of animal No. 1 obtained for sparfloxacin calculation of dosage regimen of sparfloxacin after i.v. administration in goat has been used as an example for calculation of dosage regimen for maintaining MIC C_p^{∞} min of 0.125 μg/ml at the dosage interval (γ) of 12 h. The calculation is as follows :

Calculation of loading or priming dose (D*) :

For calculation of D*, the following formula is used :

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

Where,

$C_p^{\infty}(\min)$ = 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.

e = Base of natural logarithm.

For $C_p^{\infty}(min)$ of 0.125 μg/ml and γ of 12 h.

$D^* = 0.125 \times 2.88 \ (e^{0.826 \times 12}) = 0.970$ mg/kg.

Calculation of maintenance dose (Do) :

For calculation Do, the following formula is employed :

$$Do = C_p^{\infty}(min) \cdot Vd_{area} \ (e^{\beta.\gamma} - 1)$$

$$= 0.125 \times 2.88 \times (e^{0.826 \times 12} - 1)$$

$$= 0.610 \text{ mg/kg.}$$

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