Pharmacokinetics and Immunological Effects of Ketoprofen 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

Smita Sinha

Reg. No. - M/V Pharma/27/2003-04

DEPARTMENT OF VETERINARY PHARMACOLOGY AND TOXICOLOGY

BIHAR VETERINARY COLLEGE

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Dedicated to

MY ADORABLE

PARENTS

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

Dr. C. Jayachandran
Ph.D.
University Professor & Chairman
Deptt. of Pharmacology & Toxicology
Bihar Veterinary College, Patna-800014

CERTIFICATE - I

This is to certify that the thesis entitled **"PHARMACOKINETICS AND IMMUNOLOGICAL EFFECTS OF KETOPROFEN 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. SMITA SINHA**, 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.

C. Jayachandran
19/12/05

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Major Advisor

Endorsed :

C. Jayachandran
19/12/05

(Chairman of the Department)

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

CERTIFICATE - II

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

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CERTIFICATE - III

This is to certify that the thesis entitled **"PHARMACOKINETICS AND IMMUNOLOGICAL EFFECTS OF KETOPROFEN IN GOATS"** submitted by **DR. SMITA SINHA**, in partial fulfillment of the requirement for the degree of **Master of Veterinary Science (Veterinary Pharmacology & Toxicology)** of the faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar was examined and approved on ....25.02.06..... .

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Date : 19.12.05

Smita Sinha

Place : Patna

(Smita Sinha)

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Introduction

INTRODUCTION

Ketoprofen is a potent non-steroidal anti-inflammatory drug (NSAID) of aryl-propionic acid class. It acts by blocking the synthesis of prostaglandins preferably by inhibiting cyclo-oxygenase (COX) and there by exerting its potent anti-inflammatory, analgesic and antipyretic actions. Ketoprofen is widely used in veterinary and human medicine to treat a number of musculo-skeletal conditions (Al Katheeri *et al.*, 2000).

Inflammation of different tissues is the commonest single problem faced by clinicians. Inflammatory disorders are due to the presence of mediators of inflammation, particularly prostaglandins. Although inflammation may be caused by infectious and non-infectious agents, but irrespective of causative factors, the response of inflammation is more or less similar and mediated through a number of biochemical cascades including prostaglandins as a major mediator. Administration of anti-inflammatory agents to alleviate signs of inflammation is a standard therapeutic approach.

A number of anti-inflammatory drugs are known to exert the desired action by inhibiting the synthesis of prostaglandin (Brander *et al.*, 1991). Aryl-propionic acid derivatives represent a group of effective useful NSAIDs that includes ibuprofen, fenoprofen, naproxen, benoxaprofen and carprofen. They may offer significant

advantage over older generation of NSAIDs such as aspirin and indomethacin, since they usually are better tolerated with least side effects.

Ketoprofen is also a more potent anti-oedematous agent in laboratory animal models of acute inflammation (Hutt and Caldwell, 1983; Evans, 1992). Ketoprofen is a chiral compound and exists in two enantiomeric forms [S (+) and R (-)]. Only S (+) enantiomer inhibits the enzyme cyclo-oxygenase (Kean *et al.*, 1989; Suesa *et al.*, 1993). Although it is a cyclo-oxygenase inhibitor, ketoprofen is said to stabilize lysosomal membranes and may antagonize the action of bradykinin (Williams and Upton, 1988). In human patient suffering from rheumatoid arthritis, ketoprofen has been shown to be as efficacious as aspirin, indomethacin, ibuprofen, diclofenac and piroxicam (Avouac and Teule, 1988). In control of post-operative pain, ketoprofen has proven as efficacious as pentazocine and meperidine (Avouac and Teule, 1988). It is recently approved by the united states FDA for use in horses for the alleviation of inflammation and pain associated with musculo-skeletal disorders.

Goat (*Capra hircus*), is mainly reared in tropical countries. It is the source of income for about 40% of the Indian rural population living below poverty line. Apart from milk and meat, it provides valuable fur and hides and even manure for the farmers. Thus, it plays a significant role in improving the socio-economic status of weaker sections of the society and finally, it increases the net agrarian economy of the country. Considering the importance of

goats in nation's economy, its proper and effective health coverage is essential.

Before using a drug in therapy it is essential to study its pharmacokinetic behaviour and immunological effects in detail. It is well established that many drugs either stimulate or suppress the immune system and there by alter the course of the disease. Therefore it is highly desirable to carry out immunological studies of NSAIDs. Brown *et al.* (1978) observed in human being that sodium salicylate, aspirin, phenylbutazone and indomethacin did not affect the cell mediated immunity. Diclofenac was reported to suppress the lymphocyte proliferation at supratherapeutic doses (Spiers *et al.*, 1988).

Pharmacokinetic study of ketoprofen has been carried out in cattle, horse, camel, sheep and goat but little work has been done in goat particularly on the immunological effect of ketoprofen. Keeping in view of the above mentioned facts the present investigation has been carried out with the following specific aims and objectives :-

(1) Estimation of concentration of ketoprofen at different time intervals in body fluids following intravenous administration in goat.

(2) Determination of kinetic parameters of ketoprofen.

(3) To study the immunlogical effects of ketoprofen after intramuscular administration.

Chapter - 2

Review of Literature

REVIEW OF LITERATURE

HISTORY

Sodium salicylate, one of the oldest non-steroidal anti-inflammatory drug (NSAID), was the first agent used for the treatment of rheumatic fever and as an antipyretc in 1875. The discovery of its uricosuric effects and of its usefulness in the treatment of gout soon followed. After demonstration of its anti-inflammatory effects, this compound was introduced in medicine in 1899 by Dresser under the name of Aspirin.

The synthetic salicylates soon displaced the more expensive compounds obtained from natural sources. Toward the end of the nineteenth century, other drugs were discovered that shared some or all of these actions.

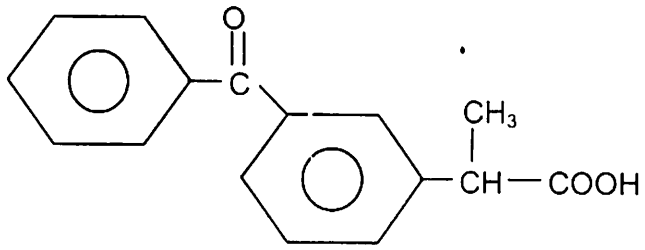
Aryl propionic acid derivatives represent a group of effective useful NSAID. It has significant advantages over aspirin and indomethacin. The intensity of untoward effects is less than that associated with the ingestion of indomethacin or high doses of aspirin.

Ketoprofen is potent non-steroidal anti-inflammatory drug (NSAID) which is widely used in human and veterinary medicine. It is a 2-aryl propionic acid derivative. It has a potent

analgesic antipyretic and anti-inflammatory effect apart from its anti oedematogenic property.

CHEMISTRY

Chemically, Ketoprofen is a [2(3-benzoyl phenyl) propionic acid. Its chemical structure is as follows :-



Molecular Formula – $C_{16}H_{14}O_3$

Molecular Weight – 254.29

MECHANISM OF ACTION

Ketoprofen has excellent analgesic, antipyretic and anti-inflammatory properties. It is an inhibitor of cyclo-oxygenase in the metabolism of arachidonic acid and thus exerts its anti-inflammatory action by blocking the synthesis of prostaglandin, prostacyclins and thromboxane products. Ketoprofen is said to stabilize lysosomal membranes and may antagonise the action of bradykinin (Williams and Upton, 1988). Efficacy of Ketoprofen has also been attributed to its ability of inhibit some lipo-oxygenases and thus formation of leukotrienes (Williams and Upton, 1988).

PHARMACOKINETICS AND METABOLISM

Ketoprofen is rapidly absorbed after oral administration and maximal concentrations in plasma are achieved within 1 to 2 hours. Food reduces the rate but not the extent of absorption. The drug is extensively bound to plasma protein (99%) and it has a half life in plasma of about 2 hours and slightly longer half-lives are observed in elderly subjects. Ketoprofen is conjugated with glucuronic acid in the liver, and the conjugate is excreted in the urine.

THERAPEUTIC USE

It is used in the treatment of rheumatoid arthritis, osteorthritis, ankylosing spondylitis and acute gouty arthritis. They are also used as analgesics for acute tendinitis and bursitis and for primary dysmenorrhea.

PHARMACOKINETIC STUDIES

Pharmacokinetic studies of ketoprofen were carried out in different species. These are as follows :-

Young children :-

Kokki *et al.* (2001) conducted pharmacokinetics of ketoprofen (KTP) following oral and intramuscular (i.m.) administration in young children. The maximal plasma concentration

of KTP ranged between 3.6 to 7.4 μg/ml in the i.m. group and between 2.8 to 8.2 μg/ml in tablet group. The terminal half-life was ranging between 0.8 to 2.2 h in the i.m. group and between 0.3 to 2.1 h in tablet group. The extrapolated area under the plasma concentration time were (AUC) ranged between 8.8 and 14.6 μg ml^{-1}.h in i.m. group and between 8.7 and 14.1 μg.ml^{-1}.h in the tablet group.

Asian Elephant :-

In Asian elephant, Hunter *et al.* (2003) conducted kinetic study. Harmonic mean half-life ranged from 3.8 to 5.5 hr. Area under the concentration time curve, mean residence time, apparent volume of distribution, Plasma clearance and maximum plasma concentration values were all significantly different between the two enantiomers for both routes of administration. Ketoprofen has a long terminal half-life.

Camel :-

In camel, Al Katheeri *et al.* (2000) conducted kinetic study after i.v. administration of racemic ketoprofen. In male camel, the elimination half life ($t_{1/2}\beta$) of 2.11 h (R-) & 2.33 h (S+), volume of distribution (Vd_{area}) of 215.6 ml/kg (R-) & 229.1 ml/kg (S+) and total body clearance (Cl_B) of 62 ml/kg/h (R-) & 69.6 ml/kg/h (S+) were

noted. In female camel, elimination half life ($t_{1/2}\beta$) of 1.88 h (R-) & 1.83 h (S+), Volume of distribution (Vd_{area}) of 118.5 ml/kg (R-) & 137.6 ml/kg (S+) and total body clearance (Cl_B) of 44.6 ml/kg/h (R-) & 50.6 ml/kg/h (S+) were noted.

Llama :-

In Llama, Navarre *et al.* (2001) conducted stereo selective pharmacokinetics of ketoprofen (KTP) after i.v. administration. The half-life of S-KTP and R-KTP were 5.49 ± 1.27 h and 5.41 ± 0.94 h. respectively. AUC value of 168.9 ± 22.4 μg/ml.h for S-isomers and 176.4 ± 25.5 μg/ml.h for R-isomer. Total body clearance of S and R isomers were 13.20 ± 1.68 and 12.67 ± 1.68 ml/h/kg. The volume of distribution of 100.1 ± 10.6 and 94.7 ± 10.9 ml/kg for S-KTP and R-KTP, respectively. Mean residence time of 7.72 ± 1.56 h. for S-KTP and 7.56 ± 1.13 h for R-KTP.

Horse :-

In mare, Sam *et al.* (1995) conducted kinetic study of ketoprofen after multiple i.v. doses. The harmonic mean of the terminal elimination half life of ketoprofen after the 1st and last doses was 98.2 and 78.0 min, respectively. The median values of total

plasma clearance and the renal clearance after the 1st dose were 4.81 and 1.93 ml/min/kg, respectively.

In horse, mean distribution half life ($t_{1/2}\ \alpha$) of 0.11 h, elimination half life ($t_{1/2}\ \beta$) of 1.02 h, volume of distribution (Vd_{area}) of 276.30 ml/kg and total body clearance (Cl_B) of 184.72 ml/kg/h were noted after i.v. administration of ketoprofen (Owens *et al.*, 1995).

In horse, distribution half life ($t_{1/2}\ \alpha$) of 0.103 h (S^+) and 0.097 h (R-), elimination half life ($t_{1/2}\ \beta$) of 1.09 h (S+) and 1.98 h (R-), volume of distribution (Vd_{area}) of 0.637 L/Kg (S+) and 0.534 L/kg (R-) and total body clearance (Cl_B) of 0.397 L/kg/h (S+) and 0.346 L/kg/h (R-) were noted after i.v. administration of ketoprofen enantiomers (Landoni *et al.*, 1996).

Cattle :-

In Cattle, volume of distribution at steady state (Vd_{SS}) was 0.11 litre/kg, elimination half life ($t_{1/2}\beta$) of 0.49 h and total body clearance (Cl_B) of 0.17 litre/kg/h were noted after i.v. administration of ketoprofen (De Graves *et al.*, 1996).

Cow Calf :-

In Cow Calf, Landoni *et al.* (1995) conducted kinetic study of ketoprofen (KTP) after i.v. administration. The elimination half life

($t_{1/2}\beta$) of 0.42 ± 0.08 h (R-) KTP & 0.42 ± 0.09 h (S+) KTP, volume of distribution (Vd_{area}) of 0.20 ± 0.06 L/Kg (R-) and 0.22 ± 0.06 L/kg (S+) KTP, Distribution half life ($t_{1/2}\alpha$) of 0.64 ± 0.61 h (R-) and 0.04 ± 0.01 (S+) and total body clearance (Cl_B) 0.33 ± 0.03 L/Kg/h (R-) & 0.33 ± 0.04 L/Kg/h (S+) were noted.

Foal :-

In healthy foal, Wilcke *et al.* (1998) conducted kinetic study. Clearance was significantly lower than that determined for adult horses. Volume of distribution was larger than that determined for adult horses. Mean plasma half-life was 4.3 hours.

Sheep :-

Arifah *et al.* (2001) conducted kinetic study of ketoprofen enantioners after i.v. administration. Both ketoprofen enantiomers had elimination half life ($t_{1/2}\beta$) and mean residence time (MRT) measurements that were short and volume of central compartment (Vd_C) and steady state volume of distribution (Vd_{SS}) that were very low. Clearance was rapid particularly for R-KTP.

Landoni *et al.* (1999) conducted enantiospecific pharmacokinetic and pharmacodynamic of ketoprofen. S-KTP volume of distribution (Vd_{area}) was higher and clearance (Cl_B) faster than

those of R-KTP. S & R KTP achieved relatively low concentration in exudates and transudate.

Goat :-

Musser *et al.* (1998) conducted kinetic study of ketoprofen in lactating goat after i.v. administration. The elimination half life of 0.32 ± 0.14 h., systemic clearance of 0.74 ± 0.12 litre/kg/h and volume of distribution at steady state of 0.23 ± 0.51 litre / kg were noted.

Arifah *et al.* (2003) conducted kinetic study of ketoprofen in goat. The pharmacokinetics of both enantiomers were characterized by rapid clearance, short mean residence time (MRT) and low volume of distribution.

Cat :-

In Cat, Lees *et al.* (2003) conducted kinetic study of ketoprofen. After i.v. dosing clearance was more than 5 times greater and elimination half life and mean residence time were 3 times shorter for R-KTP than for S-KTP. Plasma AUC of 20.25 S-KTP and 4.06 R-KTP μg h/ml after i.v. and 6.36 S-KTP and 1.83 R-KTP μg.h/ml after oral dosing were observed.

Table showing important pharmacokinetic parameters of ketoprofen in different species

Species	Dose (mg/kg)	Route of administration	Distribution half-life ($t_{1/2}\alpha$) (h)	Elimination half-life ($t_{1/2}\beta$) (h)	Volume of distribution (L/kg)	Total body clearance (L/kg/h)	References
Camel	2	i.v.	-	2.11	0.2156	0.062	Al Katheeri *et al.* (2000)
Llama	4.4	i.v.	-	-	0.1 ± 0.0106	0.013	Navarre *et al.* (2001)
Horse	2.2	i.v.	0.11	1.02	0.2763	0.1847	Owens *et al.* (1995)
	2.2	i.v.	0.103	1.09	0.637	0.397	Landoni *et al.* (1996)
Mare	2.2	i.v.	-	1.63	-		Sam *et al.* (1995)
Cattle	3.31	i.v.	-	0.49	0.11	0.17	De Graves *et al.* (1996)
Calf	3	i.v.	0.64 ± 0.61	0.42 ± 0.08	0.20 ± 0.06	0.33 ± 0.03	Landoni *et al.* (1995)
Goat	3	i.v.	-	0.32 ± 0.14	0.23 ± 0.51	0.74 ± 0.12	Musser *et al.* (1998)

IMMUNOLOGICAL STUDY OF NSAIDs

Human being :-

In human being, aspirin, acetaminophen and paracetamol suppressed the serum neutralizing antibodies (Graham *et al.* 1990).

Bull :-

Ting *et al.* (2003) Studied the effect of repeated ketoprofen administration during surgical castration of bulls on immune function and showed that a repeated ketoprofen dose 24 h after treatment (3mg/kg of BW) has no influence on changes in acute - phase proteins and immune response.

Beef Cattle :-

Ting *et al.* (2003) Studied the effect of ketoprofen during castration of beef cattle on stress response and immunity and showed that there was no difference between ketoprofen treated group and control on concanavalin-A induced interferon-gamma production.

Bull Calf :-

In bull calves, Earley *et al.* (2002) Studied immunological effect of ketoprofen on immune response and showed that concanavalin – A induced gamma interferon production was lower in ketoprofen treated group than in control.

Rabbit :-

In Rabbit, Jose *et al.* (1999) conducted immunological study of pefloxacin and diclofenac sodium. There was an apparent but not significant reduction in agglutinating antibody titres and in absolute lymphocyte count. The agglutinating antibody titres in antigen control were in the range of 1 : 160 to 1 : 640 from day seven to day 21. From day 28 to day 42, the antibody titres ranged from 1 : 320 to 1 : 140. IN pefloxacin, diclofenac and antigen treated group, it was in the range of 1 : 80 to 1 : 320 from day seven to day 21 and from day 28 to day 42, it ranged from 1 : 160 to 1 : 10. Total immunoglobulin concentration, total serum protein concentration and total leukocyte count were not affected. Cell mediated immunity was not affected.

Guinea Pig :-

In guinea pig, Raisov *et al.* (1990) conducted immunological study after administration of diclofenac and mebendazole. It has been reported that simultaneous administration of diclofenac, a NSAID and mebendazole, an anthelmintic did not affect the immune response.

GENERAL PHARMACOKINETICS

Pharmacokinetics is well known as disposition kinetics, which helps in knowing absorption, distribution, metabolism and excretion of drugs (Dost, 1953). The main objectives of pharmacokinetics of drugs are to study in respect of drug concentrations versus time course, their metabolites in various body fluids, tissues and excretion and interpretation of such data based on suitable pharmacokinetic compartment models (Wagner, 1968).

The compartment model is hypothetical structure and is used to characterise with reproducibility of behaviour and fate of a drug 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 pharmacokinetics of a drug is described either by one compartment, two compartment or multi compartment open model. In an open compartment model, body distributes the drug in all tissues at widely varying rates. In an open compartment model, the drug moves freely from one compartment to another (i.e. from blood to tissues and vice-versa).

One Compartment Open Model :

The drug is said to follow one compartment open model when the distribution of drug is instantaneous between central and peripheral compartment. Any change in drug concentration in blood reflects directly the quantitative change in its tissue level. The rate of drug elimination from the body is directly proportional to concentration of drug in blood (Baggot, 1974). When the plasma concentration time profile is plotted from the peak concentration onwards on a semilogarithmic scale, a straight line is obtained in case of one compartment open model (Sams, 1978). The plasma drug levels decline according to the following equation.

$$C_P = Be^{-\beta t} \quad \text{.....Eq. 1}$$

Where,

C_P = Concentration of drug in plasma

B = Extrapolated zero time intercept of mono exponential curve.

β = Overall elimination rate constant.

t = time elapsed after drug administration

e = Base of natural logarithm.

Baggot (1977) reported that the one compartment open model is particularly useful in describing the time course of most

drugs in plasma following extra vascular (oral/i.m./s.c.) administration.

Two Compartment Open Model :-

The pharmacokinetics of most of the drugs following intravenous administration is accurately described by two compartment open model. Baggot (1974) described that in two compartment open model, the drug distribution is quick and homogeneous into the central compartment (blood and other readily accessible tissues like liver and kidney) and comparatively more slowly into peripheral compartment (less perfused organ and tissue such as muscle and fat). This clearly shows that elimination mainly takes place from central compartment and distribution and elimination process follow the first order kinetic. Semilogarithmic plot of plasma log drug concentration against time shows a biphasic curve in two compartment open model. This biphasic curve shows initial steep decline in plasma drug concentration, which is mainly due to distribution of drug from central to peripheral compartment and the gradual decline is obtained in later phase 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.

$$C_P = Ae^{-\alpha t} + Be^{-\beta t} \quad \text{.....Eq. 2}$$

Where,

C_P = Plasma concentration of the drug

A = Zero time intercept of distribution phase.

B = Zero time intercept of elimination phase

α = Distribution rate constant

β = Elimination rate constant

t = time elapsed after drug administration

e = Base of natural logarithm

The values of A, B, α and β are essential in calculating the kinetic rate constants (K_{12}, K_{21} and K_{el}) in two compartment open model.

The value 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 multi compartment. A semilogarithmic plot of plasma drug concentration against time shows triphasic or multiphasic curve. The initial steep decline in plasma drug concentration against time is drug

to distribution of drug from blood to highly perfused tissue known as peripheral-I. After that, gradual decline is due to distribution of a drug from central to less perfused tissue known as peripheral-II. The drug concentration in plasma following single i.v. administration is expressed by following triexponential mathematical formula as a function of time.

$$C_P = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t} \quad \text{.....Eq. 3}$$

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

Clinical Importance of Pharmacokinetic Study :

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 with drawl period for drug residues in milk and tissues of food producing animals.

Some important pharmacokinetic parameters :-

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

These denote the rate of absorption (faster or slower) of a drug from its site after its extra vascular (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 drug from plasma to body fluids and tissues following i.v. administration.

(3) Elimination rate constant (β) :

The rate of elimination from the body for most of drugs follows first order of reaction process and is a hybrid or composite constant describing a rate of fall to which more than one process is contributing.

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

(a) The elimination half-life ($t_{1/2}$ β)

(b) The total body clearance (Cl_B)

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

(d) The drug with drawl period for drug residues in milk and tissues of food producing animals.

4. Elimination or biological 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 clearly shows 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. The elimination half-life follows the first order process and hence is not dependent on the dose of the drug as well as the route of administration. It is used to calculate the duration of drug action in the body and designing the rational dosage regimen.

5. *Volume of distribution*

The apparent volume of distribution is a hypothetical volume of body fluid that would be required to dissolve the total amount of the drug to attain the same concentration as that found in the blood. Riegelman *et al.* (1968) stated that the calculated value of volume of distribution is not dependent upon the method used for its calculation, if the drug distributes truly according to one compartment. 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 (>1L/Kg) indicates wide distribution throughout the body or extensive tissue binding or rapid excretion of drug or combination of all the above. A small volume of distribution is due to high protein binding or low lipid solubility of a drug and indicates that the drug is restricted to certain fluid compartment, like plasma water, extra cellular fluids etc. This is due to high protein binding or low solubility of drug.

6. *Total Body Clearance (Cl_B) :-*

Total body clearance is another important pharmacokinetic parameter, which is the sum of the clearance of each eliminating organ, particularly liver and kidney. The half life and total body clearance of a drug are different in the sense that half life depends upon the process of drug distribution, biotransformation and excretion, whereas Cl_B is independent of these processes and indicates the rate of drug removal from the body unlike B and $t_{1/2}$ β that are hybrid constant and depend upon K_{12}, K_{21} and K_{el}, the total body clearance changes exactly in proportion to Kel (Jusko and Gibaldi, 1972; Rowland *et al.*, 1973).

7. *Bioavailability*

The extent of absorption (F) is generally known as bioavailability. Bioavailability of a drug indicates the rate of drug absorption as well as the amount of absorption of a drug in pharmacologically active form. It is a measure of the fraction of administered dose of a drug that reaches the systemic circulation in the unchanged form. It is calculated experimentally by the ratio of the area under 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 a drug with plasma protein

affects drug distribution, drug effects and drug elimination. The protein bound drug also acts as a reservoir.

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

Chapter – 3

Materials and Methods

MATERIALS AND METHODS

In the present study, clinically healthy female goats of non-descript breed age ranging from 2-2.5 years and weighing between 18 to 25 kg body weight were used. The animals were housed in animal shade of the department and kept on pre-experimental period of one month before the commencement of experiment to acclimatize them to the new environment. Proper physical and clinical examination was done before the start of experiment. The animals were kept on *ad-libitum* stall feeding of green fodder supplemented with concentrate ration and partial grazing. The animals had free access to clean fresh drinking water.

Experimental design :

A. Pharmacokinetic study :

Ketoprofen was administered in each of five 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 three animals in

each group. Details of treatment given to different groups are given below :-

Group I : Saline control

Group II : Antigen (Ag) control – 2ml of 7% sheep red blood cell (SRBC) suspension i.v. given in each goat on the first day of treatment (sensitizing dose) and on the 10^{th} day of experiment (challenging dose).

Group III : Ketoprofen + Ag – Apart from antigen (SRBC) given as in group II, Ketoprofen was administered @ 3mg/kg, i.m. daily for 5 days during sensitizing and challenging period.

Sterilization of Glasswares :

For immunological study only, sterilization process is adopted. All glasswares were washed properly with detergent 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 drugs and for collection of blood, sterile disposable needles were used.

Drug used :

Ketoprofen – an injectable commercial preparation under the trade name of Neoprofen containing ketoprofen in concentration of 100 mg/ml marketed by Ranbaxy India was used in the present study.

Collection of biological fluids and their timings :

1. Pharmacokinetic Study :

The samples of various biological fluids were collected after i.v. administration of drugs in healthy goats. 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) Blood :

Before collection of blood, the sites around the jugular vein on either side of the neck of the animals were aseptically prepared. The site was sterilized prior to each collection with rectified spirit. Blood samples were collected in sterilized centrifuge tubes containing appropriate amount of sodium oxalate by vene puncture with disposable 18 G needles at various above noted time intervals after drug administration. The blood samples were centrifuged at 3000 rpm for 10 min for the separation of plasma. The plasma

samples were then kept under refrigeration until assay 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 glycerine through urethra into the urinary bladder of the experimental goats with the aid of a flexible metal probe. The balloon of the catheter was inflated by injecting 25-30 ml of water through a syringe to keep the catheter in position. The opening of the catheter was blocked with a pressure clip to check dripping of urine. Prior to drug administration urine sample was collected in a sterile test tube for the preparation of standards . After administration of the drug, urine samples were collected in sterile test tube at various above noted time intervals. The samples were kept in a refrigerator and were analysed in successive days.

2. Immunological study :

Blood samples only were collected on day 1, 7, 10, 14, 21, 28, 35, and 42 days of the experiment without anticoagulant for serum separation and upto 28 days with heparin for Lymphocyte transformation test. The separated serum samples were used for HA (Haemagglutination) test.

Administration of Drugs :

1. Pharmacokinetic study :

Neoprofen infusion, containing 100 mg of ketoprofen per ml was injected at the dose rate of 3mg/kg body weight by i.v. route in each of five healthy goats.

2. Immunological study :

Ketoprofen (3mg/kg) was administered i.m. for 5 days in healthy goats of group III during period of pre and post challenge.

Estimation of ketoprofen by Reverse phase HPLC method :

The concentration of ketoprofen in plasma and urine were estimated by HPLC method as described by Proniuk *et al.*, 1998 with slight modification. The details of the procedure are as follows :-

Apparatus :

The HPLC equipment used comprised of a HPLC pump, a dual wavelength absorbance detector, a rheodyne manual injector with a 200 µl loop size and a data module (integrator). Chromatographic separations were performed by using C_{18} column (3.9 × 150 mm size).

Chromatographic conditions :

For HPLC analysis of ketoprofen in biological samples, the flow rate was 1 ml/min, the effluent was monitored at 257 nm,

loop size was 200 µl, injection volume was 400 µl, chart speed was 0.25 mm/min and the detector sensitivity was monitored at 2.000 area under full scale (A.U.F.S.).

Reagents :

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

Mobile phase :-

Water with orthophosphoric acid (pH 3.2) – 52%

Acetonitrile _ 35%

Methanol _ 13%

Preparation of standards of ketoprofen in biological samples

Neoprofen, an injectable commercial preparation containing ketoprofen in concentration of 100 mg/ml was used in the present study. Ketoprofen was diluted in tripple distilled water to have different strengths *viz.*, 40, 20, 10, 5, 2, 1, 0.5, µg/ml.

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 ketoprofen standards of 4, 2, 1, 0.5, 0.2, 0.1, 0.05 µg/ml in the above noted biological fluids. Blank plasma/

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

Analytical method :-

(1) In a clean dry centrifuge tube, 1 ml of plasma samples was taken and 1 ml of acetonitrile and 1 ml of methanol were added for precipitation of plasma proteins.

(2) The mixture was shaken on a vortex mixer for 1 min and centrifuged for 15 min at 3000rpm.

(3) The supernatant was transferred to a clean tube.

(4) An aliquot of this mixture (up to 400 µl) was injected directly into the loop of injector and the integrator print out retention time and area.

(5) From various concentrations of standards versus area, standard curve was plotted on a graph paper for ketoprofen.

(6) Using these standard graph and the area obtained from test plasma and urine samples collected at various time intervals, the concentrations were obtained separately for plasma as well as for urine samples.

Analysis of immunological parameters :-

1. Preparation of buffer :-

(a) Phosphate buffer saline (Aziz, 1985) :-

The composition of buffer used for reconstitution and preparation of sheep red blood cells (SRBC) is as follows :-

NaCl	-	2.0 gm
KCl	-	0.05 gm
$Na_2 HPO_4.2H_2O$	-	0.14 gm
KH_2PO_4	-	0.05 gm
Double distilled water	-	250 ml
pH	-	7.2 to 7.4

This solution was autoclaved at 15 lb pressure for 15 minutes and stored at refrigerated temperature till use. This buffer was used for reconstitution and preparation of red blood cells suspension.

(b) Alsever's solution :

The equal volume of following composition was used as 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 days.

(2) Mitogen used for delayed type hypersensitivity :-

(a) DNCB (1 chloro-2, 4 – dinitrobenzene) –

One percent DNCB solution was prepared in acetone (10 mg/ml).

(b) PHA - P (phytohaemagglutinin –P) –

It is a plant lectin and is prepared from red kidney bean (*Phaseolus vulgaris*) and was obtained from IVRI, Izatnagar as a gift sample. It was used as mitogen for cutaneous basophilic hypersensitivity reaction. A concentration of 1mg/ml of PHA-P in PBS solution was prepared.

(C) PPD (Tuberculin) :-

A commercial preparation of tuberculin (purified protein derivative) available in the diluted form of 10 TU/0.1 ml manufactured by Beacon diagnostics Ltd., 424 New GIDC, Kabilpore, Navsari was procured.

(D) Concanavalin-A

It is a plant mitogen and is obtained from RMRI, Patna. It is used in Lymphocyte transformation test (LTT).

(3) ***Heparin*** : An injectable commercial preparation under the trade name of Beparine (5000 I.U. in 5 ml) marketed by Biological E. Ltd, Azamabad, Mumbai was used in the present study.

(4) ***RPMI-1640 Growth Media*** : It is obtained from HiMedia Laboratories Pvt. Ltd, Mumbai. The composition is as follows –

Tripple distilled water	-	80.0 ml
Medium 199 (10x)	-	9.0 ml
Tryptose phosphate broth (10x)	-	10.0 ml
Calf serum	-	10.0 ml
Sodium bicarbonate (2.8% Sol.)	-	3.0 ml
Penicillin	-	200.0 IU/ml
Streptomycin	-	200.0 µg/ml

In maintenance medium the composition was the same as that of the GM except that it contained 2 percent calf serum in place of 10 percent.

(5) ***Fetal bovine serum*** : It is used in LTT. It is obtained from Hi Media Laboratories Pvt. Ltd, 23, Vadhani Ind. Est. LBS Marg, Mumbai.

(6) ***MTT Solution*** (3-4, 5-Dimethyl-thiazole 2,5-Diphenyl tetrazolium bromide) – It is used in LTT in the concentration of 5 mg/ml. It is obtained from Madras Veterinary College, Chennai.

7. Preparation of SRBC suspension :-

Ten ml of blood was collected from jugular vein of sheep in clean sterilized test tube containing equal volume of Alsever's solution as anticoagulant and left it for 24 hours. The blood was pooled and mixed with equal amount of phosphate buffer saline (PBS) and centrifuged at 800 r.p.m. 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) which could be used for 3 days after which fresh suspension was prepared as and when required.

8. Collection of Serum Samples from Goats :

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

9. Assessment of Immune Response After Administration of Drug :

(A) Humoral Immune response (HIR) :- For assessing HIR, HA test was done which is stated below-

(i) Haemagglutination (HA) test :-

Humoral immune response (HIR) was assessed by HA test. Antigen used for haemagglutination reactions is sheep red blood cell (SRBC). HA test performed in the test and control goats of sera. The anti-SRBC antibody titres were measured using micro-titration technique by the procedure described by Beard (1980).

HA test was performed in 'U' shaped micropersplex plate. By taking 0.5 ml of serum, two fold serial dilutions were 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 erythrocytes and left at room temperature for 40 min. The goat's serum produced a diffused sheet of agglutination RBC covering the bottom of wells whereas, 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 erythrocytes) and expressed as log 2. HA titre/0.5 ml of goat's serum.

(B) Cell mediated immune response (CMIR): -

Cell mediated immune response was assessed by DTH reactions. Mitogens used for cutaneous basophilic hypersensitivity reactions are as follows :-

(a) DNCB (1 chloro – 2, 4 – dinitrobenzene)

(b) PHA-P (phytohaemagglutinin- P)

(c) PPD (purified protein derivative /tuberculin)

(1) DNCB skin sensitivity test :-

The test was done as per the method described by Chauhan and Verma (1983) with minor modifications. 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 vehicle 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 of 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 CMI response was calculated by substracting the thickness of right side from left side.

2. PHA-P Skin sensitivity test :

The test was done as per the method described by Corrier and De Loach (1990) with minor modifications. Area of about 10-15 cm^2 was shaved below the DNCB area on left and right lateral neck for PHA-P application. These areas were cleaned with acetone and dried. After drying the skin, 0.1 ml of PHA-P (1 mg/ml) in 0.1 ml of PBS was injected intradermally on the right side of neck. The left side received 0.1 ml of sterile PBS and served as control. On 15^{th} day of experiment, a second injection of PHA-P (0.1 ml) was given on right side and left side was injected with only PBS. The PHA-P stimulation index was calculated at the difference in swelling on PHA-P injected and PBS injected site with the help of slide caliper on 4, 8, 12, 24, 48, 72, and 96 h.

3. Purified Protein derivative (PPD) skin sensitivity test :-

The test was done as per the method described by Singh (1987). About 10-15 cm^2 areas of neck were shaved and 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 below the PHA-P area 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 0.1ml PBS. Measurement of skin thickness was made by

the aid of slide caliper on 4, 8, 12, 24, 48, 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 periods.

Measurement of skin thickness by slide caliper :-

Main scale × Vernier scale × Limit factor (0.01)

Diameter measured on three different position.

4. Lymphocyte Transformation Test (LTT) :

LTT is synonymously known as lymphocyte proliferation assay. LTT was done for evaluating *in-vitro* cell mediated immune response in goats as described by Bounous *et al.* (1992) with slight modification.

To obtain peripheral blood lymphocytes (PBL) from control and experimental goats, 5 ml of blood was drawn from jugular vein into sterile syringe containing heparin (10 IU/ml of blood). The blood samples were transferred in a centrifuge tube containing 1 ml phosphate buffer saline (PBS). The tubes were centrifuged at 1500 rpm for 20 min. 2 ml of histopaque (1.077 g/ml, sigma, USA) was taken in a sterile tapering bottomed, screw capped 15 ml plastic tubes and buffy coat was collected and layered over the histopaque. Tubes were centrifuged at 1900 rpm for 20 min. Mononuclear cells were

collected carefully from interface layer ring and washed three times with PBS by centrifugation at 1000 rpm for 10 min each time. Washed cells were collected in RPMI-1640 growth medium (RPMI-GM) and adjusted to give 2×10^6 viable cells/ml in RPMI-1640 GM.

In 3 sets of triplicate wells of a 96 well, flat bottomed tissue culture plates (Nunclone) fifty microlitre of cell suspension was added under sterilized condition. First set of triplicate wells (control) received 50 µl of RPMI-GM whereas the second set received 50 µl of RPMI-GM containing 10 µg/ml of Con-A, thus making final concentration of Con-A to 5 µg/ml. The plates were incubated at 37°C is a humidified chamber at 5% CO_2 tension. After 72 hrs., 20 µl of MTT solution (3-4, 5-Dimethyl-Thiazole 2,5-Diphenyl tetrazolium-bromide), at the concentration of 5 mg/ml, was added to all the wells and incubated further for 4 hr. The MTT formazan was extracted from the cells using Dimethylsulfoxide (150 µl/well) and optical density (OD) of well was measured at wavelength of 510 nm and a reference wavelength of 650 nm. Mean was calculated and stimulation index (SI) was determined using the following formula :-

$$SI = \frac{OD\ stimulated}{OD\ unstimulated}$$

Calculation of Pharmacokinetic parameters :-

The following pharmacokinetic parameters of ketoprofen was calculated after its single i.v. administration from semi log plot of

plasma drug concentration versus time curve. The experimental data was analysed using two compartment (for i.v. route) open model as described by Gibaldi and Perrier (1975) and Notari (1980).

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

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

Where,

C_P = drug concentration in plasma at time 't'

e = base of natural logarithm

The description of different kinetic parameters are as follows :-

(a) A, the zero time concentration of the drug in plasma during distribution phase (μg/ml).

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

(c) B, the zero time concentration of the drug in plasma during elimination phase (μg/ml).

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

(e) C_p^0 (A+B), the theoretical zero time concentration of the drug in plasma (μg/ml).

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

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

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

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

(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})

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

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

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

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

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

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

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

(o) T≈P, the approximate tissue to plasma concentration ratio

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

(p) Vdc, the volume of distribution, based on distribution and elimination (L/kg)

$$Vdc = \frac{D}{C_p^0}$$

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

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

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

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

(s) Vd_{SS}, the volume of distribution at steady state (L/kg)

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

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

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

Statistical Analysis :

Comparison of effects of Ketoprofen in immune response at different time intervals and on various days of post treatment in three groups were done by two way analysis of variance with interaction (Snedecor and Cochran, 1967).

Chapter - 4

Results

RESULTS

PHARMACOKINETIC STUDY AFTER A SINGLE INTRAVENOUS ADMINISTRATION :-

Pharmacokinetic study of ketoprofen was conducted in five healthy female goats following single i.v. dose of 3 mg/kg and the results are presented below.

(1) Plasma levels :-

Concentrations of ketoprofen in plasma at various time intervals following its single i.v. administration at the dose rate of 3 mg/kg have been shown in Table-1 and Fig-1. The mean peak plasma concentration of 9.87 ± 0.48 μg/ml was attained at 0.042 h. The drug was detectable upto 5 h in all the animals with the mean of 0.04 ± 0.005 μg/ml. The drug was detected only in two out of five animals at 6 h with the mean of 0.015 ± 0.005 μg/ml and none at 8 h.

(2) Urine levels :-

Table-2 and Fig-2 reveal urine concentrations of ketoprofen after its single i.v. administration (3 mg/kg). The drug appeared in urine of three out of five animals at 0.042 h with a mean of 0.29 ± 0.03 μg/ml but appeared in all animals at 0.083 h with a mean of 7.01 ± 1.21 μg/ml. The mean peak urine drug concentration

Table – 1

Plasma concentrations ($\mu g.ml^{-1}$) of ketoprofen in healthy goats following i.v. administration of ketoprofen (3 $mg.kg^{-1}$)

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	8.45	9.76	11.35	10.39	9.41	9.87 ± 0.48
0.083	5.42	5.82	8.23	8.41	6.23	6.82 ± 0.63
0.167	4.73	4.80	6.12	5.65	4.95	5.25 ± 0.27
0.25	3.80	3.96	4.92	4.82	4.00	4.30 ± 0.24
0.333	3.48	3.35	3.93	3.62	3.26	3.53 ± 0.12
0.50	3.00	2.96	3.01	3.05	2.85	2.97 ± 0.03
0.75	2.06	1.92	2.02	1.97	1.95	1.98 ± 0.025
1	1.31	1.02	1.35	1.22	0.82	1.14 ± 0.10
1.5	1.07	0.90	1.08	1.00	0.70	0.95 ± 0.07
2	0.75	0.65	0.80	0.75	0.50	0.69 ± 0.05
3	0.40	0.35	0.42	0.39	0.35	0.38 ± 0.01
4	0.10	0.12	0.15	0.14	0.11	0.12 ± 0.009
5	0.04	0.06	0.04	0.05	0.03	0.04 ± 0.005
6	N.D.	0.02	N.D.	0.01	N.D.	0.015 ± 0.005

N.D. = Non-detectable

Fig. 1

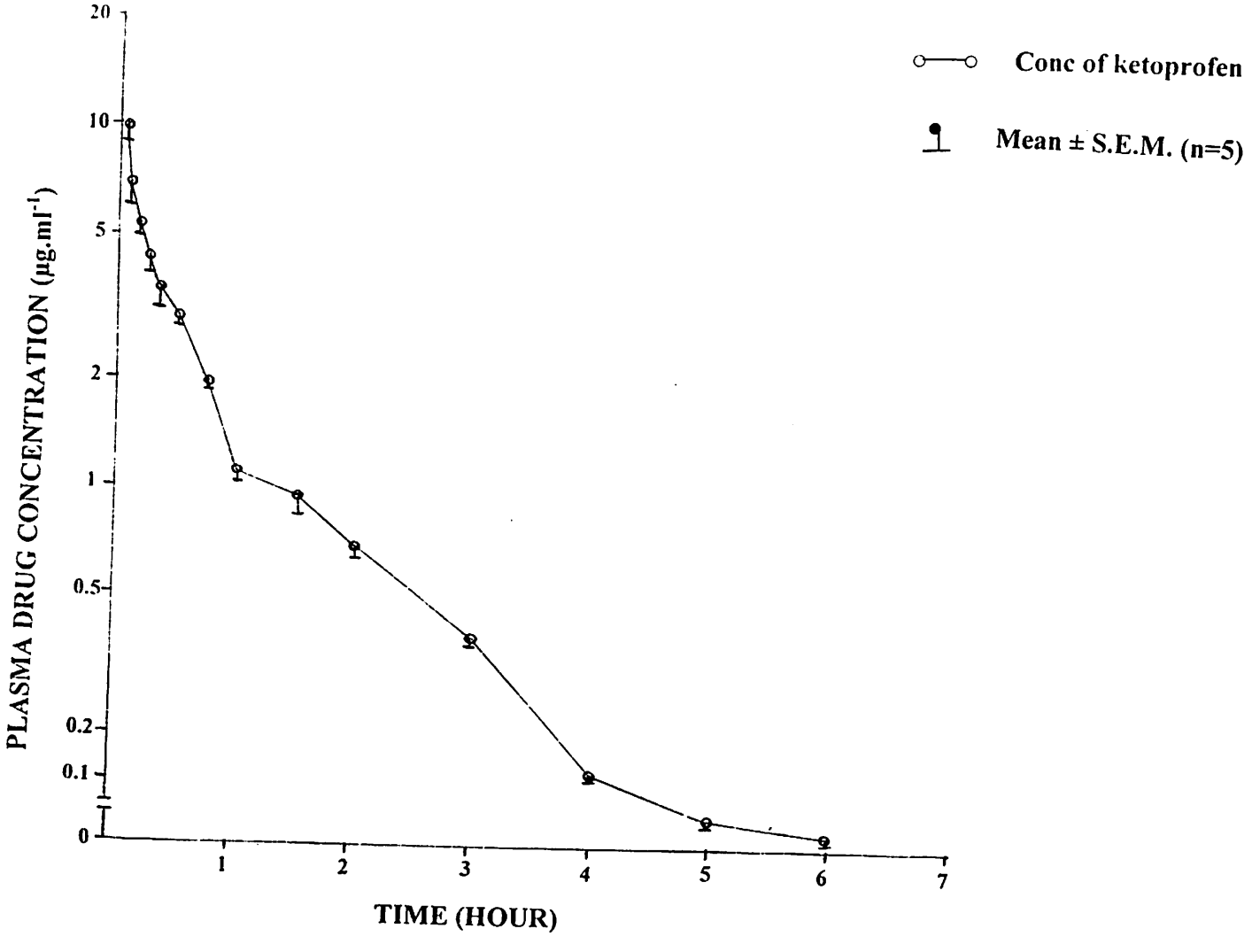
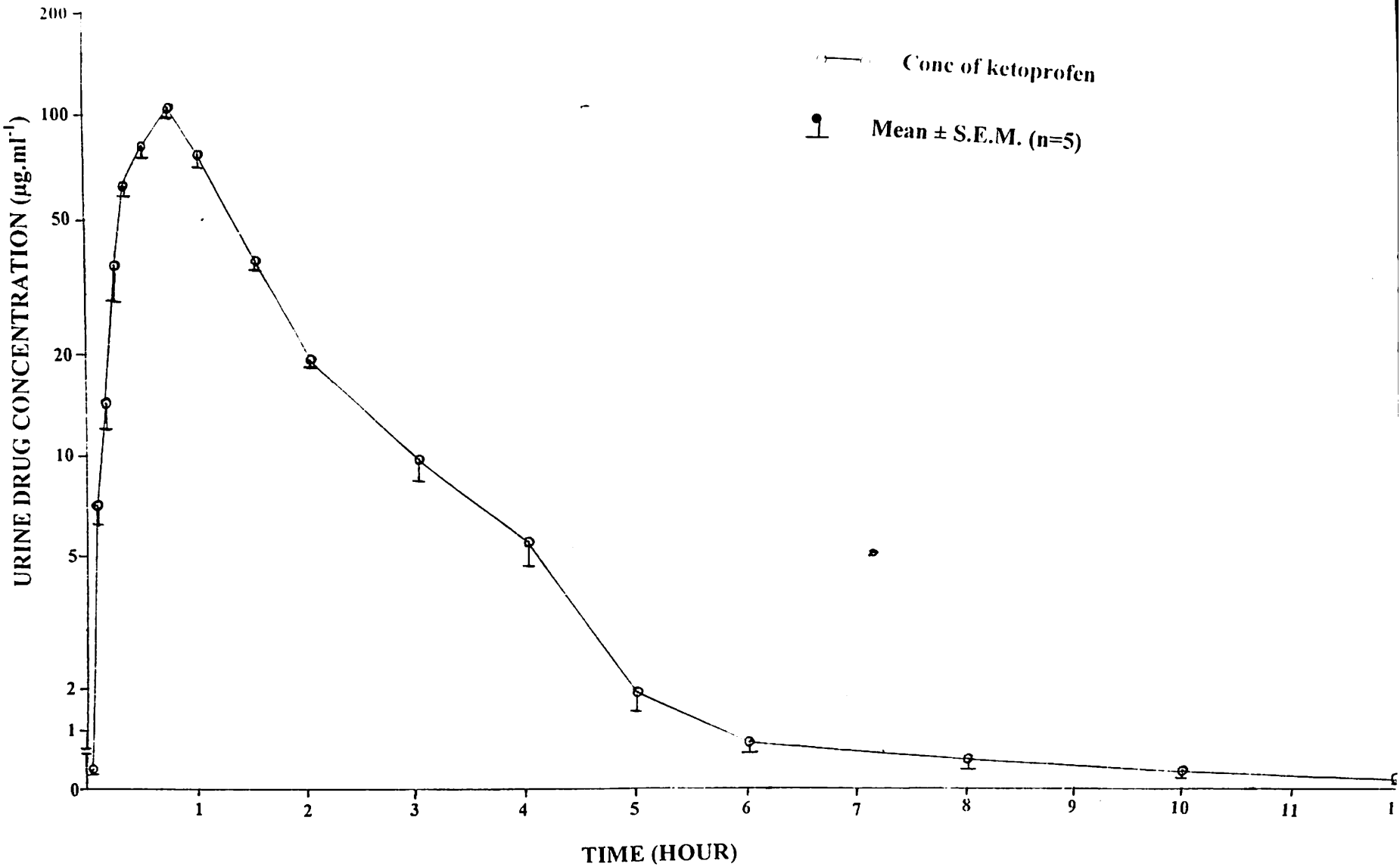

Conc of ketoprofen
Mean ± S.E.M. (n=5)
PLASMA DRUG CONCENTRATION (µg.ml-1)
20
10
5
2
1
0.5
0.2
0.1
0
1
2
3
4
5
6
7
TIME (HOUR)

Table – 2

Urine concentrations ($\mu g.ml^{-1}$) of ketoprofen in goats following single intravenous dose of 3 $mg.kg^{-1}$

Time (h)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
0.042	0.28	0.23	N.D.	0.35	N.D.	0.29 ± 0.03
0.083	3.89	10.65	7.45	8.24	4.82	7.01 ± 1.21
0.167	4.50	20.25	12.25	16.25	18.96	14.44 ± 2.84
0.25	7.07	48.21	39.45	44.30	45.20	36.85 ± 7.58
0.333	56.56	60.65	65.20	64.20	70.15	63.35 ± 2.28
0.50	64.85	83.29	89.21	80.51	90.21	81.61 ± 4.56
0.75	78.10	106.39	110.20	120.54	115.52	106.15 ± 7.40
1	57.88	72.20	82.20	84.23	85.25	76.35 ± 5.17
1.5	35.14	39.96	35.51	41.21	40.47	38.46 ± 1.30
2	19.27	17.96	20.22	20.02	22.12	19.92 ± 0.68
3	6.23	12.17	10.12	9.85	11.09	9.90 ± 1.00
4	2.96	9.74	5.45	3.26	6.45	5.57 ± 1.23
5	0.92	3.45	1.20	1.05	2.96	1.92 ± 0.53
6	0.42	1.08	0.50	0.92	0.95	0.77 ± 0.13
8	0.16	0.96	0.20	0.43	0.42	0.43 ± 0.14
10	0.05	0.57	0.08	0.15	0.18	0.21 ± 0.09
12	N.D.	0.20	N.D.	0.06	0.08	0.11 ± 0.04

N.D. = Non-detectable


Conc of ketoprofen
Mean ± S.E.M. (n=5)
URINE DRUG CONCENTRATION (µg.ml-1)
200
100
50
20
10
5
2
1
0
1
2
3
4
5
6
7
8
9
10
11
TIME (HOUR)

of 106.15 ± 7.40 μg/ml was achieved at 0.75 h. The drug was detectable upto 10 h in all the animals with a mean of 0.21 ± 0.09 μg/ml. The drug was detectable only in three out of five animals at 12 h with a mean of 0.11 ± 0.04 μg/ml.

(3) ***Kinetic parameters :-***

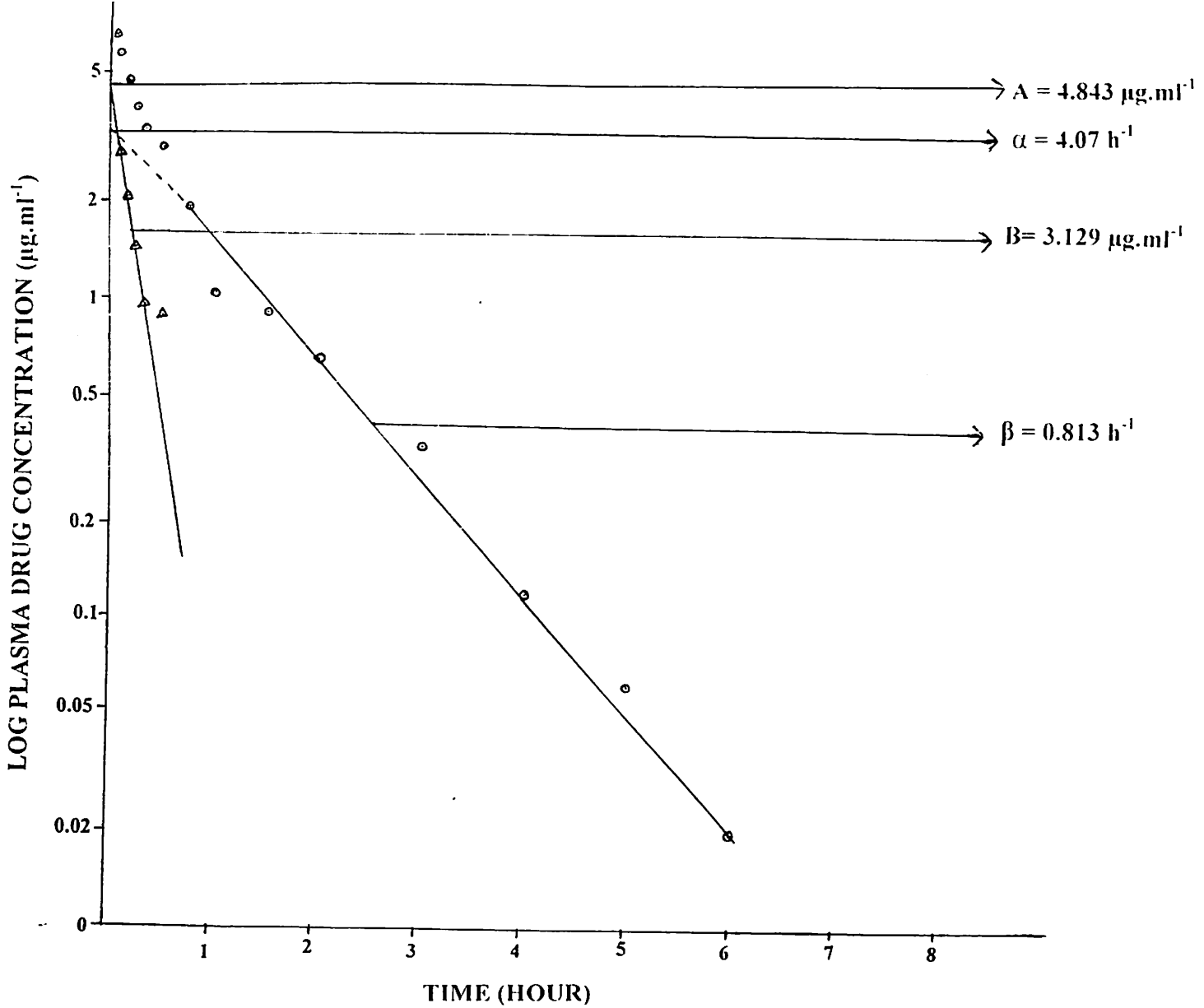
Plasma drug concentration versus time profile has confirmed a two compartment open model for ketoprofen as depicted in Fig-3. Table-3 shows the values of different kinetic parameters calculated by the above noted compartment model.

The mean extrapolated zero time concentration during distribution phase (A), elimination phase (B) and theoretical zero-time concentration (C_p^0) were noted to be 5.65 ± 0.89, 3.56 ± 0.35 and 9.23 ± 1.02 μg/ml, respectively. The distribution ate constant (α) ranged from 3.05 ± 6.63 h^{-1} with a mean of 4.98 ± 0.66 h^{-1} while its elimination rate constant (β)ranged from 0.808 to 0.928 h^{-1} with a mean of 0.86 ± 0.02 h^{-1}. The mean distribution half-life ($t_{1/2}$ α) and elimination half-life ($t_{1/2}$ β) were noted to be 0.15 ± 0.02 and 0.81 ± 0.02 h, respectively. The mean area under curve (AUC) of 5.31 ± 0.22 mg/L.h, area under first moment curve (AUMC) of 5.06 ± 0.21 mg/L.h^2 and mean residential time (MRT) of 0.95 ± 0.02 h were noted in the present study. The average rate of transfer of drug from central

Table – 3

Kinetic parameters of ketoprofen in healthy goats after single intravenous administration at the dose rate of 3 mg.kg^{-1} (calculated by 2-compartment open model)

Parameter (Unit)	Animal Number					Mean ± S.E.M.
	1	2	3	4	5	
A (µg/ml)	3.00	4.843	8.124	6.99	5.27	5.65 ± 0.89
B (µg/ml)	4.10	3.129	3.99	4.29	2.43	3.56 ± 0.35
C_p^o (µg/ml)	7.10	7.972	12.11	11.28	7.70	9.23 ± 1.02
α (h^{-1})	4.93	4.07	6.20	6.63	3.05	4.98 ± 0.66
$t_{1/2}\alpha$ (h)	0.14	0.17	0.11	0.105	0.23	0.15 ± 0.02
β (h^{-1})	0.898	0.813	0.86	0.928	0.808	0.86 ± 0.02
$t_{1/2}\beta$ (h)	0.77	0.85	0.81	0.75	0.86	0.81 ± 0.02
AUC (mg/l.h)	5.168	5.03	5.95	5.67	4.73	5.31 ± 0.22
AUMC (mg/l.h^2)	5.209	5.032	5.60	5.15	4.31	5.06 ± 0.21
MRT (h)	1.007	1.00	0.94	0.91	0.91	0.95 ± 0.02
K_{12} (h^{-1})	1.233	1.213	2.40	2.48	0.72	1.61 ± 0.35
K_{21} (h^{-1})	3.225	2.09	2.62	3.10	1.52	2.51 ± 0.32
Kel (h^{-1})	1.37	1.58	2.04	1.98	1.62	1.72 ± 0.13
Fc	0.66	0.51	0.42	0.47	0.50	0.51 ± 0.04
T ≈ P	0.53	0.95	1.36	1.14	1.01	1.00 ± 0.14
Vdc ($L.kg^{-1}$)	0.42	0.38	0.25	0.27	0.39	0.34 ± 0.03
Vd_B ($L.kg^{-1}$)	0.73	0.96	0.75	0.70	1.23	0.87 ± 0.10
Vd_{area} ($L.kg^{-1}$)	0.65	0.733	0.59	0.57	0.79	0.67 ± 0.04
Vdss ($L.kg^{-1}$)	0.58	0.60	0.48	0.49	0.57	0.54 ± 0.02
Cl_B ($ml.kg^{-1}min^{-1}$)	9.75	9.95	8.47	8.83	10.66	9.53 ± 0.39


A = 4.843 µg.ml-1
α = 4.07 h-1
B= 3.129 µg.ml-1
β = 0.813 h-1
LOG PLASMA DRUG CONCENTRATION (µg.ml-1)
5
2
1
0.5
0.2
0.1
0.05
0.02
0
1
2
3
4
5
6
7
8
TIME (HOUR)

to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central (Kel) compartment were calculated to be 1.61 ± 0.35 h^{-1}, 2.51 ± 0.32 h^{-1}, 1.72 ± 0.13 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 0.51 ± 0.04 and 1.00 ± 0.14. Various values of volume of distribution obtained by different methods are shown in Table 3. A mean Vd_{area} of 0.67 ± 0.04 L/kg was noted. The total body clearance (Cl_B) value ranged from 8.47 to 10.66 with a mean of 9.53 ± 0.39 ml/kg/min.

IMMUNOLOGICAL STUDY AFTER MULTIPLE INTRAMUSCULAR ADMINISTRATION :

(A) ***Humoral immune response :-***

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

Table-4 shows humoral immune response of ketoprofen against SRBC antigen (mean ± S.E) to HA antibody titre (log 2 value) in goats. The HA antibody titre were recorded in ketoprofen treated group (1.467513 ± 0.172197) produced significantly higher titre as compared to antigen treated group (0.990882 ± 0.122196). The antibody forming responses against SRBC were gradually

Table – 4

HA antibody titre (log 2 value) of control and ketoprofen against SRBC antigen in goats

Days	Saline control	Antigen control	Ketoprofen +Ag	Mean ± S.E.M.
1	0.30103 ± 0	0.30103 ± 0	0.30103 ± 0	0.30103^{a} ± 0
7	0.30103 ± 0	0.30103 ± 0	0.30103 ± 0	0.30103^{a} ± 0
10	0.30103 ± 0	0.80273 ± 0.100343	1.50514 ± 0.173799	0.86963^{b} ± 0.183961
14	0.30103 ± 0	1.50514 ± 0.173799	2.1072 ± 0.173799	1.30445^{c} ± 0.27480
21	0.30103 ± 0	2.00685 ± 0.265483	2.60891 ± 0.100343	1.63893^{d} ± 0.355159
28	0.30103 ± 0	1.30445 ± 0.100343	2.20754 ± 0.100343	1.27100^{c} ± 0.278339
35	0.30103 ± 0	1.00342 ± 0.100343	1.70582 ± 0.100343	1.00342^{b} ± 0.206861
42	0.30103 ± 0	0.70239 ± 0.100343	1.00342 ± 0.100343	0.66894^{a} ± 0.109664
	0.30103^{A} ± 0	0.990882^{B} ±0.122196	1.467513^{C} ± 0.172197	

* Each value is mean ± S.E. of 3 samples tested.

** Means with common superscript did not differ significantly ($P<0.05$).

significantly increased from 10th day (0.86963 ± 0.183961) and the highest HA titre was observed on 21st day (1.63893 ± 0.355159) and then significantly declining trends noted till 42 days (0.66894 ± 0.109664). The study reveals immunopotentiating effect on humoral immunity by this drug.

(B) ***Cell mediated immune response :-***

Effect of ketoprofen on cell mediated immune response in goats after multiple intramuscular administration were assessed through delayed type hypersensitivity (DTH) reaction *in-vivo* using three different mitogens (DNCB, PHA-P and PPD/tuberculin) and through lymphocyte transformation test (LTT) *in-vitro*.

Table-5 to 9 shows delayed type hypersensitivity reaction of ketoprofen against SRBC using DNCB mitogen (mean ± S.E) in increase in skin thickness (mm) in goats after multiple i.m. administration. Table-5 indicates that there is significant decrease in skin thickness in ketoprofen treated group (0.9195 ± 0.026371 mm) as compared to both the control group (1.0319 ± 0.034445 mm-saline and 1.0795 ± 0.039397 mm-antigen group) in pre-challenging period i.e., fifth day of experiment. DTH response to DNCB was gradually increased significantly from 4 h (0.9955 ± 0.031715 mm) and thereafter with the highest DTH reaction was found at 12 h

Table – 5

DTH reaction of ketoprofen due to DNCB in goats in pre-challenging period

Hours	Saline control	Antigen control	Ketoprofen +Ag	Mean ± S.E.M. (mm)
4	1.02 ± 0.01763	1.07 ± 0.03605	0.89 ± 0.040414	0.9955^{a} ± 0.031715
8	1.19 ± 0.03214	1.27 ± 0.02603	1.01 ± 0.03929	1.1566^{b} ± 0.04096
12	1.26 ± 0.01732	1.34 ± 0.03179	1.07 ± 0.03179	1.2255^{c} ± 0.042266
24	1.08 ± 0.02081	1.09 ± 0.04255	1.01 ± 0.037118	1.0633^{d} ± 0.021015
48	0.99 ± 0.012018	1.04 ± 0.04163	0.86 ± 0.04509	0.9644^{a} ± 0.032451
72	0.87 ± 0.03282	0.926 ± 0.04667	0.826 ± 0.01452	0.8733^{e} ± 0.022360
96	0.806 ± 0.01763	0.816 ± 0.02185	0.76 ± 0.01201	0.7933^{f} ± 0.012801
	1.0319^{A} ± 0.034445	1.0795^{B} ± 0.039397	0.9195^{C} ± 0.026371	

* Each value is mean ± S.E. of 3 samples tested.

** Means with common superscript did not differ significantly ($P<0.05$).

Table – 6

DTH reaction of ketoprofen due to DNCB in goats in post-challenging period

Hours	Saline control	Antigen control	Ketoprofen +Ag	Mean ± S.E.M. (mm)
4	1.31 ± 0.01452	1.37 ± 0.02403	0.88 ± 0.04582	1.18667^{a} ± 0.078598
8	1.4 ± 0.011547	1.48 ± 0.024037	0.95 ± 0.04255	1.27667^{a} ± 0.084623
12	1.59 ± 0.02333	1.63 ± 0.01732	1 ± 0.0233	1.40889^{b} ± 0.102095
24	1.376 ± 0.11609	1.4 ± 0.09539	0.89 ± 0.03929	1.22333^{a} ± 0.093956
48	1.13 ± 0.11921	1.15 ± 0.101707	0.87 ± 0.03844	1.05333^{c} ± 0.064828
72	1 ± 0.07211	0.973 ± 0.02962	0.826 ± 0.0088191	0.93^{d} ± 0.0364767
96	0.663 ± 0.0448	0.623 ± 0.02962	0.54 ± 0.01855	0.60777^{e} ± 0.024876
	1.21142^{A} ± 0.0677118	1.23285^{A} ± 0.073524	0.85^{B} ± 0.032870	

* Each value is mean ± S.E. of 3 samples tested.

** Means with common superscript did not differ significantly ($P<0.05$).

Table – 7

DTH reaction due to DNCB in normal saline treated group in pre and post-challenging period

Hour	Pre-challenge	Post-challenge	Mean ± S.E.M. (mm)
4	1.02 ± 0.01763	1.31 ± 0.01452	1.170^{ab} ± 0.064910
8	1.19 ± 0.03214	1.4 ± 0.011547	1.295^{c} ± 0.049379
12	1.26 ± 0.017320	1.59 ± 0.02333	1.4266^{d} ± 0.075660
24	1.08 ± 0.02081	1.376 ± 0.11609	1.22833^{ac} ± 0.084751
48	0.99 ± 0.012018	1.13 ± 0.11921	1.06333^{b} ± 0.062057
72	0.87 ± 0.03282	1 ± 0.07211	0.9333^{e} ± 0.046308
96	0.806 ± 0.01763	0.663 ± 0.0448	0.7350^{f} ± 0.038622
	1.031904^{A} ± 0.034445	1.211428^{B} ± 0.067711	

* Each value is mean ± S.E. of 3 samples tested.

** Means with common superscript did not differ significantly ($P<0.05$).

Table – 8

DTH reaction due to DNCB in SRBC treated group in pre and post-challenging period

Hour	Pre-challenge	Post-challenge	Mean ± S.E.M. (mm)
4	1.07 ± 0.03605	1.37 ± 0.02403	1.21833^{a} ± 0.069109
8	1.27 ± 0.02603	1.48 ± 0.024037	1.375^{b} ± 0.050973
12	1.34 ± 0.03179	1.63 ± 0.01732	1.48667^{c} ± 0.066114
24	1.09 ± 0.042557	1.4 ± 0.09539	1.24667^{a} ± 0.082972
48	1.04 ± 0.04163	1.15 ± 0.101707	1.09667^{d} ± 0.055297
72	0.926 ± 0.04666	0.973 ± 0.02962	0.950^{e} ± 0.026832
96	0.816 ± 0.02185	0.623 ± 0.02962	0.72^{f} ± 0.046260
	1.07952^{A} ± 0.039397	1.232857^{B} ± 0.073524	

* Each value is mean ± S.E. of 3 samples tested.

** Means with common superscript did not differ significantly ($P<0.05$).

Table – 9

DTH reaction due to DNCB in ketoprofen and SRBC treated group in pre and post-challenging period

Hour	Pre-challenge	Post-challenge	Mean ± S.E.M. (mm)
4	0.89 ± 0.040414	0.88 ± 0.04582	0.8850^{a} ± 0.027416
8	1.01 ± 0.03929	0.95 ± 0.04255	0.980^{bc} ± 0.02988
12	1.07 ± 0.03179	1 ± 0.0233	1.0383^{b} ± 0.023582
24	1.01 ± 0.037118	0.89 ± 0.03929	0.9550^{c} ± 0.036674
48	0.86 ± 0.04509	0.87 ± 0.03844	0.8667^{a} ± 0.02667
72	0.826 ± 0.01452	0.816 ± 0.008819	0.8216^{a} ± 0.007923
96	0.76 ± 0.01201	0.54 ± 0.01855	0.6467^{d} ± 0.050177
	0.9195^{A} ± 0.026371	0.85^{B} ± 0.032870	

* Each value is mean ± S.E. of 3 samples tested.

** Means with common superscript did not differ significantly ($P<0.05$).

(1.2255 ± 0.042266 mm) post SRBC inoculation and it significantly declined till 96 h (0.7933 ± 0.012801 mm) during pre-challenging period. Table-6 indicates that there is significant decrease in skin thickness in ketoprofen treated group (0.85 ± 0.032870 mm) as compared to both the control group (1.21142 ± 0.0677118 mm-saline and 1.23285 ± 0.073524 mm-antigen group) in post-challenging period i.e., 15th day of experiment. Table - 7, 8 and 9 shows significant increase in skin thickness in post-challenging period in both the saline control and antigen control group as compared to pre-challenging period. But there is decrease in skin thickness in post-challenging period in ketoprofen treated group as compared to pre-challenging period. The study indicates immunosuppressive effect on cell mediated immunity by ketoprofen as evidenced by lowered DTH reaction.

Table-10 to 14 shows DTH response of ketoprofen against SRBC using PHA-P mitogen (mean ± S.E.) in increase in skin thickness (mm) in goats after multiple i.m. administration. Table-10 shows that there is significant decrease in skin thickness in ketoprofen treated group (0.97 ± 0.47288 mm) as compared to antigen control (1.031904 ± 0.041348 mm) and significant increase in skin thickness as compared to saline control (0.848095 ± 0.033256 mm) in pre-challenging period. Significant increase in DTH response to

(1.2255 ± 0.042266 mm) post SRBC inoculation and it significantly declined till 96 h (0.7933 ± 0.012801 mm) during pre-challenging period. Table-6 indicates that there is significant decrease in skin thickness in ketoprofen treated group (0.85 ± 0.032870 mm) as compared to both the control group (1.21142 ± 0.0677118 mm-saline and 1.23285 ± 0.073524 mm-antigen group) in post-challenging period i.e., 15th day of experiment. Table - 7, 8 and 9 shows significant increase in skin thickness in post-challenging period in both the saline control and antigen control group as compared to pre-challenging period. But there is decrease in skin thickness in post-challenging period in ketoprofen treated group as compared to pre-challenging period. The study indicates immunosuppressive effect on cell mediated immunity by ketoprofen as evidenced by lowered DTH reaction.

Table-10 to 14 shows DTH response of ketoprofen against SRBC using PHA-P mitogen (mean ± S.E.) in increase in skin thickness (mm) in goats after multiple i.m. administration. Table-10 shows that there is significant decrease in skin thickness in ketoprofen treated group (0.97 ± 0.47288 mm) as compared to antigen control (1.031904 ± 0.041348 mm) and significant increase in skin thickness as compared to saline control (0.848095 ± 0.033256 mm) in pre-challenging period. Significant increase in DTH response to

Table –10

DTH reaction of ketoprofen due to PHA-P in goats in pre-challenging period

Hour	Saline control	Antigen control	Ketoprofen +Ag	Mean ± S.E.M. (mm)
4	0.59 ± 0.011547	1.04 ± 0.02962	0.99 ± 0.04932	0.87444^{a} ± 0.073505
8	0.78 ± 0.011547	1.21 ± 0.01732	1.09 ± 0.05897	1.02778^{bc} ±0.066682
12	1.04 ± 0.02905	1.31 ± 0.02027	1.21 ± 0.05859	1.18889^{d} ± 0.044015
24	0.98 ± 0.03282	1.08 ± 0.02516	1.116 ± 0.06887	1.06^{b} ± 0.030550
48	0.936 ± 0.04176	0.986 ± 0.00819	0.986 ± 0.07264	0.97^{e} ± 0.025712
72	0.856 ± 0.01201	0.836 ± 0.01452	0.79 ± 0.05567	0.82777^{a} ± 0.019633
96	0.746 ± 0.02027	0.753 ± 0.02905	0.60 ± 0.056075	0.70111^{e} ± 0.031066
	0.848095^{A} ± 0.033256	1.031904^{B} ± 0.041348	0.97^{C} ± 0.047288	

* Each value is mean ± S.E. of 3 samples tested.

** Means with common superscript did not differ significantly ($P<0.05$).

Table -11

DTH reaction of ketoprofen due to PHA-P in goats in post-challenging period

Hour	Saline control	Antigen control	Ketoprofen + Ag	Mean ± S.E.M. (mm)
4	1.09 ± 0.0233	1.42 ± 0.011547	1.126 ± 0.014529	1.21333^{a} ± 0.52599
8	1.24 ± 0.02603	1.44 ± 0.01201	1.28 ± 0.02645	1.32444^{b} ± 0.032918
12	1.33 ± 0.01763	1.47 ±0.008191	1.306 ± 0.01763	1.36889^{b} ± 0.025897
24	1.16 ± 0.0850	1.21 ± 0.062449	1.006 ± 0.09562	1.12556^{c} ± 0.051237
48	1.05 ± 0.05364	1.07 ±0.078810	0.84 ± 0.03382	0.99^{d} ± 0.046963
72	0.88 ± 0.01527	0.906 ± 0.03711	0.79 ± 0.025166	0.85888^{e} ± 0.022326
96	0.56 ± 0.02603	0.603 ± 0.02962	0.55 ± 0.032145	0.57222^{f} ± 0.016731
	1.047142^{A} ± 0.055015	1.160952^{B} ± 0.068312	0.986190^{C} ± 0.058839	

* Each value is mean ± S.E. of 3 samples tested.

** Means with different superscript differ significantly ($P<0.05$).

Table – 12

DTH reaction due to PHA-P in normal saline treated group in pre and post-challenging period

Hour	Pre-challenge	Post-challenge	Mean ± S.E.M. (mm)
4	0.59 ± 0.011547	1.09 ± 0.0233	0.84166^{a} ± 0.113149
8	0.78 ± 0.011547	1.24 ± 0.02603	1.01333^{bc} ± 0.105124
12	1.04 ± 0.02905	1.33 ± 0.01763	1.18333^{d} ± 0.066604
24	0.98 ± 0.03282	1.16 ± 0.0850	1.07166^{b} ± 0.056769
48	0.936 ± 0.04176	1.05 ± 0.05364	0.9950^{c} ± 0.040062
72	0.856 ± 0.01201	0.88 ± 0.01527	0.86833^{a} ± 0.010137
96	0.746 ± 0.02027	0.56 ± 0.02603	0.6550^{e} ± 0.043569
	0.84809^{A} ± 0.033256	1.04714^{B} ± 0.055015	

* Each value is mean ± S.E. of 3 samples tested.

** Means with different superscript differ significantly ($P<0.05$).

Table – 13

DTH reaction due to PHA-P in SRBC treated group in pre and post-challenging period

Hour	Pre-challenge	Post-challenge	Mean ± S.E.M. (mm)
4	1.04 ± 0.02962	1.42 ± 0.011547	1.23167^{a} ± 0.085417
8	1.21 ± 0.01732	1.44 ± 0.01201	1.32833^{b} ± 0.053753
12	1.31 ± 0.02027	1.47 ± 0.008191	1.39^{b} ± 0.035683
24	1.08 ± 0.02516	1.21 ± 0.062449	1.145^{c} ± 0.041852
48	0.986 ± 0.00819	1.07 ± 0.078810	1.03^{d} ± 0.040414
72	0.836 ± 0.01452	0.906 ± 0.03711	0.87167^{e} ± 0.023722
96	0.753 ± 0.02905	0.603 ± 0.02962	0.67833^{f} ± 0.038333
	1.03190^{A} ± 0.041217	1.16095^{B} ± 0.068312	

* Each value is mean ± S.E. of 3 samples tested.

** Means with different superscript differ significantly ($P<0.05$).

Table – 14

DTH reaction due to PHA-P in ketoprofen and SRBC treated group in pre and post-challenging period

Hour	Pre-challenge	Post-challenge	Mean ± S.E.M. (mm)
4	0.99 ± 0.04932	1.126 ± 0.014529	1.05833^{a} ± 0.038246
8	1.09 ± 0.05897	1.28 ± 0.02645	1.18667^{b} ± 0.50771
12	1.21 ± 0.05859	1.306 ± 0.01763	1.25833^{b} ± 0.034872
24	1.116 ± 0.06887	1.006 ± 0.09562	1.06167^{a} ± 0.058161
48	0.986 ± 0.07264	0.84 ± 0.03382	0.915^{c} ± 0.048079
72	0.79 ± 0.05567	0.79 ± 0.025166	0.79^{d} ± 0.027325
96	0.60 ± 0.056075	0.55 ± 0.032145	0.5767^{e} ± 0.031269
	0.97^{NS} ± 0.047288	0.986^{NS} ± 0.058839	

* Each value is mean ± S.E. of 3 samples tested.

** NS – Non-significant ($P<0.05$).

*** Mean with common superscript did not differ significantly ($P<0.05$).

PHA-P was noted in initial hours and mean peak skin thickness was noted at 12 h (1.18889 ± 0.044015 mm followed by significantly declining trend was observed from 24 h upto 96 h (0.70111 ± 0.031066 mm) during pre-challenging period. Table-11 indicates that there is significant decrease in skin thickness in ketoprofen treated group (0.986190 ± 0.058839 mm) as compared to both the control group (1.047142 ± 0.055015 mm saline and 1.160952 ± 0.068312 mm-antigen group) in post-challenging period. Table 12 and 13 indicated that there is significant increase in skin thickness in post-challenging period as compared to pre-challenging period in saline control and antigen control. Table-14 indicates non-significant increase in skin thickness in post-challenging period as compared to pre-challenging period in ketoprofen treated group. The study indicates immunosuppressive action on cell mediated immunity as evidenced by lowered DTH reaction.

Table-15 to 19 show DTH response of ketoprofen against SRBC using PPD/tuberculin. Mean ± S.E. in increase in skin thickness (mm) in goats after multiple i.m. administration was noted. Table-15 shows non-significant observations of ketoprofen in comparison to both negative and positive control. Table-16 shows significant increase in skin thickness in ketoprofen treated group (0.761428 ± 0.043645 mm) as compared to saline control

Table -15

DTH reaction of ketoprofen due to PPD in goats in pre-challenging period

Hour	Saline control	Antigen control	Ketoprofen +Ag	Mean ± S.E.M. (mm)
4	0.73 ± 0.008819	0.67 ± 0.020816	0.49 ± 0.09837	0.6333^{ab} ± 0.03
8	0.776 ± 0.012018	0.77 ± 0.011547	0.67 ± 0.09643	0.7388^{ac} ± 0.021669
12	0.816 ± 0.01452	1.067 ± 0.02403	0.94 ± 0.16072	0.94111^{d} ± 0.059335
24	0.706 ± 0.014529	0.856 ± 0.02962	0.88 ± 0.17473	0.81444^{cd} ± 0.058073
48	0.61 ± 0.02309	0.686 ± 0.03844	0.67 ± 0.15409	0.65444^{ab} ± 0.047729
72	0.52 ± 0.011547	0.476 ± 0.018559	0.583 ± 0.13568	0.52667^{bc} ± 0.042589
96	0.466 ± 0.018559	0.43 ± 0.03282	0.43 ± 0.06641	0.44444^{c} ± 0.022737
	0.661428^{NS} ± 0.027836	0.708571^{NS} ± 0.045925	0.6671428^{NS} ± 0.056967	

* Each value is mean ± S.E. of 3 samples tested.

** NS – Non-significant ($P<0.05$).

*** Mean with common superscript did not differ significantly ($P<0.05$).

Table –16

DTH reaction of ketoprofen due to PPD in goats in post-challenging period

Hour	Saline control	Antigen control	Ketoprofen +Ag	Mean ± S.E.M. (mm)
4	0.536 ± 0.02728	0.766 ± 0.0088197	0.71 ± 0.008819	0.67333^{a} ± 0.035978
8	0.62 ± 0.012018	1.02 ± 0.017638	1.02 ± 0.04702	0.89222^{b} ± 0.068043
12	0.696 ± 0.012018	1.2 ± 0.02309	1.04 ± 0.05174	0.98111^{c} ± 0.076329
24	0.67 ± 0.014529	1.106 ± 0.066916	0.776 ± 0.018559	0.85222^{b} ± 0.068470
48	0.623 ± 0.014529	0.973 ± 0.05044	0.64 ± 0.04055	0.74666^{d} ± 0.059884
72	0.53 ± 0.011547	0.85 ± 0.086216	0.59 ± 0.0233	0.65777^{a} ± 0.055396
96	0.52 ± 0.011547	0.72 ± 0.058118	0.526 ± 0.01452	0.59^{e} ± 0.037712
	0.600952^{A} ± 0.015749	0.949047^{B} ± 0.061299	0.761428^{C} ± 0.043645	

* Each value is mean ± S.E. of 3 samples tested.

** Mean with different superscript differ significantly ($P<0.05$).

Table – 17

DTH reaction due to PPD in normal saline treated group in pre and post-challenging period

Hour	Pre-challenge	Post-challenge	Mean ± S.E.M. (mm)
4	0.73 ± 0.008819	0.536 ± 0.02728	0.635^{a} ± 0.045807
8	0.776 ± 0.012018	0.62 ± 0.012018	0.70167^{b} ± 0.034391
12	0.816 ± 0.01452	0.696 ± 0.012018	0.75667^{c} ± 0.028126
24	0.706 ± 0.014529	0.67 ± 0.014529	0.69^{b} ± 0.011832
48	0.61 ± 0.02309	0.623 ± 0.014529	0.6167^{a} ± 0.012560
72	0.52 ± 0.011547	0.53 ± 0.011547	0.525^{d} ± 0.00637
96	0.466 ± 0.018559	0.52 ± 0.011547	0.4933^{d} ± 0.015420
	0.66142^{A} ± 0.027836	0.60095^{B} ± 0.015479	

* Each value is mean ± S.E. of 3 samples tested.

** Mean with common superscript did not differ significantly ($P<0.05$).

Table – 18

DTH reaction due to PPD in SRBC treated group in pre and post-challenging period

Hour	Pre-challenge	Post-challenge	Mean ± S.E.M. (mm)
4	0.67 ± 0.020816	0.766 ± 0.0088197	0.71333^{a} ± 0.023863
8	0.77 ± 0.011547	1.02 ± 0.017638	0.89667^{b} ± 0.057426
12	1.067 ± 0.02403	1.2 ± 0.02309	1.1333^{c} ± 0.0333
24	0.856 ± 0.02962	1.106 ± 0.066916	0.98167^{d} ± 0.064777
48	0.686 ± 0.03844	0.973 ± 0.05044	0.83^{b} ± 0.070095
72	0.476 ± 0.018559	0.85 ± 0.086216	0.66333^{a} ± 0.092327
96	0.43 ± 0.03282	0.72 ± 0.058118	0.57833^{e} ± 0.071387
	0.70857^{A} ± 0.045958	0.94904^{B} ± 0.040130	

* Each value is mean ± S.E. of 3 samples tested.

* Mean with different superscript differ significantly ($P<0.05$).

Table – 19

DTH reaction due to PPD in ketoprofen and SRBC inoculated group in pre and post-challenging period

Hour	Pre-challenge	Post-challenge	Mean ± S.E.M. (mm)
4	0.49 ± 0.9837	0.71 ± 0.008819	0.60667^{a} ± 0.066114
8	0.67 ± 0.09643	1.02 ± 0.04702	0.84834^{bc} ± 0.093074
12	0.94 ± 0.16072	1.04 ± 0.05174	0.99333^{c} ± 0.079190
24	0.88 ± 0.17473	0.776 ± 0.018559	0.82834^{bc} ± 0.081911
48	0.67 ± 0.15409	0.64 ± 0.04055	0.655^{ab} ± 0.71449
72	0.583 ± 0.13568	0.59 ± 0.0233	0.58833^{a} ± 0.061612
96	0.43 ± 0.06641	0.526 ± 0.01452	0.48^{a} ± 0.036878
	0.66714^{NS} ± 0.056967	0.76142^{NS} ± 0.043645	

* Each value is mean ± S.E. of 3 samples tested.
* NS - Non-significant ($P<0.05$).
* Mean with common superscript did not differ significantly ($P<0.05$).

(0.600952 ± 0.015749 mm) and significant decrease in skin thickness as compared to antigen control (0.949047 ± 0.061299 mm) in post-challenging period. Significant gradual increase in DTH response to PPD was noted at initial hours and mean peak skin thickness was noted at 12 h during pre-challenge (0.94111 ± 0.059335 mm) and post-challenge (0.98111 ± 0.076329 mm) period of experiment. Later on, significantly declining trend was noted. Table-17 indicates significant decrease in skin thickness in post-challenge as compared to pre-challenge in saline control. Table-18 shows significant increase in skin thickness in post-challenge as compared to pre-challenge in antigen control. Table-19 indicates no significant observations in ketoprofen treated group in both pre and post-challenge period.

The study indicates immunosuppressive action of ketoprofen on cell mediated immunity as assessed by lowered DTH reaction.

The result of lymphocyte transformation test (LTT), expressed as stimulation index (SI) values, for peripheral blood lymphocytes are depicted in Table-20. The SI values ranged between 0.942 and 1.174. The perusal of the table revealed a significant ($P<0.05$) rise in SI index on 14 days post SRBC inoculation as compared to ketoprofen and saline control treated group thereafter a

Table – 20

Comparative mean stimulation index (SI) values of PBLs of different groups to Con-A at various intervals post SRBC and ketoprofen inoculation

Group	Treatment	SRBC/Saline inoculation (days)	Drug inoculation post SRBC administration (days)	Mean stimulation index*				
				Days post SRBC/Saline inoculation				
				7	10	14	21	28
I	Normal saline solution	1 and 10	--	$0.942^{a} \pm 0.019$	$1.058^{a} \pm 0.024$	$1.035^{a} \pm 0.0059$	$1.008^{a} \pm 0.007$	$0.998^{a} \pm 0.003$
II	SRBC	1 and 10	--	$0.993^{a} \pm 0.026$	$1.150^{a} \pm 0.024$	$1.174^{b} \pm 0.022$	$1.121^{a} \pm 0.019$	$1.045^{a} \pm 0.018$
III	SRBC + Ketoprofen	1 and 10	1 to 5 and 10 to 15	$0.969^{a} \pm 0.032$	$1.087^{a} \pm 0.029$	$1.125^{a} \pm 0.008$	$1.033^{a} \pm 0.006$	$1.031^{a} \pm 0.008$

* Each value is mean of 3 samples tested.

** Means with common superscript in a column did not differ significantly ($P<0.05$).

decline in SI values were registered. For all periods post SRBC inoculation in general a higher SI values were recorded for SRBC treated group (Gr. II) followed by ketoprofen treated group (Gr. III) and NSS treated group (Gr. I) for corresponding period post SRBC inoculation. In addition a gradual rise in SI value was recorded till 14 days post SRBC inoculation and further the SI values declined till 28 days post SRBC inoculation.

Chapter - 5

Discussion

DISCUSSION

Ketoprofen is a potent non-steroidal anti-inflammatory drug (NSAID) having analgesic, antipyretic and anti-inflammatory properties (Adams, 2001). It is being preferred clinically over older groups of NSAIDs against various inflammatory conditions due to its better tolerance and accompanied with least side effects.

It is well established that many drugs either stimulate or suppress the immune system and thereby alter the course of the disease. Therefore it is highly desirable to carry out immunological studies of NSAIDs. It has been reported that ketoprofen suppresses the immunity by lowering the production of cytokines such as interferon gamma and also acute phase protein production (Ting *et al.*, 2003). However, the literature is meager and limited in respect of ketoprofen towards the humoral and cell mediated immune response.

Pharmacokinetic study of ketoprofen has been carried out in cattle, horse, camel, sheep and goat but little work has been done in goat particularly on the immunological effect of ketoprofen. With the above mentioned aims and objectives the present study has been undertaken in goats.

PHARMACOKINETIC STUDY :

(a) Distribution in plasma :-

Ketoprofen was present with a mean peak plasma concentration of 9.87 ± 0.48 μg/ml at 0.042 h on its intravenous administration @ 3mg/kg (Table-1). The drug declined with time and was traced upto 5 h in all the animals with the mean of 0.04 ± 0.005 μg/ml. The drug was detected only in two out of five animals at 6h with the mean of 0.015 ± 0.005 μg/ml and none at 8 h.

(b) Urinary excretion :-

In case of urine, the drug was present only in three out of 5 animals with a mean concentration of 0.29 ± 0.03 μg/ml at 0.042h while it was detectable in all animals at 0.083 h (7.01 ± 1.21 μg/ml). Mean peak urine drug concentration of 106.15 ± 7.40 μg/ml was obtained at 0.75 h. Thereafter, the drug was found to decline with time and it was detectable in all animals upto 10 h post administration. At 12 h, the drug was detectable in urine of three out of five animals with a mean of 0.11 ± 0.04 μg/ml (Table-2).

(c) Kinetic parameters :-

In the present study, the mean value of 5.65 ± 0.89 μg/ml for zero time concentration during distribution phase (A) while low mean value of 3.56 ± 0.35 μg/ml for zero time concentration during

elimination phase (B) and mean value of 9.23 ± 1.02 μg/ml for theoretical zero time concentration (C_p^o =A+B) were obtained after its single intravenous administration (3mg/kg). A moderately high value for distribution rate constant (α) of 4.98 ± 0.66 h^{-1} and low distribution half-life ($t_{1/2}$ α) of 0.15 ± 0.02 h denote that the drug is distributed to peripheral tissues at faster rate. The elimination half-life ($t_{1/2}$ β) of 0.81 ± 0.02 h obtained in the present study denotes that the drug is removed at a faster rate. This has lead to a lower mean residential time (MRT) of 0.95 ± 0.02 h in goat. A comparatively lower $t_{1/2}$ β of 0.32 h in goat (Musser *et al.*, 1998), 0.42 h in calf (Landoni *et al.*, 1995) and 0.49 h in cattle were observed. On the other hand, a-higher $t_{1/2}$ β of 1.63 h in mare (Sam *et al.*, 1995) and 2.11h in camel (Al Katheeri *et al.*, 2000) were noted. As compared to other NSAIDs, the elimination half-life ($t_{1/2}$ β) of ketoprofen was found to be lower in ruminants. An elimination half-life ($t_{1/2}$ β) of diclofenac was noted to be 2.4 h in pig (Oberle *et al.*, 1994) and 4.06 ± 0.59 h in buffalo calf (Nitesh Kumar *et al.*, 2003). Similarly, paracetamol also showed higher $t_{1/2}$ β in many ruminants. For paracetamol, elimination half-life ($t_{1/2}$ β) of 8.69 ± 0.83 h in buffalo calf (Sindhu *et al.*, 1993), 4.84 ± 1.26 h in cross bred calf (Sharma *et al.*, 1995), and 3.56 ± 0.13 h in goat. (Sudha kumari *et al.*, 1998) were noted.

The above facts clearly indicate that ketoprofen is remained in the body of ruminants for a shorter period as compared to older group of NSAIDs. The shorter $t_{1/2}$ β obtained with ketoprofen led to a lower mean residential time (MRT) of 0.95 ± 0.02h in the present study in goat. Quicker elimination of this drug is supported by high total body clearance of 9.53 ± 0.39 ml/kg/min.

A moderately high value of volume of distribution of 0.67 ± 0.04 L/kg and a high value of tissue to plasma concentration ration (T≈P) of 1.00 ± 0.14 indicate that the drug may be distributed to a greater extent in peripheral tissue, which may be beneficial in the treatment of arthritis and other inflammatory conditions in goats.

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 a particular antigen (Kolmer *et al.*, 1952) whereas CMIR can be judged by DTH and LTT. Sheep red blood cell (SRBC) is more often used as an antigen for the evaluation of humoral immune response in different experimental animals by assessing the antibody titre in the sensitized candidate (Kabat *et al.*, 1961).

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The cell mediated immune response (CMIR) was evaluated through delayed type hypersensitivity (DTH) reaction through different mitogens as well as *in-vitro* culture of lymphocyte that are exposed to mitogen.

In the present study, the serum antibody titre to SRBC was evaluated using haemagglutination test for the assessment of humoral immune response (HIR) as per Beard *et al.* (1980). Likewise, CMIR was determined by DTH (Chauhan *et al.*, 1983) reaction *in-vivo* and lymphocyte transformation test (LTT) *in-vitro* (Bounous *et al.*, 1993).

Humoral Immune Response :

In the present investigation, the haemagglutinating antibody titres in ketoprofen treated group were recorded significantly higher titre (1.467513 ± 0.172197) as compared to antigen treated group (0.990882 ± 0.122196), which indicates immunopotentiating effect (Table-4). Moreover, the results of seroconversion also showed a higher HA antibody titres in ketoprofen inoculated group as compared to SRBC without ketoprofen treated group. Therefore, it may be inferred that ketoprofen elicits immunopotentiating effect on humoral immune response. It seems that no literature is available particularly pertaining to the present finding. However, some conflicting reports are available on the effect

of individual administration of NSAIDs on immune response. It has been documented that aspirin and acetaminophen suppressed the serum neutralizing antibodies in human being (Graham *et al.*, 1990). Furthermore, Valera *et al.* (1995) reported that humoral immune response by fluoroquinolone is a complex phenomenon which is affected by various factors like dose of the drug, duration of therapy and time of administration of antigen. In the present study, the seroconversion was observed on 10^{th} days of antigen exposure and the highest HA titre was recorded on 21 day followed by declining trend till 42 days post SRBC inoculation. The above finding corroborates the finding of Jose et al. (1999) who observed similar observation in diclofenac treated rabbits upto 42 days.

Cell Mediated immune response :

DTH reaction is considered as a valid measure for the *in-vivo* assessment of CMIR (Thompson *et al.*, 1975). T-cells, 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-lymphocytes at the site of mitogen inoculation (Reuben *et al.*, 1979).

In the present study the skin in-contact sensitivity due to DNCB, PHA-P and PPD was used as one of the parameter of CMIR.

The inoculated area was reddened, oedematous, swollen and subsequently indurated. The reactions were more prominent in DNCB inoculated group (Table – 5) followed by PHA-P and PPD. The DNCB and PHA-P skin sensitivity test showed significant variation in the mean skin thickness between the ketoprofen treated group and their respective positive controls. In contrast, PPD skin sensitivity did not show any significant variation in the mean skin thickness between ketoprofen treated group and their respective positive or negative control (Table-5 to 19). In the present study, DNCB and PHA-P showed immunosuppressive action on cell mediated immunity as evident by the significant decrease in mean skin thickness in ketoprofen treated group as compared to antigen group. PPD. (tuberculin) also showed immunosuppressive action on cell mediated immune response as statistically non-significant decrease in mean skin thickness was observed in ketoprofen treated group as compared to both the control group (Table – 16).

The lymphocyte transformation test (LTT) synonymously called lymphocyte proliferation assay has been invariably employed as a method of detection of CMIR *in-vitro*. The technique is preferred due to its low cost and accuracy as compared to other sophisticated techniques used for the evaluation of CMIR.

In the present experiment, the LTT was employed as one of the technique for the evaluation of CMIR in different experimental

groups such as SRBC inoculated group (Gr. II), SRBC and Ketoprofen treated group (Gr. III), normal saline inoculated group (Group I) by using the lymphocytes from peripheral blood lymphocytes (PBLs) of the experimental goats.

The perusal of the table-20 showed that the stimulation index (SI) of the lymphocytes stimulated with concanavalin-A were significantly higher in SRBC inoculated group at 14 days post SRBC inoculation in comparison to the SI values of group I and group III for respective period. The values for the rest of the period in all the three treatment group were non-significant. However, the values for SRBC inoculated group without drug (Group II) revealed a numerically higher values as compared to SRBC and ketoprofen treated group. The corresponding values were least for the normal saline treated group (Group I). The results were indicative of that ketoprofen suppressed the T-lymphocyte activity as reflected in terms of SI values. Furthermore, the result showed that the ketoprofen lowered the cell mediated immune response. The perusal of the data obtained for DTH reaction and LTT revealed a similar trend of immunosuppressive effect of ketoprofen on CMIR. Earley *et al.* (2002) also observed the immunological effect of ketoprofen on immune response and showed that concanavalin - A induced gamma interferon production was lower in ketoprofen treated group than in control.

The present finding is in agreement with the results of Spiers *et al.* (1988) who observed that diclofenac suppressed the lymphocyte proliferation, at supratherapeutic doses.

Jose *et al.* (1999) reported that simultaneous administration of pefloxacin and diclofenac did not affect the total leucocyte values, but caused an apparent decrease in lymphocyte count.

In contrast to this Brown and Collins (1978) observed that sodium salicylate, aspirin, phenylbutazone and indomethacin did not affect the cell mediated immunity.

Based on the pharmacokinetic and immunological studies of ketoprofen in the present investigation, it is concluded that ketoprofen can be safely and effectively given in pyrexia, pain and other inflammatory conditions.

Chapter - 6

Summary

SUMMARY

A detailed pharmacokinetic and immunological studies of ketoprofen was carried out in goats weighing between 18-25 kg. Concentrations of the drug in plasma and urine as well as various kinetic parameters were calculated by using two compartment models following intravenous administration. Effects of ketoprofen on humoral and cell mediated immunity were estimated in goats following multiple intramuscular administration. The salient findings of the present study are as follows :-

[I] Pharmacokinetic study :-

(i) Following single intravenous administration, the mean peak plasma concentration of 9.87 ± 0.48 μg/ml and mean peak urine concentration of 106.15 ± 7.40 μg/ml were found at 0.042 and 0.75 h, respectively.

(ii) The drug was detectable in all animals upto 5 h in plasma and upto 10 h in urine.

(iii) 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 5.65 ± 0.89, 3.56 ± 0.35 and 9.23 ± 1.02 μg/ml, respectively.

(iv) A high value of distribution rate constant (α) of 4.98 ± 0.66 h^{-1} and low value of distribution half-life ($t_{1/2}\,\alpha$) of 0.15 ± 0.02 h denote that the drug is expected to be distributed to peripheral tissues at a faster rate.

(v) The elimination half-life ($t_{1/2}\,\beta$) of 0.81 ± 0.02 h obtained in the present study denotes that the drug is removed at a faster rate as compared to older group of NSAIDs. This has led to low value of mean residential time (MRT) of 0.95 ± 0.02h. The quicker elimination of this drug is further supported by high total body clearance (Cl_B) of 9.53 ± 0.39 ml/kg/min.

(vi) A moderately high value of volume of distribution of 0.67 ± 0.04 L/kg and a high value of tissue to plasma concentration ratio (T≈P) of 1.00 ± 0.14 denote better distribution of the drug in different body fluids and tissues of goats.

[II] Immunological study :-

(i) ***Humoral immune response*** : The agglutinating antibody titre recorded in ketoprofen treated group produced significantly higher titre value (1.467513 ± 172197) as compared to antigen treated group (0.990882 ± 0.122196). Hence it is assumed that immunopotentiating effect was shown by ketoprofen on humoral immune response. The

antibody formation was observed on 10th day of antigen exposure and highest antibody titre was observed on 21st day followed by declining trend upto 42nd day in all groups except saline control group.

(ii) ***Cell mediated immune response*** : Cell mediated immune response (CMIR) was evaluated *in-vivo* through delayed type hypersensitivity (DTH) reaction using three different mitogens as well as in vitro culture of lymphocytes that are exposed to mitogen. Lymphocyte transformation test (LTT) has been employed as a method of detection of CMIR *in-vitro*. In the present study, three mitogens such as DNCB, PHA-P and PPD were used for estimating the delayed type hypersensitivity (DTH) response. The DNCB and PHA-P skin sensitivity test showed significant variation in the mean skin thickness in ketoprofen treated group as compared to positive control (Table 5 and 10). There was significant decrease in DNCB and PHA-P mean skin thickness in ketoprofen treated group (0.9195 ± 0.026371 and 0.97 ± 0.47288 mm) as compared to antigen control (1.0795 ± 0.039397 and 1.031904 ± 0.041348 mm), respectively. On the other hand, PPD delayed type hypersensitivity test showed non-significant variation in the mean skin thickness between treated groups and their respective control (Table 15).

In lymphocyte transformation test, the stimulation index (SI) value of the lymphocytes stimulated with con-A were significantly higher in SRBC inoculated group at 14 days post SRBC inoculation in comparison to the SI values of saline control and ketoprofen and SRBC treated group, for respective period. The values for the rest of the period in all the three treatment group were non-significant. However, the value for SRBC inoculated group without drug revealed a numerically higher values as compared to SRBC, and ketoprofen treated group. The result showed that T-lymphocyte activity was suppressed by ketoprofen as reflected in terms of SI values.

From DTH reaction and LTT, it is concluded that ketoprofen suppressed the cell mediated immune response.

By taking into account of various advantages and its least immunomodulatory effect shown by this drug, it is concluded that ketoprofen can be safely and effectively given in clinical cases associated with pyrexia, pain and other inflammatory conditions.

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Appendix

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

Where, x = time; y = drug concentration; n = number of samples.

$$b = \frac{(8 \times 1.67413) - (23.25 \times 3.76148)}{(8 \times 93.8125) - 540.5625}$$

$$= \frac{13.39304 - 87.45441}{750.5 - 540.5625}$$

$$= \frac{-74.06137}{209.9375}$$

$$= -0.352778$$

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

$$= -0.352778 \times (-2.303)$$

$$= 0.813\ h^{-1}$$

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,

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

$$= \log (0.47018) - (-0.352778 \times 2.90625)$$

$$= \log 0.47018 + 1.02526$$

$$= \log 1.49544$$

APPENDIX

CALCULATION OF KINETIC PARAMETERS :

Kinetic parameters were calculated from the plasma log drug concentration versus time profile. An example is noted below from the data of animal no. 2 obtained after i.v. injection of Ketoprofen (3 $mg.kg^{-1}$) given alone in goat. The data showed a biphasic curve and hence, fits well into two compartment open model. Here, elimination phase starts from 0.75 h.

Sl. No.	Time (h) X	X^2	Plasma drug concentration (Y) $\mu g.ml^{-1}$	Log (Y×10)	XY
1	0.75	0.5625	1.92	1.28330	0.96247
2	1	1	1.02	1.00860	1.00860
3	1.5	2.25	0.90	0.95424	1.43136
4	2	4	0.65	0.81291	1.62582
5	3	9	0.35	0.54406	1.63218
6	4	16	0.12	0.07918	0.31672
7	5	25	0.06	-0.22184	-1.1092
8	6	36	0.02	-0.69897	-4.19382
Σn = 8	ΣX = 23.25 $(\Sigma X)^2$ = 540.5625 $\overline{X}$ = 2.90625	ΣX^2 = 3.8125		ΣY = 3.76148 $\overline{Y}$ = 0.47018	ΣXY = 1.67413

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

Where, x = time; y = drug concentration; n = number of samples.

$$b = \frac{(8 \times 1.67413) - (23.25 \times 3.76148)}{(8 \times 93.8125) - 540.5625}$$

$$= \frac{13.39304 - 87.45441}{750.5 - 540.5625}$$

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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,

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

$$= \log (0.47018) - (-0.352778 \times 2.90625)$$

$$= \log 0.47018 + 1.02526$$

$$= \log 1.49544$$

Zero time concentration (B) = antilog of 1.49544 = 31.29248 $\mu g.ml^{-1}$

Since plasma concentration is multiplied earlier by 10 in the above calculation, the value of 31.29248 $\mu g.ml^{-1}$ should be divided by 10 to get the actual zero time concentration. Hence, zero time concentration (B) = 3.129248 $\mu g.ml^{-1}$.

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).

Substracting the theoretical values from the 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 the method adopted for calculation of B and β. The calculated values are –

$$\alpha = 4.07\ h^{-1}$$

$$A = 4.843\ \mu g.ml^{-1}$$

C_p^0. theoretical plasma concentration at zero time

$$C_p^0 = A + B$$

$$= 4.843 + 3.129$$

$$= 7.972\ \mu g.ml^{-1}$$

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

$$t_{1/2}\ \alpha = \frac{0.693}{\alpha} = \frac{0.693}{4.07}$$

$$= 0.1702\ h$$

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

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

$$= \frac{0.693}{0.813} = 0.8523\ h$$

AUC, Area under curve

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

$$= \frac{4.843}{4.07} + \frac{3.129}{0.813} = 1.19 + 3.85$$

$$= 5.03\ mg.L^{-1}.h$$

AUMC, area under the first moment of plasma drug concentration time curve

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

$$= \frac{4.843}{16.56} + \frac{3.129}{0.66}$$

$$= 0.292 + 4.740 = 5.032\ mg.L^{-1}.h^2$$

MRT, mean residential time

$$MRT = \frac{AUMC}{AUC} = \frac{5.032}{5.03} = 1.00$$

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

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

$$= \frac{4.843 \times 0.813 + 3.129 \times 4.07.}{7.972}$$

$$= 2.09\ h^{-1}$$

Kel, the elimination rate constant of the drug from central compartment

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

$$= \frac{4.07 \times 0.813}{2.09} = 1.58\ h^{-1}$$

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

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

$$= 4.07 + 0.813 - 2.09 - 1.58$$

$$= 1.213\ h^{-1}$$

F_C, the fraction of drug available for elimination from central compartment

$$F_C = \frac{\beta}{Kel} = \frac{0.813}{1.58}$$

$$= 0.51$$

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

$$T \approx P = \frac{K_{12}}{K_{21} - \beta} = \frac{1.213}{2.09 - 0.813}$$

$$= 0.95$$

Vd_C, the volume of distribution based on distribution and elimination

$$Vd_C = \frac{D}{A + B}, \text{ where D = dose rate (mg.kg}^{-1})$$

$$= \frac{3}{4.843 + 3.129}$$

$$= 0.376 \text{ L.kg}^{-1}$$

Vd_B, the volume of distribution based on elimination

$$Vd_B = \frac{D}{B} = \frac{3}{3.129}$$

$$= 0.958 \text{ L.kg}^{-1}$$

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

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

$$= \frac{3}{5.03 \times 0.813} = 0.733 \text{ L.kg}^{-1}$$

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

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

$$= \frac{1.213 + 2.09}{2.09} \times 0.376$$

$$= 0.60 \text{ L.kg}^{-1}$$

Cl_B, the total body clearance

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

$$= 0.733 \times 0.813$$

$$= 0.5959 \text{ L.kg}^{-1}.\text{h}^{-1}$$

$$= 9.95 \text{ ml.kg}^{-1}.\text{min}^{-1}$$