Pharmacokinetics of Gentamicin and its Interaction with Diclofenac 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

Deepak Kumar Prasad

Reg No. - M/V. Phar./41/2002-2003

DEPARTMENT OF VETERINARY PHARMACOLOGY AND TOXICOLOGY

BIHAR VETERINARY COLLEGE

PATNA - 800 014

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

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

CERTIFICATE - I

This is to certify that the thesis entitled "PHARMACOKINETICS OF GENTAMICIN AND ITS INTERACTION WITH DICLOFENAC 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. DEEPAK KUMAR PRASAD**, under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

Jayachandran 22/5/04

(C. Jayachandran)
Major Advisor

Endorsed :

22/05/04

(Chairman / Head of the Department)

DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY

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

CERTIFICATE - II

We, the undersigned, members of the Advisory Committee of **DR. DEEPAK KUMAR PRASAD**, 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 OF GENTAMICIN AND ITS INTERACTION WITH DICLOFENAC IN GOATS**" may be submitted by **DR. DEEPAK KUMAR PRASAD** in partial fulfillment of the requirements for the degree.

22/5/04

[Dr. C. Jayachandran]
Chairman
Advisory Committee

Members of the Advisory Committee :

(i) **Dr. S. D. Singh**
Associate Professor and Head,
Department of Veterinary Pharmacology & Toxicology,
Bihar Veterinary College, Patna-14.

22/05/04

(ii) **Dr. S. R. P. Sinha**
Associate Professor,
Department of Veterinary Parasitology
Bihar Veterinary College, Patna-14

22/5/04

(iii) **Dr. S. R. Singh**
Associate Professor and Head,
Department of Animal Breeding & Genetics
Bihar Veterinary College, Patna-14
[Nominee of DRI-cum-Dean, P.G. Studies]

22/5/04

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

CERTIFICATE - III

This is to certify that the thesis entitled "**PHARMACOKINETICS OF GENTAMICIN AND ITS INTERACTION WITH DICLOFENAC IN GOATS**" submitted by **DR. DEEPAK KUMAR PRASAD**, 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 ..... 11.09.04 .

11/9/04

[Dr. C. Jayachandran]
Chairman
Advisory / Examination
Committee

Members of the Advisory / Examination Committee :

(i) **Dr. S. D. Singh** 11/09/04

(ii) **Dr. S. R. P. Sinha** 11/9/04

(iii) **Dr. S. R. Singh** 11/9/04

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

Dedicated to

my benevolent
and
adorable

PARENTS

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Place - Patna
Date - 22.05.04

Deepak Kumar Prasad
(Deepak Kumar Prasad)

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Chapter - 1

Introduction

INTRODUCTION

The last decade has witnessed a constant and untiring effort to make the anti-microbial therapy more specific, more effective, with least side effects and affordable for the human and veterinary clinicians. During recent years the anti-microbials have attracted further more attention and curiosity. It has also been found that efficacy and the pharmacokinetics of antibiotics may vary when they are given alone and concurrently with other drugs. Now during the recent time when the combined and multidrug therapy has gained much popularity it has become more essential to explore the detailed pharmacokinetics of the drugs on their separate and con-current administrations. Although the original observations about such interactions stemmed from fundamental research, subsequent knowledge of drug interactions, acquired from experiments on animals, has been used to therapeutics advantages in animals and man to enable a physician to minimize or prevent drug toxicity by adjustment of the dosage schedule.

Gentamicin, a member of aminoglycoside group of antibiotics constitutes a very important weapon in the armamentarium of clinicians against infections caused by aerobic gram negative bacteria such as *Escherichia coli, Salmonella, Shigella,*

Klebsiella, Proteus, Haemophillus, Pasturella, Enterobacter, Campylobacter, Pseudomonas and Serratia. This antibiotic is highly preferred in veterinary practice because of its low cost and reliable efficacy against many susceptible organisms. It is used for treating systemic as well as local infections such as respiratory tract infections, urinary tract infections, skin, burn and soft tissue infections. Gentamicin is also effective for treating various poultry diseases like colibacillosis, staphylococosis, necrotic dermatitis etc. The disposition kinetic data of Gentamicin in cattle, equine, sheep, goat, cat, dog, rabbit and poultry were explored by different workers.

The systemic microbial infections is generally associated with pyrexia, pain and inflammation as well ; hence, non-steroidal anti-inflammatory drugs (NSAIDs) are usually administered along with antimicrobials to overcome these problems. Diclofenac is a potent NSAID with good analgesic anti-inflammatory, antipyretic and uricosuric properties (Maier *et al.*, 1979). It has also been reported to possess antibacterial activity. It is one of the most potent inhibitors of prostaglandin synthetase, which is the mediator of inflammation. It is mostly used against degenerative joint diseases, rheumatoid arthritis ankylosing spondylitis and allied conditions (Brodgen *et al.*, 1980).

Antimicrobials and NSAIDs are frequently used concomitantly and pharmacokinetic interactions between them have

been described by various workers (Kampmann *et al.*, 1972; Carbon *et al.*, 1981, 1984; Nitesh Kumar *et al.*, 2002, 2003; Mukta, 2002; Baxla, 2004; Mukesh, 2004). Joly *et al.* (1988) showed enhancement of the therapeutic effects of cephalosporins (Cefotiam, Cefmenoxime and Ceftriaxone) in experimental endocarditis by altering their pharmacokinetics when simultaneously used with the NSAID, diclofenac. In experimental staphylococcal osteomyelitis, ibuprofen given concomitantly with Oxacillin significantly increased the antibiotic efficacy but the mechanism of interaction was not studied (Khurana and Deddish, 1986). Concurrent administration of anti-inflammatory drugs with antimicrobials may change their disposition characteristics (Joly *et al.*, 1988; Nitesh Kumar 2003; Mukta, 2002; Baxla, 2004) and thereby changing their dosage regimen. Baxla (2004) established definite kinetic interactions between gentamicin and paracetamol as noted by significant variations in drug concentrations in body fluids and kinetic parameters of both the drugs.

Goat (*Capra hircus*) is mainly reared in tropical countries, including India for meat and milk purposes apart from its valuable hide. Goat farming is an important tool to over come poverty and unemployment in rural folk. Goat farming has now assumed a key position in rural development programme in developing countries like India. Hence, it is essential that proper health coverage should be

given to this species, which is proving to be an asset for the poor mass of the country.

Pharmacokinetic studies on gentamicin were carried out in different species of animals but studies on interaction of gentamicin with diclofenac is very scanty.

Keeping in view of the aforesaid facts, the present investigation was carried out in goats with the following specific aims and objectives :

1. Estimation of concentrations of gentamicin and diclofenac at different time intervals in body fluids following their separate parental administration.
2. Determination of kinetic parameters of gentamicin and diclofenac when given alone.
3. Calculation of dosage regimen of gentamicin when administered alone.
4. Estimation of drug(s) concentrations in biological fluids and calculations of kinetic parameters of gentamicin and diclofenac as well as calculation of dosage regimen of gentamicin when the drugs are given concurrently to know the interaction of the drugs following their parentral administration.

Chapter - 2

Review of Literature

REVIEW OF LITERATURE

Aminoglycoside antibiotics constitute a very important weapon in the armamentarium against gram-negative infections in animals and human. Members of aminoglycoisides are streptomycin, gentamicin, neomycin, kanamycin, tobramycin, amikacin, netilmicin etc. Gentamicin is a highly preferred aminoglycoside in veterinary practice because of its low cost and reliable activities against many susceptible organisms. It is used for treating systemic as well as local infections.

GENTAMICIN

Gentamicin is an important agent for the treatment of many serious gram-negative bacillary infections such as *Escherichia coli, Salmonella, Klebsiella, Proteus, Haemophilus, Pasteurella, Campylobacter* and *Pseudomonas*. It binds to the 30S ribosomal subunit; however, it also appears to bind to several sites on the 50S ribosomal subunit as well (Davies, 1988). This antibiotic is frequently used in combination with β-lactam antibiotics such as penicillins or cephalosporins for the therapy of proven or suspected serious gram-negative microbial infections. It is therapeutically used in cases of

urinary tract infections, bacteremia, infected burns, osteomyelitis, pneumonia, peritonitis and otitis. However, β-lactam antibiotics and aminoglycosides must never be mixed in the same bottle because penicillins inactivate the aminoglycosides to a significant degree.

1. History

Gentamicin is broad-spectrum aminoglycoside antibiotic derived form species of the actinomycete *Micromonospora*. Gentamicin was first studied and described by Weinstein and co-workers in 1963. It was isolated, purified and characterized by Roselot and co-workers (1964).

2. Chemistry

Chemically, aminoglycosides consist of two or more amino sugars joined in glycosidic linkage to a hexose nucleus, which is usually in a central position. This hexose or aminocyclitol is 2-deoxystreptamine in Gentamicin. The gentamicin family which includes gentamicin C_1, C_{1a} and C_2, sisomicin and netilmicin contains a different 3-amino sugar (garosamine). Variations in methylation of the other amino sugars result in the different components of gentamicin. These modifications appear to have little effect on biological activity. The structural formula of gentamicin is shown in Fig. 1.

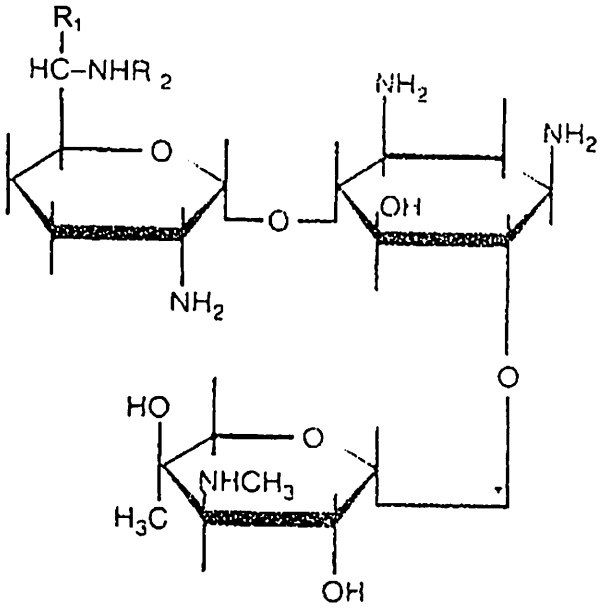


Fig. I. *Chemical structure of Gentamicin.*

	R_1	R_2
Gentamicin C_1	CH_3	CH_3
Gentamicin C_2	CH_3	H
Gentamicin C_{1a}	H	H

3. Mechanism of Action

The aminoglycoside antibiotics are rapidly bactericidal. Bacterial killing is concentration dependent; the higher the concentration, the greater the rate at which bacteria are killed (Kapushik *et al.*, 1988; Blaser, 1991). Aminoglycosides are characterized by post antibiotic effect.

The primary intracellular site of action the gentamicin is the 30 S ribosomal subunit, which consists of 21 proteins and a single 16 S molecule of RNA (Mitssuhashi, 1975). However,it also appears to bind to several sites on the 50 S ribosomal subunit as well (Davies, 1988). Gentamicin disrupts the normal cycle of ribosomal function by interfering with the initiation of protein synthesis. It also can induce misreading of the mRNA template causing incorrect amino acids to be incorporated into the growing polypeptide chains (Tai *et al.*, 1978). It remains to be established that this is the primary mechanism of aminoglycoside induced cell death.

4. Antibacterial activity

The antibacterial activity of gentamicin is primarily against aerobic gram-negative bacilli such as *Escherichia coli*, *Salmonella, Klebsiella, Proteus, Haemophilus, Enterobacter, Campylobacter* and *Pseudomonas*. It has limited activity against anaerobic microorganisms or facultative bacteria under anaerobic conditions.

Single intramuscular dose of gentamicin has been effective in curing over 90% of uncomplicated infections of the lower urinary tract (Ronald *et al.*, 1976; Varese *et al.*, 1980). In meningitis,

direct administration of gentamicin into cerebral cortex has been suggested using 0.03 mg of gentamicin every 24 hours (McGee and Baringer, 1990).

The antibacterial activity of aminoglycosides is markedly reduced by low pH (Strausbaugh and Sande, 1978) and hyperosmolarity (Papapetropoulou *et al.*, 1983); however, the very high concentrations achieved in urine in patinets with normal renal function usually are sufficient to eradicate sensitive microorganisms. The prolonged release of gentamicin from the renal cortex following discontinuation of therapy has been shown to produce a therapeutic effect for several months in experimental pyelonephritis in rats (Bergeron *et al.*, 1982). Aminoglycoside alone is not very effective because therapeutic concentrations are difficult to achieve owing to relatively poor penetration of the drug into inflamed tissues and the associated conditions of low oxygen tension and low pH, both of which interfere with the aminoglycoside antibacterial activity. Aminoglycoside in combination with a β-lactam antibiotic is recommended for the treatment of pneumonia caused by *P. aeruginosa.*

The aerobic gram-negative bacilli vary in their susceptibilities to gentamicin as shown in the following table.

Typical minimum inhibitory concentrations of gentamicin that will inhibit 90% (MIC_{90}) of clinical isolates for several species.

Sl. No.	*Species*	*MIC_{90} (μg/ml)*
1.	*Citrobacter freundii*	0.5
2.	*Enterobacter spp.*	0.5
3.	*Escherichia coli*	0.5
4.	*Klebsiella pneumoniae*	0.5
5.	*Proteus mirabilis*	4
6.	*Providencia stuartii*	8
7.	*Pseudomonas aeruginosa*	8
8.	*Serratia spp.*	4
9.	*Enterococcus faecalis*	32
10.	*Staphylococcus aureus*	0.5

Reference : Wiedmann, B., and Atkinson, B.A. Suceptibility to antibiotics : species incidence and trends. In, *Antibiotics in Laboratory Medicine*, 3rd ed. (Lorian, V., ed.) Williams & Wilkins, Baltimore, 1991, pp. 962-1208.

5. Pharmacokinetics Study

(1) Cow

Gentamicin (4 mg/kg body weight) was administered i.v. to 7 Holstein bull calves between 12 and 24 hours of age and 5, 10 and 15 days after birth, and was administered once i.v. to 7 Holstein cows.

Serum was collected from each animal before administration and at 22 different time intervals from 2 to 400 minutes after injection. Decay of serum gentamicin concentrations was best described by a 2-compartment phamacokinetic model. Elimination half life of gentamicin decreased from day 1 (149 min) to day 5 (119 min), but did not change between days 5 and 15 (111 min). Compared with the half life in calves, that in cows was shorter (76 min). In the calves, apparent volume of distribution did not change between 1 and 5 days of age, and decreased on day 10 and day 15. Total body clearance of gentamicin in cows (1.29 $ml.min^{-1}.kg^{-1}$) was lower than that seen in calves on day 1 (1.92 $ml.min^{-1}.kg^{-1}$) and on day 15 (2.10 $ml.min^{-1}.kg^{-1}$). The decrease in apparent volume of distribution of gentamicin was mirrored by a large decrease in the extracellular fluid volume, as measured by insulin space. The percentage protein binding of gentamicin was < 30%. (Clarke *et al.*, 1985).

Kinetic disposition and dosage regimen of gentamicin in pregnant cows were investigated after single i.v. administration (5 mg/kg). the distribution half life ($t_{1/2}\alpha$) and elimination half life ($t_{1/2}\beta$) were calculated to be 0.05 ± 0.01 and 1.12 ± 0.25 h, respectively. The values of Vd_{area} and total body clearance (Cl_B) were 0.37 ± 0.13 $L.kg^{-1}$ and 213.7±28.4 $ml.kg^{-1}.h^{-1}$, respectively. To maintain the therapeutic plasma concentration (1.0 μg/ml) in pregnant cows, the dosage

regimen of gentamicin would be 5 mg/kg body weight repreated at the 4 h interval (Satish *et al.*, 1989).

Six healthy mature lactating cows were given gentamicin (5 mg/kg of body weight) by i.v. route and another dose 19 days later by i.m. route. Serum gentamicin concentrations were determined over a period of 48 hours after each drug dosing, using radio-immunoassay. The distribution phase half life ($t_{1/2}\ \alpha$) was 0.25 ± 0.12 hour, and post distribution half-life was 1.83 ± 0.18 hours. The volume of the central compartment was 0.10 ± 0.02 $L.kg^{-1}$, volume of distribution at steady state was 0.16 ± 0.03 $L.kg^{-1}$ and the total body clearance was 1.32 ± 0.17 $ml.min^{-1}.kg^{-1}$. Intramuscular absorption was rapid, with a half life for absorption of 0.63 ± 0.28 hour. The extent of i.m. absorption was 92% ± 15%. The percentage of the i.m. dose eliminated in urine during the first 8 hours was 83 ± 8. Gentamicin was detected in milk for 48 hours. (Haddad *et al.*, 1986).

(2) Buffalo

Grewal *et al.* (2002) studied the disposition kinetics of gentamicin following single i.m. administration (3 mg/kg) in buffaloes suffering from clinical mastitis revealed that the drug was detectable in plasma and milk upto 12 and 24 h, respectively. The minimum

therapeutic concentration of gentamicin achieved in milk is probably effective against few organisms, susceptible at low drug concentration. The peak plasma level was 6.36 ± 0.09 μg/ml at 2 h. The drug was detectable in plasma upto 12 h.

In buffalo calves, phamacokinetic study was done after i.v. administration of gentamicin (5mg/kg). The kinetic parameters distribution half life ($t_{1/2}$ α) elimination half life ($t_{1/2}$ β) area under curve (AUC), mean residential time (MRT), Vdarea and total body clearence was found to be 0.69 ± 0.08h, 5.05 ± 0.30h, 45.90 ± 3.10 mg. L^{-1}.h, 5.79 ± 0.34h, 0.80 ± 0.04 $L.kg^{-1}$ and 1.86 ± 0.16 $ml.kg^{-1}.min^{-1}$, respectively. Vd_{area} of 0.80 ± 0.04 $L.kg^{-1}$ obtained for gentamicin in buffalo calves denotes good distribution of drug which is supported by the value of 1.54 ± 0.16 obtained for approximate tissue to plasma concentration (T ≈ P) ratio (Baxla, 2004).

(3) Horse

Serum gentamicin levels and pharmacokinetic parameters were studied in 14 horses after i.v. administrationof 3 and 6 mg/kg body weight. The microbiological cylinder plate assay, using *Staphylococcus epidermidis* (ATCC 12228) as the test organism was used to determine serum gentamicin concentrations. Samples were

collected 0, 5, 10, 15, 20, 30, 45, 60, 120, 240, 360 and 720 min after antibiotic administration. The results were best fitted in two compartment open model with a central volume of distribution (Vdc) of 0.13 ± 0.08 and 0.19 ± 0.07 $L.kg^{-1}$, elimination half-life of 3.43 ± 0.84 and 3.78 ± 0.84 h and total body clearance of 0.65 ± 0.45 and 0.99 ± 0.55 $L.min^{-1}.kg^{-1}$ for doses of 3 and 6 mg/kg, respectively. It is concluded that gentamicin has an adequate Vdc to be of use against systemic infections and that the concentrations persist long enough to allow drug therapy in the horse (Zurich *et al.*, 1995).

The disposition of drugs may differ between pregnant and non-pregnant animals, necessiating a change in dosage. Gentamicin was administered to 7 Throughbred and Quarterhorse mares on two occasions, followed by plasma drug gentamicin assay and pharmacokinetic analysis. The first dose was administered 1-4 weeks before parturition (mean weight 578 kg) and the second dose was administered in the period 1-4 weeks after parturition (mean weight 518 kg). The dose administered at each time was approximately 6.6 mg/kg, intravenously. Mean volume of distribution of steady state was 0.15 ± 0.02 and 0.16 ± 0.03 $L.kg^{-1}$, systemic clearance was 1.06 ± 0.17 and 1.11 ± 0.17 $ml.kg^{-1}.min^{-1}$ and mean (harmonic) elimination half life was 2.02 and 2.1 h, for pregnant and non pregnant mares, respectively (Santschi *et al.*, 2000).

Pharmacokinetics of gentamicin C_1, C_{1a} and C_2 components following i.v. administration of total gentamicin at 6.6 mg/kg body weight to 6 healthy mature horses was determined. Significant difference in clearance, half life ($t_{1/2}$) and mean residence time (MRT) between the gentamicin C_{1a} and the two other components were found. The total body clearance (Cl) of gentamicin C_{1a} was 1.62 ± 0.50 ml/min/kg and similar to the glomerular filteration rate (GFR) reported for horses. The Cl of gentamicin C_1 and C_2 were 1.03 ± 0.08 $mg.min^{-1}.kg^{-1}$ and 1.10 ± 0.15 $ml.min^{-1}.kg^{-1}$, respectively, and significantly slower than that of gentamicin C_{1a}. The values of apparent volume of distribution of steady state were 0.22 ± 0.05, 0.26 ± 0.12 and 0.23 ± 0.05 $L.kg^{-1}$ for gentamicin C_1, C_{1a} and C_2, respectively. The MRT values were (mean ± s.d.) 3.6 ± 0.05, 2.7 ± 0.3 and 3.5 ± 0.4 h and $t_{1/2}$ values were 3.1 (2.5 – 4.0), 2.4 (2.0 – 3.2) and 3.3 (2.4 – 4.3) h (hormonic mean and range) for gentamicin C_1, C_{1a} and C_2, respectively. It was concluded that the difference in pharmacokinetics between the gentamicin components has potential pharmacological and toxicological implications (Steinman *et al.*, 2002).

4. Camel

Kinetics of gentamicin (3 mg/kg body weight) were determined in 6 camels (*Camlus dromedarius*) after i.v. and i.m.

administration. After i.v. administration, the overall elimination rate constant (β) was 0.24 ± 0.01 h^{-1} and the half life was 2.92 ± 0.12 h. The mean residence time (MRT) was 4.20 ± 0.15 h. The volume of distribution at steady state (Vd_{SS}) was 260.6 ± 12.8 $ml.kg^{-1}$ and the total body clearance (Cl_B) was 62.7 ± 5.0 ml $kg^{-1}.h^{-1}$. Following i.m. administration, gentamicin reached a peak serum concentration of 9.36 ± 0.5 $\mu g.ml^{-1}$, in post injection hour (PIH) of 0.5 ± 0.05. The elimination half life was 2.80 ± 0.09 h, not significantly different from that obtained by the i.v. route. V_{dss} was 254.1 ± 17.0 $ml.kg^{-1}$ and Cl_B was 62.9 ± 5.0 $ml.kg^{-1}.h^{-1}$, neither were significantly different from values obtained by the i.v. route. The mean absorption time (MAT) was 0.37 ± 0.22 h and the absorption rate constant (Ka) was 0.091 ± 0.03 min^{-1}. Gentamicin bio availability (F) was 89.1 ± 6.6% (Wasfi *et al.*, 1992).

5. Llama

Single intravenous and multiple dose pharmacokinetics of gentamicin was studied in 19 healthy llamas. Gentamicin was given (5 mg/kg i.v.) as a single bolus, and serum gentamicin concentrations were monitored over the next 48 h. Two months later, 10 of these llamas were given gentamicin (2.5 $mg.kg^{-1}$) i.v. for the first day, then

i.m. every 8 h for 7 days. Serum gentamicin concentrations and indices of renal function and damage were monitered during the 7 days. There were no significant dose or time related difference in clearance of the drug, volume of distribution, apparent coefficients of the distribution and elimination phases, mean residence time, or distribution ($t_{1/2}$ α) and elimination phase ($t_{1/2}$ β) half-lives. The 5 mg/kg i.v. kinetic study revealed $t_{1/2}$ α of 14.5 ± 5.06 min and $t_{1/2}$ β of 166 ± 20.5 min. The 2.5 mg/kg i.v. kinetic study revealed $t_{1/2}$ α of 17.7 ± 6.59 min and $t_{1/2}$ β of 165 ± 40.3 min. Peak serum gentamicin concentration averaged 10.10 $\mu g.ml^{-1}$ in the multiple dose trial and trough concentration average 1.50 $\mu g.ml^{-1}$. There was no evidence of renal impairment in the llamas. It is concluded that gentamicin pharmacokinetic variable in llamas resembles those in other ruminant species. (Lackey *et al.*, 1996).

6. Sheep

The pharmacokinetics of gentamicin (3 mg/kg i.v. bolus) was evaluated in 6 adult ewes before and after fever induced with *Escherichia coli* endotoxin (1 $\mu g.kg^{-1}$). In ewes with endotoxin induced fever, increased gentamicin concentrations in plasma occurred at 15 and 40 minutes and at 6 hours after injection of gentamicin. Changes

were not observed in the apparent volume of distribution calculated by the area method, the volume of distribution at steady state, the overall biological half life, or body clearance. Significant reduction occurred in the zero time intercept for distribution, the distribution rate constant, the concentration in plasma at the time of injection, the volume of the peripheral compartment, and the first order transfer rate constants; only the volume of the central compartment was increased. Total amounts of gentamicin were increased in the central compartment and decreased in the peripheral or tissue compartment (Wilson *et al.*, 1984).

7. Swine

Gentamicin was administered to 6 pigs in single i.m. dose of 5 mg/kg. Serum concentrations were 50 to 100 μg/ml by 30 minutes. From the second hour after administration, the serum concentration fell considerably until the 12th hour. In milk, only low concentrations were found. In urine however, more than 150 μg/ml was detected 12 hours after administration. This value decreased within 48 hours to concentrations between 7.5 to 2.5 μg/ml. Serum was free from gentamicin by 24 hours after administration as shown by the agar diffusion method (plate hole-test). No symptoms of incompatibility were observed. (Glawisching *et al.*, 1985).

A single i.v. bolus injection of gentamicin (5 mg/kg) was administered to 6 newborn male piglets, aged from 4 to 12 h at the time of drug administration and six 42 day old castrated male piglets, that had been weaned for 2 weeks. Gentamicin was measured in serum and in urine by a fluorescence polarization immunoassay. The serum concentration time data was best described by a 3-compartment open model. A rapid initial distribution phase was observed in every animal. The serum half – life was significantly longer in the newborn piglets (5.19 ± 0.30 h) than in the older group (3.50 ± 0.23 h). Mean residence time was similarly longer in younger piglets (6.62 ± 0.57 h) than in older animals (2.86 ± 0.11h). The steady state volume of distribution (Vd_{SS}) was significantly larger for younger pigs (0.785±0.036 L/kg) than older pigs (0.474±0.029 L/kg). Urinary half-life was 72.66 ± 10.79 h in the newborn piglets and 69.20 ± 14.77 h in the 42 day old animals. A urinary phase was observed in 3 of the 42-day old piglets with a mean urinary half life of 232 .01 ± 14.55 h. Percentage of urinary recovery of the administered dose after 144 h were 94.18 ± 1.01 and 94.04 ± 1.12 in the newborn and 42 day old pigs, respectively. Serum gentamicin clearance was significantly lower in newborn pigs (0.121 ± 0.007 $L.h^{-1}.kg^{-1}$) than in the 42 day old group (0.166 ± 0.010 $L.h^{-1}.kg^{-1}$) (Giroux *et al.*, 1995).

8. Rabbit

The bio availability of gentamicin following i.m. and s.c. administration was studied in 6 rabbits that were each given 3.5 mg/kg of gentamicin sulphate in random sequence of administration through i.v., i.m. and s.c. routes. Gentamicin analysis was performed on serial blood samples using fluorescence polarization immunoassay. The elimination half-life, mean residence time and serum gentamicin concentrations were not significantly different between the routes of injection. It is concluded that in this study the bioavialability of gentamicin slightly exceeded 100% and that the i.v. dose appeared to be less bioavailable than i.m. and s.c. doses (Ogden *et al.*, 1995).

Phamacokinetics and dosage regimen of gentamicin were investigated in rabbits following a single i.m. administration of 4 mg/kg. The absorption and elimination half-lives and apparent volume of distribution were 4.8 ± 0.6 min, 44.4 ± 9.0 min and 0.45 ± 0.11 $L.kg^{-1}$, respectively. Therapeutic plasma level of 1 μg/ml was maintained upto 2 h. A satisfactory intramuscular dosage regimen would be 3.48 mg/kg and 9.10 mg/kg as priming and maintenance dosage respectively, to be repeated at 2 h intervals (Uppal *et al.*, 1992).

The plasma disposition, bio-distribution and dosage regimens of gentamicin were studied in rabbits folowing i.v. administration (4 mg/kg). The distribution and elimination half-life values were calculated to be 0.38 ± 0.07, and 2.88 ± 0.33 h, respectively. The concentration of gentamicin was found to be highest in kidneys, both at 10 min and 1 h while adrenals revealed absence of the drug. Based on kinetic parameters, satisfactory i.v. dosage regimens of gentamicin in rabbits would be 10.02 and 8.71 mg/kg as the loadig and maintenance doses, respectively, to be repeated at 8 h intervals (Uppal *et al.*, 1992).

9. Goat

The disposition kinetics of parenterally administered gentamicin (5 mg/kg) was studied in Gaddi goats. The serum concentration time profile was described by bi-exponential and mono-exponential equation following i.v., i.m. and s.c. administration with elimination half-life values of 0.96 ± 0.09, 2.37 ± 0.47 and 3.56 ± 0.39 h, respectively. The apparent volume of distribution following i.v. administration was 0.26 ± 0.04 $L.kg^{-1}$. The bioavailability was higher following i.m. administration (96.30%) as compared to s.c. (76.97%). It is suggested that a suitable dosage for gentamicin in goats would be 3.35 mg/kg body weight given s.c. at 12 h intevals (Garg *et al.*, 1995).

A pharmacokinetic study of gentamicin was conducted in goats following single dose i.m. administration (5 mg/kg). The effect of fever (induced by *E. coli* endotoxin) was observed on absorption, distribution and elimination of gentamicin. Serum concentrations of gentamicin were detectable upto 6 hr in both groups of goats. Significant difference in serum concentrations of gentamicin were not observed at any time after injection between 2 groups of animals. The data were adequately described by a one compartment model both in normal and febrile goats. Elimination half life of 104.8 min in febrile goats was calculated. The volume of distribution (Vd/F) was 192.5 ml/kg in febrile goats whereas in normal goats it was 158.0 ml/kg. Based on these pharmacokinetic parameters, dosage regimen for gentamicin was calculated. (Ahmad *et al.*, 1997).

The pharmacokinetics of gentamicin was studied in goats given a single intravenous dose (5 mg/kg) of uranyl nitrate for renal impairment. Gentamicin was injected intravenously (5 mg/kg) on the 7^{th} day after injection of uranyl nitrate, when the serum creatinine and blood urea nitrogen concentrations rose significantly. An elimination half life ($t_{1/2}\,\beta$) of 425 min was observed. the plasma clearance (Cl) in renally-impaired goats was 0.3 $ml.min^{-1}.kg^{-1}$. The volume of distribution was 187 ml/kg. The area under the curve was

16625 $\mu g.ml^{-1}.h$. A dosage of 2.5 mg/kg every 24 h was calculated in renally impaired goats based on the phamacokinetic parameters in the present study (Ahmad *et al.*, 2001).

10. Dog

Pharmacokinetic studies was conducted in the juvenile dog. Half life (elimination phase) was 60.9 ± 7.8 min. volume of distribution calculated from the area under the curve was 35.4 ± 3.6 L/100 kg of body weight, and clearance of drug from the body was 4.08 ± 0.62 $ml.min^{-1}.kg^{-1}$ of body weight. The 24 hour creatinine renal clearance was 3.82 ± 0.92 $ml.min^{-1}.kg^{-1}$, consistent with gentamicin being eliminated mainly by glomerular filtration. (Riviere *et al.*, 1981).

The pharmacokinetics of gentamicin C_1, C_2 and C_{1a} were studied in six beagles after administration of gentamicin at 4 mg/kg of body weight as a single intravenous bolus dose. Plasma concentrations of the gentamicin components were analysed with a novel high-performance liquid chromatography method capable of identifying and quantifying each of the component. The pharmacokinetics analysis of the plasma concentration versus time data was performed using the non compartment approach. The

results indicated significant difference in the pharmacokinetic characteristics between the gentamicin component C_1, C_{1a} and C_2. The mean residence times of gentamicin C_1, C_{1a} and C_2 were 81 ± 13, 84 ± 12 and 79 ± 13 min (mean ± S.E.) respectively. The half-lifes of the respective components were 64 ± 12, 66 ± 12 and 63 ± 12 min. Clearance (Cl) of gentamicin C_1, 4.62 ± 0.71 $ml.min^{-1}.kg^{-1}$ and C_2, 1.82 ± 0.25 $ml.min^{-1}.kg^{-1}$. Similarly, the volume of distribution at steady state (V_{SS}) of gentamicin C_1, 0.36 ± 0.04 $L.kg^{-1}$ was significantly higher ($p = 0.0156$) than the (V_{SS}) of gentamicin C_{1a}, 0.14 ± 0.01 $L.kg^{-1}$ and C_2, 0.15 ± 0.012 $L.kg^{-1}$. Tissue binding was considered the most likely cause for the difference (Isoherranen *et al.*, 2000).

11. Chicken

The pharmacokinetics of gentamicin following repeated i.m. or i.v. administration (4 mg/kg) was studied in White leghorn chickens. The kinetics profile of gentamicin following repeated i.m. or i.v. administration was best described by one and two compartment open models, respectively. The absorption and distribution half life were 24.15 ± 9.47 and 17.36 ± 4.64 min, respectively. The elimination half life following repeated i.m. or i.v. administration were found to be 179.12 ± 39.18 and 97.4 ± 10.54 min, respectively.

The values for apparent volume of distribution and total body clearence following repeated i.v. injection were 0.32 ± 0.05 $L.kg^{-1}$ and 2.27 ± 0.17 $ml.min^{-1}.kg^{-1}$, respectively. Statistical comparison of the values of disposition kinetic parameters generated in the present study with the corresponding values for single i.m. or i.v. administration in chickens reported previously revealed that on repeated administration (i.v. or i.m.) the kinetic behaviour of gentamicin has changed significantly. However, no accumulating tendency of the drug was evidenced (Grag *et al.*, 1989).

Following intravenous administration of gentamicin sulphate (4 mg/kg) is white leghorn chickens, the disposition kinetics was best described by a two compartment open model. The distribution and elimination half life of gentamicin were 10.25 ± 1.42 and 131.60 ± 115.14 min, respectively. The values for apparent volume of distribution and the total body clearence were 0.97 ± 0.18 $L.kg^{-1}$ and 5.01 ± 1.08 $min^{-1}.kg^{-1}$, respectively. The concentration of gentamicin was highest in kidneys both at 15 min and 90 min after single administration (4 mg/kg). White thigh muscles and brain revealed only traces of drug. On the basis of kinetic disposition data, a satisfactory dosage regimen of gentamicin sulfate has been computed for fowls (Garg *et al.*, 1989).

TABLE I : IMPORTANT KINETIC PARAMETERS OF GENTAMICIN IN DIFFERENT SPECIES

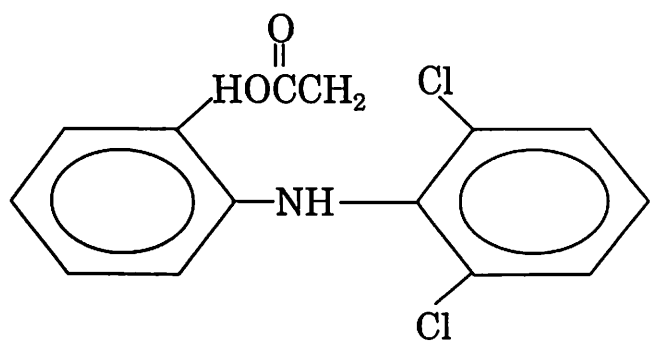
Species	Absorption half-life ($t_{1/2}$ Ka) (h)	Distribution half-life ($t_{1/2}$ α) (h)	Elimination half-life ($t_{1/2}$ β) (h)	Volume distribution (L/kg)	Total body clearance (mg/kg/min)	Dose (mg/kg)	Route of administration	References
Cow (pregnant)	-	0.05±0.01	1.12±0.25	0.37±0.13	213.7±28.5	5	i.v.	Satish *et al.*, (1989)
Cow calf	-	-	149 min (day1) 119 min (day 15)	-	1.92 (day 1) 2.10 (day 15)	4 5	i.v.	Clarke *et al.*, (1985)
Buff calf	-	0.69 ± 0.08	5.05 ± 0.30	0.80 ± 0.04	1.86 ± 0.16	5	i.v.	Baxla (2004)
Equine Horse	-	-	3.43 ± 0.84 3.78±0.84	0.13 ± 0.08 0.19 ± 0.07	0.65 ± 0.45 0.99 ± 0.55 $L.kg^{-1}.min^{-1}$	3 6	i.v.	Zurich *et al.*, (1995)
Camel	-	-	2.92±0.12	Vd_{SS} 260.6±12.8	62.7 ±5.0	3	i.v.	Wasfi *et al.*, (1992)
Llama	-	14.5±5.06	166±20.5 min	-	-	5	i.v.	Lackey *et al.*, (1996)
Goat " "	- - -	-	0.96±0.09 2.37±0.47 3.56±0.39	0.26±0.04	-	5 " "	i.v. i.m. s.c.	Garg *et al.*, (1995)
Rabbit	4.8±0.6	-	44.4±9.0	0.45 ± 0.11	-	4	i.m.	Uppal *et al.*, 1992
Birds chicken	24.15±947 min	17.36±4.64 min	179.21±3918 97.41±10.54	0.32 ± 0.05	2.27 ± 0.17	4 "	i.m. i.v.	Garg *et al.*, (1989)
Dog	-	-	60.9 ± 7.8 min.	35.4 ± 3.6 L/100 kg	4.08 ± 0.62	-	i.v.	Riviere *et al.* (1981)
Swine (42 day old male)	-	-	3.50 ± 0.23	Vd_{SS} 0.474±0.029	0.166 ± 0.010	6	i.v.	Giroux *et al.* (1995)

DICLOFENAC

Diclofenac is a potent nonsteroidal anti-inflammatory drug (NSAID), which is widely used in human and veterinary practice. It is a phenylacetic acid derivative that was developed specifically as an anti-inflammatory agent. It is also an analgesic compound with good antipyretic and urisosuric propeties (Maier *et al.*, 1979).

1. Chemistry

Chemically, diclofenac is a phenyl acetic acid derivative. The chemical structure is as follows :



Empirical formula = $C_{14} H_{23} O_2 Cl_2 N$

Molecular weight = 307.

2. Mechanism of Action

Diclofenac possess analgesic, antipyretic and anti-inflammatory properties. It is an inhibitor of cycloxygenase in the metabolism of arachidonic acid and thus exerts its anti-inflammatory

action by blocking the synthesis of prostaglandins, prostacycline and thromboxane products. Diclofenac also inhibits the lipo-oxygenase pathway and thereby reducing the production of leukotrienes and monohydroxy acids, which are associated with the inflammatory processes. It also reduces polymorph chemotaxis and production of lysosomal enzymes and super oxide radicles, thereby reducing tissue destruction in inflammatory reactions. Diclofenac suppresses hyperthermia through its action on the thermoregulatory centre in hypothalamus.

3. Pharmacokinetics and metabolism

Diclofenac is rapidly and completely absorbed after oral administration and peak concentrations in plasma are reached within 2 to 3 hours. Administration with food slows the rate but does not alter the extent of absorption. It is extensively bound to plasma proteins (99%) and its half life in plasma is 1 to 2 hours. Cmax and AUC are dose related in range of 25-150 mg. Diclofenac accumulat es in synovial fluid after oral administration, that may be the possible reason behind the longer duration of therapeutic effect than the plasma half life. Diclofenac is metabolized in the liver to 4-hydroxy diclofeanc, the principal metabolite and other hydroxylated forms. The metabolites are excreted in urine (65%) and bile (35%). Apart from liver, bile and kidney, high levels of diclofenac are found in blood, heart and lungs.

4. Therapeutic uses

Diclofenac is used in veterinary practice for long term symptomatic treatment of rheumatoid arthritis, osteoarthritis, non descriptive pyrexia, painful conditions due to acute and chronic inflammation, muscular pain, neuralgia, soft tissue injuries such as sprain or strain and ankylosing spondylitis. It as also useful for short term treatment of acute musculoskeletal injury, acute painful shoulder, post operative pain and dysmenorrhea.

5. Pharmacokinetic studies

Pharmacokinetic studies of diclofenac were conducted in differed species. These are notes as follows :

1. Man

Willis *et al.* (1979) noted in man that the lag time between dosing and appearance of drug in plasma varied between 1.0 and 4.5h after oral doses. Peak plasma levels ranged from 1.4 to 3.0 μg/ml. The mean terminal drug half life in plasma was 1.8 h after oral dose and 1.1 h after i.v. dose. They noted availability (oral) 54 ± 2%, urinary excretion less than 1%, bound in plasma more than 99.5%, clearance 4.2 ± 0.9 $ml.min^{-1}.kg^{-1}$ and volume distribution 0.17 ± 0.11 $L.kg^{-1}$. After i.v. injection, plasma levels of diclofenac fell rapidly and were below the limits of detection at 5.5 h post dosing.

Kurowski (1988) noted oral bio availability of 72.9% with on average lag time of 2.2 h. Peak plasma concentrations amounted to 2.9 μg/ml after 3.1 h as compared to 2.15 μg/ml after 20-30 min following an intramuscular injection of 75 mg. Diclofenac was excreted with an average half-life of 1.15 h. The bio-availability of the three i.m. injectable solutions, as calculated from the area under the curve (AUC), did not differ significantly.

2. Minipig

The pharmacokinetics and metabolism of diclofenac was studied in Yucatan minipigs after i.v. administration of 25 and 50 mg and after oral administration of 50 mg in a solution of 50 ml buffer 50 ml water and 200 ml water and the results were compared to historical data in man. The absolute bio availability after oral administration of 50 ml buffer, 50 ml water and 200 ml water solutions were 107, 97 and 107%, respectively, as compared to approximately 50% in man. The total plasma clearence in mini pigs was five fold slower than in man (57 ± 17 vs 252 ± 54 $ml.h^{-1}.kg^{-1}$). The volume of distribution of the central compartment (Vdc) was 40% less in man than in pigs (39 vs 67 $ml.kg^{-1}$). The terminal half lives of the parent drug were similar in pigs (2.4 h) and man (1.8 h). The rate of oral drug absorption increased in the order of 50 ml aqueous, 200 ml aqueous and 50 ml buffered solutions (Ka = 0.52 ± 0.11, 0.59

± 0.13 and 1.2 ± 0.7 h^{-1}, respectively) as observed by Oberle *et al.* (1994).

3. Buffalo calves

In buffalo calves, pharmacokinetics study was done after i.v. administration of diclofenac (1 $mg.kg^{-1}$). The kinetic parameters distribution half life ($t_{1/2}$ α), elimination half life ($t_{1/2}$ β), area under curve (AUC), mean residential time (MRT), Vd_{area} and total body clearance was found to be 0.34 ± 0.08 h, 4.06 ± 0.59 h, 11.24 ± 0.48 $mg.L^{-1}.h$, 4.72±0.85 h, 0.54 ± 0.10 $L.kg^{-1}$ and 1.52±0.07 $ml.kg^{-1}.min^{-1}$, respectively Vd_{area} of 0.54 ± 0.10 $L.kg^{-1}$ obtained for diclofenac in buffalo calves denotes good distribution of the drug which is supported by the value of 2.43 ± 0.32 obtained for approximate tissue to plasma concentration (T ≈ P) ratio (Nitesh Kumar *et al.*, 2003).

4. Rat

In rat, biliary encretion of the drug (unchanged and conjugated) was detected in bile duct canulated rats were 27.2 and 31.2% and only 4.7 and 5.4% excreted in the bile after i.v. and intraduodenal administration, respectively. Maximum plasma concentration was reached within 2 min, after intraduodenal dosing. Bio-availability in the bile duct cannulated rats was 71% after intraduodenal dose where as in normal animal was 79% after oral dose and 106% after intraduodenal dose (Peris-Ribera *et al.*, 1991).

TABLE II : IMPORTANT KINETIC PARAMETERS OF DICLOFENAC IN DIFFERENT SPECIES

Species	Absorption half-life ($t_{1/2}$ Ka) (h)	Distribution half-life ($t_{1/2}$ α) (h)	Elimination half-life ($t_{1/2}$ β) (h)	Volume distribution (L/kg)	Total body clearance (mg/kg/min)	Dose	Route of administration	References
Man	-	-	-	0.17 ± 0.11	4.2 ± 0.9	-	Oral	Willis *et al.* (1979)
Minipig	-	-	-	67 ml/kg	57 ± 17 ml/kg/h	25 & 50 mg	i.v.	Oberle *et al.* (1994)
Buffalo calf	-	0.34 ± 0.08	4.06 ± 0.59	0.54 ± 0.10	1.52 ± 0.07	1 mg/kg	i.v.	Nitesh Kumar *et al.* (2003)

KINETIC INTERACTIONS OF ANTIMICROBIALS WITH NON STEROIDAL ANTI-INFLAMMATORY DRUGS

Anti microbials and non steroidal anti-inflammatory drugs (NSAIDs) are frequently used concomitantly and pharmacokinetic interactions between them have been described (Kampamann *et al.*, 1972; Carbon *et al.*, 1981, 1984; Joly *et al.*, 1988; Mueller *et al.*, 1993; Nergelius *et al.*, 1997; Sudha Kumari 1998; Tang *et al.*, 1999; Verma *et al.*, 2000; Nitesh Kumar, 2003; Mukta, 2002; Baxla, 2004).

The effect of diclofenac on the pharmacokinetics of the three cephalosporins viz., ceftriaxone, cefotiam and cefmenoxime was studied in rabbits by Joly *et al.* (1988). Ceftriaxone concentrations at 1, 2, 4, 6, 12 and 24 h and AUC in serum increased significantly ($p < 0.05$) when this antimicrobial was administered in conjunction with diclofenac. Diclofenac increased significantly ($p < 0.05$) the serum terminal half-life ($t_{1/2}$ β) of ceftriaxone and non-significantly that of cefotiam but not of cefmenoxime.

No effect of diclofenac on the phamacokinetics of cloxacillin was shown in man by Nergleius *et al.* (1997). Total plasma clearance of cloxacillin was with placebo 219 ± 51 (mean ± SD) and

with diclofenac 212 ± 39 ml/min/1.73m^2 (ns); renal clearence was 97 ± 21 and 96 ± 24 ml/min/1.73 m^2 respectively (ns). The terminal $t_{1/2}$ of cloxacillin was 1.03 ± 0.42 h with placebo, and 1.12 ± 0.37h with diclofenace (ns). Thus, diclofenac did not alter cloxacillin pharmacokinetics.

Pharmacokinetics of enrofloxacin (5 mg/kg) when given alone and in combination with paracetamol (50 mg/kg) by i.v. route in six goats was carried out by Sudha Kumari (1998). The study showed that the mean therapeutic concentration (0.12 μg/ml) in plasma was maintained upto 10 h for enrofloxacin and 6 h for enrofloxacin with paracetamol. Significantly higher values were obtained for zero time concentration in distribution (C_p^0), which were 19.60 ± 3.92 and 21.52 ± 4.12 μg/ml, respectively, in combined administration as compared to single administration (3.37 ± 0.79 and 5.27 ± 0.99 μg/ml, respectively). Significantly higher elimination rate constant (β) and lower elimination half life ($t_{1/2}$ β) of 0.456 ± 0.067 h-1 and 1.70 ± 0.26 h, respectively, in combination as compared to single administration (0.270 ± 0.041 h^{-1} and 2.82 ± 0.33 h, respectively). The distribution half-life (0.57 ± 0.17 h), AUC (18.90 ± 5.87 mg/L.h), K_{12} (0.251 ± 0.079 h^{-1}) Fc (0.42 ± 0.09), T ≈ P (1.96 ± 0.48), Vd_{area} (1.10 ± 0.47

L/kg) and Cl_B (9.22 ± 4.73 ml/kg/min) did not show any significant difference when enrofloxacin was given along with paracetamol as compared to enrofloxacin when given alone (0.60 ± 0.10 h, 9.85 ± 1.38 mg/L.h, 0.436 ± 0.133 h^{-1}), 0.51 ± 0.06, 1.11 ± 0.22, 2.34 ± 0.54 L/kg and 9.40 ± 1.36 ml/kg/min), respectively.

The stimulation of diclofenace metabolism by interaction with quinidine was studied in monkeys by Tang *et al.*, (1999). After a dose of diclofenac via portal vien infusion at 0.055 mg/kg/h, a steady state plasma drug concentration in three rhesus monkeys were 87, 104 and 32 ng/ml, respectively (control). When diclofenac was co-administered with quinidine (0.25 mg/kg/h) via the same route, the corresponding plasma diclofenac concentration were 50, 59 and 18 ng/ml, representing 57, 56 and 56% of control values, respectively. In contrast, steady state systemic diclofenac concentrations in the same three monkeys were elevated from 1.4 to 2.5 times, when the monkeys were pretreated with L-754, 394 (10 mg/kg, i.v.), an inhibitor of cytochrome P- 450 (CYP) 3A. Further investigation indicated that the plasma protein binding (> 99%) and blood / plasma ratio (0.7) of diclofenac remain unchanged in the presence of quinidine. Therefore, the decrease in plasma concentrations of diclofenac after a combined dose of diclofenac and quinidine are taken

to reflect increased hepatic clearance of the drug, presumably resulting from the stimulation of CYP-3A-catalyzed oxidative metabolism. Consistent with this proposed mechanism, a 2-fold increases in the formation of 5-hydroxy diclofenac derivatives was observed in monkey hepatocyte suspensions containing diclofenac and quinidine. Stimulation of diclofenac metabolism by quinidine was diminished when monkey liver microsomes were pretreated with antibodies against CYP-3A. Subsequent kinetic studies indicated that the K(m) value for the CYP-mediated conversion of diclofenac to its 5-hydroxy derivatives was little changed (75 vs 59 micro M) where as V(max) increased 2.5 fold in the presence of quinidine. These data suggest that the catalytic capacity of monkey hepatic CYP-3A toward diclofenac metabolisms is enhanced by quinidine.

The mean pharmacokinetic characteristic of cyclosporine were unchanged during co-administration with diclofenac was studied in man by Muller et al. (1993). A single oral dose of 300 mg cyclosporine was administered alone and on day 8 of multiple oral dosing of 50 mg diclofenac every 8 h. Serial blood samples were obtained over 48h after each cyclosporine dose and over a dosing interval for diclofenac on day 7 (diclofenac alone) and day 8 (co-administration of diclofenace with cyclosporine). Based on area under

the curve (AUC) comparison, lack of pharmacokinetic interaction was conclusively demonstrated for the extent of cyclosporine absorption. The diclofenace maximum plasma concentration and AUC over a dosing interval were significantly increased during co-administration, however, a straight forward interpretation of the statistical result was confounded by pronounced variability in diclofenac pharmacokinetics. The results underscore the need for continued caution when cyclosporine and diclofenac are co-administered.

Pharmacokinetics of enrofloxacin was studied in five cattle following i.m. administration (5 mg/kg) alone and along with diclofenac sodium (0.8-1.0 mg/kg). Therapeutic concentration (0.1 μg/ml) in plasma was maintained up to 12 and 24 h for enrofloxacin and enrofloxacin along with diclofenac sodium, respectively. The plasma elimination half life (9.2 h), Vd_{area} (17.3 L/kg), t_{max} (2 h), MRT (13.2 h) and total body clearance (1.4 L/kg/h) were comparatively significantly higher when enrofloxacin was given along with diclofenac sodium as compared to enrofloxacin give alone (5.9 h, 7.1 L/kg, 0.6 h, 0.4 h and 0.82 L/kg/h, respectively). The AUC (3.8 mg/L.h) and C_{max} (0.2 μg/ml) was significantly lower when enrofloxacin was administered along with diclofenac sodium as compared to enrofloxacin given alone (5 $mg.L^{-1}.h$ and 0.82 $\mu g.ml^{-1}$, respectively).

Diclofenace sodium significantly ($p < 0.1$) reduced the plasma concentration of ciprofloxacin (as metabolite of enrofloxacin). Based on the pharmacokinetic parameters calculated, an intramuscular dosage regimen of enrofloxacin (priming dose of 1.8 mg/kg followed by maintenance dose of 1.10 mg/kg every 8 h) to maintain therapeutic concentration of 0.1 μg/ml is recommended in cattle (Verma *et al.*, 2000).

Nitesh Kumar (2003) showed no effect of diclofenac in the pharmacokinetics of enrofloxacin and its active metabolism ciprofloxacin. In contrst enrofloxacin, significantly influenced the pharmacokinetics of diclofenac. Significantly higher $t_{1/2}$ β, AUMC MRT, Vd_{area} value of 12.84 ± 1.29h, 264.8 ± 58.10 mg/L.h^2, 18.07 ± 1.92 h, 1.34 ± 0.04 L/kg, respectively, were noted when diclofenac was given in combination with enrofloxacin as compared to its alone administration (4.06 ± 0.59 h, 51.78 ± 7.30 mg/L.h^2, 4.72 ± 0.85 h, 0.54 ± 0.10 L/kg, respectively) in buffalo calves following i.v. administration.

Mukta (2002) studied the pharmacokinetic of amikacin and its interaction with diclofenac in buffalo calves. Significantly ($p < 0.01$) lower A, B, C_p^0, AUC, AUMC and F_C value of 0.87 ± 0.21 μg.ml^{-1},

1.07 ± 0.06μg.ml^{-1}, 1.94 ± 0.23 μg.ml^{-1}, 10.25 ± 1.34 mg.L^{-1}.h, 100.9 ± 25.04 mg.L^{-1}.h^2 and 0.59 ± 0.06, respectively were noted when amikacin was given in combination with diclofenac as compared to its alone administration (13.23 ± 0.70 μg.ml^{-1}, 5.22 ± 0.38 μg.ml^{-1}, 18.41 ± 0.67 μg.ml^{-1}, 48.56 ± 5.84 mg.L^{-1}.h, 256.4 ± 39.72 mg.L^{-1}.h^2 and 0.39 ± 0.03, respectively) in buffalo calves following i.v. administration. In contrast $t_{1/2}$ α, $t_{1/2}$ β and Cl_B showed non-significant changes. Whereas, amikacin also significantly influence the pharmacokinetics of diclofenac. Significantly higher ($p < 0.01$) value of B, C_p^0, AUC, AUMCand F_C 18.91 ± 1.98 μg.ml^{-1}, 30.53±1.07 μg.ml^{-1}, 158 ± 9.85 mg.L^{-1}.h, 1168 ± 52.85 mg.L^{-1}.h^2 and 0.67 ± 0.06, respectively was noted when diclofenac was given along with amikacin as compared to its alone administration (1.65±0.35 μg.ml^{-1}, 7.38 ± 1.49 μg.ml^{-1}, 11.24 ± 0.48 mg.L^{-1}.h, 51.78 ± 7.30 mg.L^{-1}.h^2 and 0.30 ± 0.03, respectively). In contrast $t_{1/2}$ β, K_{21} and Vd_{area} showed non-significant change.

Baxla (2004) studied the pharmacokinetics of gentamicin and its interaction with paracetamol in buffalo calves. Significantly higher A, C_p^0, α, AUC, AUMC, K_{12} and T ≈ P value of 34.48 ± 2.35 μg.ml^{-1}, 39.03 ± 2.40 μg.ml^{-1}, 1.935 ± 0.119 h^{-1}, 62.16 ± 2.82 mg.L^{-1}.h,

433.1 ± 28.63 $mg.L^{-1}.h^2$, 1.088 ± 0.111 h^{-1} and 5.04 ± 0.16, respectively, were noted when gentamicin was administered along with paracetamol as compared to its alone administration (11.08 ± 0.86 $\mu g.ml^{-1}$, 15.89 ± 0.85 $\mu g.ml^{-1}$, 1.058 ± 0.10 h^{-1}, 45.90 ± 3.10 $mg.L^{-1}.h$, 268.2 ± 27.86 $mg.L^{-1}.h^2$ and 1.54 ± 0.16, respectively) in buffalo calves following i.v. administration. In contrast B, $t_{1/2}$ β, MRT, Vd_{area} differs non-significantly. Gentamicin also significantly influence the pharmacokinetics of paracetamol. Significantly higher B, C_p^0, $t_{1/2}$ α, $t_{1/2}$ β, AUC, AUMC and MRT value of 22.32 ± 1.44 $\mu g.ml^{-1}$, 47.18 ± 1.46 $\mu g.ml^{-1}$, 0.55 ± 0.07 h, 2.33 ± 0.14 h, 93.57 ± 6.02 $mg.L^{-1}.h$, 266.4 ± 25.79 $mg.L^{-1}.h^2$ and 2.82 ± 0.13, respectively, were noted when paracetamol was given in combination with gentamicin as compared to its alone administration (13.11 ± 1.08 $\mu g.ml^{-1}$, 22.62 ± 1.00 $\mu g.ml^{-1}$, 0.11 ± 0.01 h, 0.77 ± 0.09 h, 15.59 ± 1.07 $mg.L^{-1}.h$, 16.09 ± 2.56 $mg.L^{-1}.h^2$ and 1.00 ± 0.10, respectively).

GENERAL PHARMACOKINETICS

Pharmacokinetics often referred as disposition kinetics, which helps in knowing absorption, distribution, metabolism and excretion of drugs (Dost, 1953). According to Wagner (1968), the aim of pharmacokinetics is to study the time concentration course of

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

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

Where,

C_p = Concentration of drug plasma

B = Extrapolated zero time intercept of mono-exponential curve

β = Over all elimination rate constant

t = Time elapsed after drug administration

e = Base of natural logarithm

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

Two compartment open model

The pharmacokinetics of most of the drugs following i.v. administration are accurately described by two compartment open model. Baggot (1974) stated that in two compartment open model, the drug distribution is instantaneous and homogeneous into the central

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Two compartment open model

The pharmacokinetics of most of the drugs following i.v. administration are accurately described by two compartment open model. Baggot (1974) stated that in two compartment open model, the drug distribution is instantaneous and homogeneous into the central

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

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

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

Where,

C_p = Plasma concentration of the drug,

A = Zero time intercept of distribution phase.

B = Zero time intercept of elimination phase.

α = Distribution rate constant.

β = Elimination rate constant

e = Base of natural logarithm

t = Time elapsed after drug administration

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

Three or multi compartment open model

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

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

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

PHARMACOKINETICS OF CLINICAL IMPORTANCE

Clinically, the pharmacokinetic study consists of: -

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

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

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

SOME IMPORTANT PHARMACOKINETIC PARAMETERS

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

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

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

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

3. Elimination rate constant (β)

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

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

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

iii. The total body clearance (Cl_B)

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

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

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

5. Volume of distribution

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

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

6. Total body clearance (Cl_B)

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

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

DOSAGE REGIMEN

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

Chapter - 3

Materials and Methods

MATERIALS AND METHODS

In the present study, five clinically healthy goats of non-descript breed between 1.5 to 2 years of age and 20 to 22 kg body weight were used. The goats were housed in the animal shed with concrete floor. The animals were maintained on dry fodder, cattle feed and greens apart from routine grazing for 5 to 6 hours. Water was provided *ad lib*.

EXPERIMENTAL DESIGN

Gentamicin and diclofenac were administered separately in each of five healthy goats by intravenous (i.v.) route. An interval of 15 days was allowed to elapse before administration of next dose of the drug. After conducting kinetic study of these drugs alone, the drugs were administered together in combination by i.v. route to investigate the interaction of these drugs in goats.

DRUGS USED

Gentamicin and diclofenac were used in the present experiment. Ranbamycin® — an injectable commercial preparation containing gentamicin in concentration of 40 mg/ml marketed by Ranbaxy and Verastan® — an injectable commercial preparation containing diclofenac in concentration of 25 mg/ml marketed by Ranbaxy, supplied as gift sample were used in the present study.

COLLECTION OF BIOLOGICAL FLUIDS AND THEIR TIMINGS

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 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, the urine samples were collected in sterile test tubes at various above noted time intervals. The samples were kept in a refrigerator and were analyzed in successive days.

ADMINISTRATION OF DRUGS

Injection of gentamicin (Ranbamycin®) containing 40 mg of gentamicin per ml was injected at the dose rate of 7.5 mg/kg body weight by i.v. route in each healthy goats. Diclofenac (Verastan®) injection containing 25 mg of diclofenac per ml was administered at the dose rate of 2 mg/kg body weight by i.v. route in each healthy goats. After conducting kinetic study of gentamicin and diclofenac by i.v. route separately, both the drugs were administered together at the above stated dose rate in each animal by i.v. route to know the interactions of the drugs.

ESTIMATION OF GENTAMICIN

Estimation of Gentamicin in biological fluids was carried out by microbial assay method using *Bacillus subtilis* (ATCC 6633) as the test microorganism (Grove, 1955). Procedures adopted for the microbiological assay are given below: -

I. Sterilization of glasswares, needles and porcelain assay cylinders

All glasswares and porcelain assay cylinders were washed properly with detergent solution in running tap water. These were again rinsed with distilled water and finally air dried. Test tubes, centrifuge tubes, vials and vial containing porcelain assay cylinders were plugged with cotton wool. Assay plates, pipettes and syringes were wrapped with papers. All these materials were sterilized in hot air oven at 160°C for an hour. For administration of drug and for collection of blood, sterile disposable needles were used.

II. Preparation of media

(a) Assay agar: -

Antibiotic assay media of the following composition was used for microbiological assay of gentamicin in plasma and urine after i.v. administration in buffalo calves.

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

The media was heated to dissolve and the solution was transferred into a conical flask and pH was adjusted. The mouth of the flask was plugged with non-absorbable cotton. Wet sterilization of media was done by autoclaving at 15 pound pressure (121^0C) for 20 min.

(b) Nutrient Broth: -

Nutrient Broth of the following composition was prepared.

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

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

III. Preparation of assay agar plates

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

IV. Preparation of test organism

The test organism used for the microbiological assay technique of gentamicin was *Bacillus subtilis* (ATCC 6633) as noted by Brown *et al.* (1984) and Orsini, *et al.* (1985). The culture of *Bacillus subtilis* was obtained from National Collection of Industrial Microorganism (NCIM), Division of Biochemical Sciences, National Chemical Laboratory, Pune-8. The organism was grown on the slant of culture tube containing nutrient agar slants at 37^0C for over night. Then it was stored under refrigeration. The organism was transferred weekly to fresh media to maintain its normal activity.

V. Preparation of standards in biological samples

Gentamicin was diluted in sterile glass distilled water to have different strengths *viz.*, 80, 40, 20, 10, 5, 2, 1 and 0.5 μg/ml. From each of these solutions, 0.1 ml was taken with the aid of micropipette and added to sterile vials containing 0.9 ml of plasma or urine collected prior to drug administration. This yielded drug standards of 8, 4, 2, 1, 0.5, 0.2, 0.1, and 0.05 μg/ml in the respective biological fluid. These standard samples were stored in refrigerator and used simultaneously with test samples in assay plates for obtaining standard curve. With the aid of standard curve, concentration of gentamicin in test sample was estimated.

VI. Assay procedure

The plasma and urine levels of gentamicin were estimated by microbiological assay technique (cylinder plate diffusion

method) using *Bacillus subtilis* (ATCC 6633) as the test organism (Grove, 1955).

The test organism was inoculated in sterile nutrient broth and kept under incubation for 2 to 3 hours at 37^{0}C until the growth was seen (turbid by naked eye). The assay plates were flooded with the broth containing the organism and excess broth was drained out after 10-15 minutes. The plates were dried in the incubator at 37^{0}C for a period of half an hour. Plates were marked for different standards and biological test samples. Sterile porcelin assay cylinders of uniform size were placed against each mark at appropriate distance along the circumference in the inoculated assay plates. 50 microlitres of each of the standard solution of various strength as well as test samples of the drug were poured in separate porcelain cylinder in the assay plate. These assay plates were left horizontally on plane surface of the table for about 2 hour and then kept in the incubator at 37^{0}C for overnight to allow the growth of organisms. The mean diameters of the bacterial zones of inhibition produced by the standards as well as test samples of the drug were measured. The standard curve was plotted from the measure of zone of inhibition against each concentration of the drug on a semi log scale. With the help of this standard curve and measured zone of inhibition of different test samples, concentrations of drug in test samples were estimated.

ESTIMATION OF DICLOFENAC BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) METHOD :

The concentrations of diclofenac in plasma and urine were estimated by HPLC method as described by El- Sayed *et al.* (1988) with slight modification. The details of the procedure are as follow :

Apparatus

The HPLC equipment used comprised of a HPLC pump, a dual wavelength absorbance detector, a rheodyne manual injector with a 20 µl loop size and a data module (integrator). Chromatographic separations were performed using C_{18} column (3.9 × 300 mm size).

Chromatographic Conditions

For HPLC analysis of diclofenac in biological samples, the flow rate was 1.5 ml. min^{-1}, the effluent was monitored at 280 nm, loop size was 20 µl, injection volume was 100 µl, chart speed was 0.25 $mm.min^{-1}$ 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 triple distilled water was used for HPLC analysis.

Mobile phase

The mobile phase comprised of acetonitrile : water (50 : 50% V/V), adjusted to pH 3.3 with glacial acetic acid.

Preparation of standards of Diclofenac in biological samples

Verastan(R), an injectable commercial preparation containing diclofenac sodium in concentration of 25 $mg.ml^{-1}$ was used in the present study. Diclofenac was diluted in triple distilled water to have different strengths *viz.*, 40, 20, 10, 5, 2.5, 1, 0.5, 0.25, and 0.1 $\mu g.ml^{-1}$.

From each standard solution, 0.1 ml was added to a centrifuge tube containing 0.9 ml plasma or urine collected prior to drug administration. This yielded diclofenac standards of 4, 2, 1, 0.5, 0.25, 0.1, 0.05, 0.025 and 0.01 $\mu g.\ ml^{-1}$ in the above noted biological fluid Blank plasma / blank urine containing no drug was also prepared. These standards were used simultaneously with test samples for determination of the drug concentrations in the test samples.

Analytical method

(1) In a clean and dry centrifuge tube, 1 ml of plasma samples was taken and 4 ml of acetonitrile was added for precipitation of plasma proteins.

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

(3) The supernatant was transferred to a clean tube and evaporated to dryness in a boiling water bath.

(4) The residue was reconstituted in 400 μl HPLC eluent (mobile phase) and vortexed for 1 min.

(5) An aliquot of this mixture (up to 100 μl) was injected directly into the loop of injector and the integrator print out retention time and area.

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

(7) 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.

CALCULATION OF PHARMACOKINETIC PARAMETERS

The following pharmacokinetic parameters of gentamicin and paracetamol were calculated after its single i.v. administration from semi log plot of plasma drug concentration versus time curve. The experimental data was analyzed by using two compartment (for i.v. route) open model as described by Gibaldi and Perrier (1982) and

Notari (1980). For a two compartment model, the concentration of the drug in plasma at any time is obtained from the formula: -

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

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

(a) A, the zero time concentration of the drug in plasma and α, the regression coefficient (distribution rate constant) for distribution phase were calculated by the method of residual yield.

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

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

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

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

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

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

where α and β are described above.

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

For two compartment model

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

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

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

(g) MRT, mean residential time (h)

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

CALCULATION OF DOSAGE REGIMEN

Dosage regimen is generally calculated for an antimicrobial agent to maintain minimum inhibitory concentration (MIC) in plasma at desired dosage intervals. Leroy *et al.* (1978) reported the therapeutic plasma levels (MICs) of gentamicin to be 1-4 μg/ml. Hence, in the present study, dosage regimen of gentamicin were calculated at 1, 2 and 4 μg/ml levels for the dosage intervals (γ) 8 and 12 hours using the formulas (Saini and Shrivastva, 1997).

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

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

where

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

D_o = Maintenance dose (mg/kg)

C_p^{∞} (min) = Desired minimum plasma concentration (μg/ml)

γ = Dosage interval (h)

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

STATISTICAL ANALYSIS

Comparison of concentration of the drug in plasma and urine at various the intervals various kinetic parameters of the drugs and dosage regimen of gentamicin when the drug were given alone and when given together in combination with diclofenac in goats were compared by using paired 't' test (Snedecor and Cochran, 1967).

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Chapter - 4

Results

RESULTS

I. PHARMACOKINETIC STUDY AFTER SINGLE I.V. ADMINISTRATION

(A) GENTAMICIN

1. Plasma levels

Concentrations of gentamicin in plasma at various time intervals following its single intravenous (i.v.) administration at the dose rate of 7.5 mg/kg have been shown in Table 1 and Fig. 1. The mean peak plasma concentration of 34.39 ± 3.62 μg/ml was attained at 0.042 h. The drug was detectable upto 12 h in all animals with the mean of 0.46 ± 0.10 μg/ml. The drug was detectable in 3 out of 5 animals at 24 h. The mean therapeutic concentration (≥ 2 μg/ml) was maintained upto 5 h.

2. Urine levels

Concentrations of gentamicin in urine at different time intervals after its single i.v. administration at the dose rate of 7.5 mg/kg are presented in Table 2 and Fig. 2. The drug appeared in urine of all the animals with a mean of 13.72 ± 1.45 μg/ml at 0.042 h. The mean peak urine drug concentration of 455.43 ± 15.05 μg/ml was achieved at 45 min. The drug was detectable upto 36 h in all animals

Table - 1

Plasma concentrations (μg/ml) of gentamicin in healthy goats following single intravenous dose (7.5 mg/kg)

Time (h)	ANIMAL NUMBER					Mean ± S.E.M
	1	2	3	4	5	
0.042	22.16	43.17	31.21	39.10	36.33	34.39 ± 3.62
0.083	13.93	30.20	24.10	31.54	31.10	26.17 ± 3.34
0.167	11.06	27.95	19.33	28.91	26.11	22.67 ± 3.35
0.25	8.78	20.43	16.10	21.66	20.10	17.41 ± 2.35
0.333	7.02	19.10	14.21	17.13	19.46	15.38 ± 2.29
0.50	6.10	16.58	10.10	13.10	16.33	12.44 ± 1.98
0.75	5.38	12.51	8.21	11.63	12.12	9.97 ± 1.38
1	4.39	9.39	6.82	9.21	9.10	7.78 ± 0.97
1.5	3.48	8.66	5.00	7.10	8.20	6.49 ± 0.98
2	2.76	6.53	4.80	5.31	6.10	5.10 ± 0.66
3	2.19	5.36	3.12	4.50	4.00	3.83 ± 0.55
4	1.50	4.54	2.50	3.33	3.46	3.07 ± 0.51
5	1.10	3.59	1.99	2.30	2.60	2.32 ± 0.40
6	0.87	2.86	1.56	1.70	2.10	1.82 ± 0.33
8	0.59	1.74	1.00	0.80	1.30	1.09 ± 0.20
10	0.35	1.12	0.64	0.60	0.80	0.70 ± 0.13
12	0.16	0.76	0.38	0.48	0.50	0.46 ± 0.10
24	N.D.	0.41	0.22	N.D.	0.30	0.31 ± 0.05

N.D. = Non detectable

Fig. 1

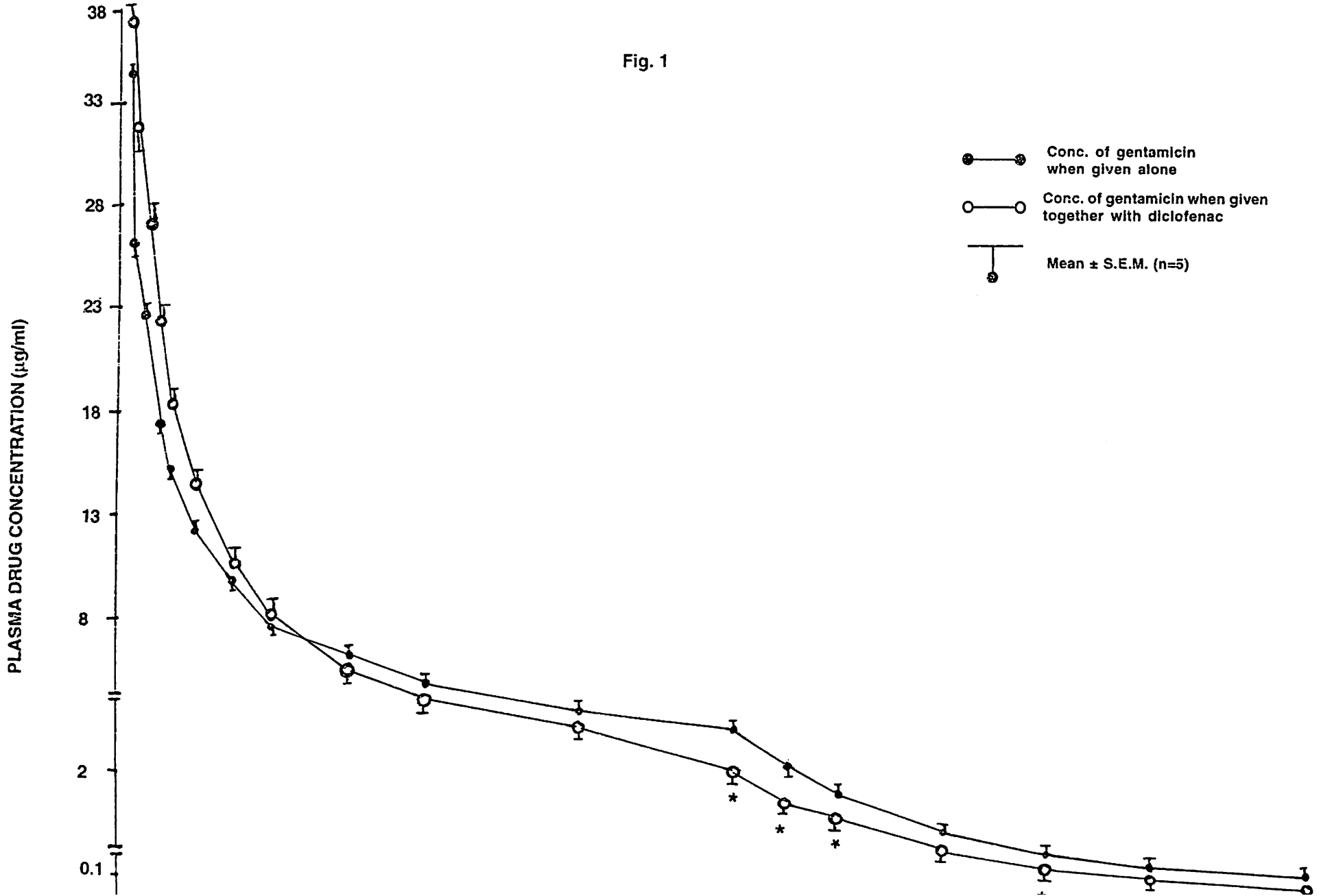

PLASMA DRUG CONCENTRATION (µg/ml)
38
33
28
23
18
13
8
2
0.1
Conc. of gentamicin when given alone
Conc. of gentamicin when given together with diclofenac
Mean ± S.E.M. (n=5)
*
*
*

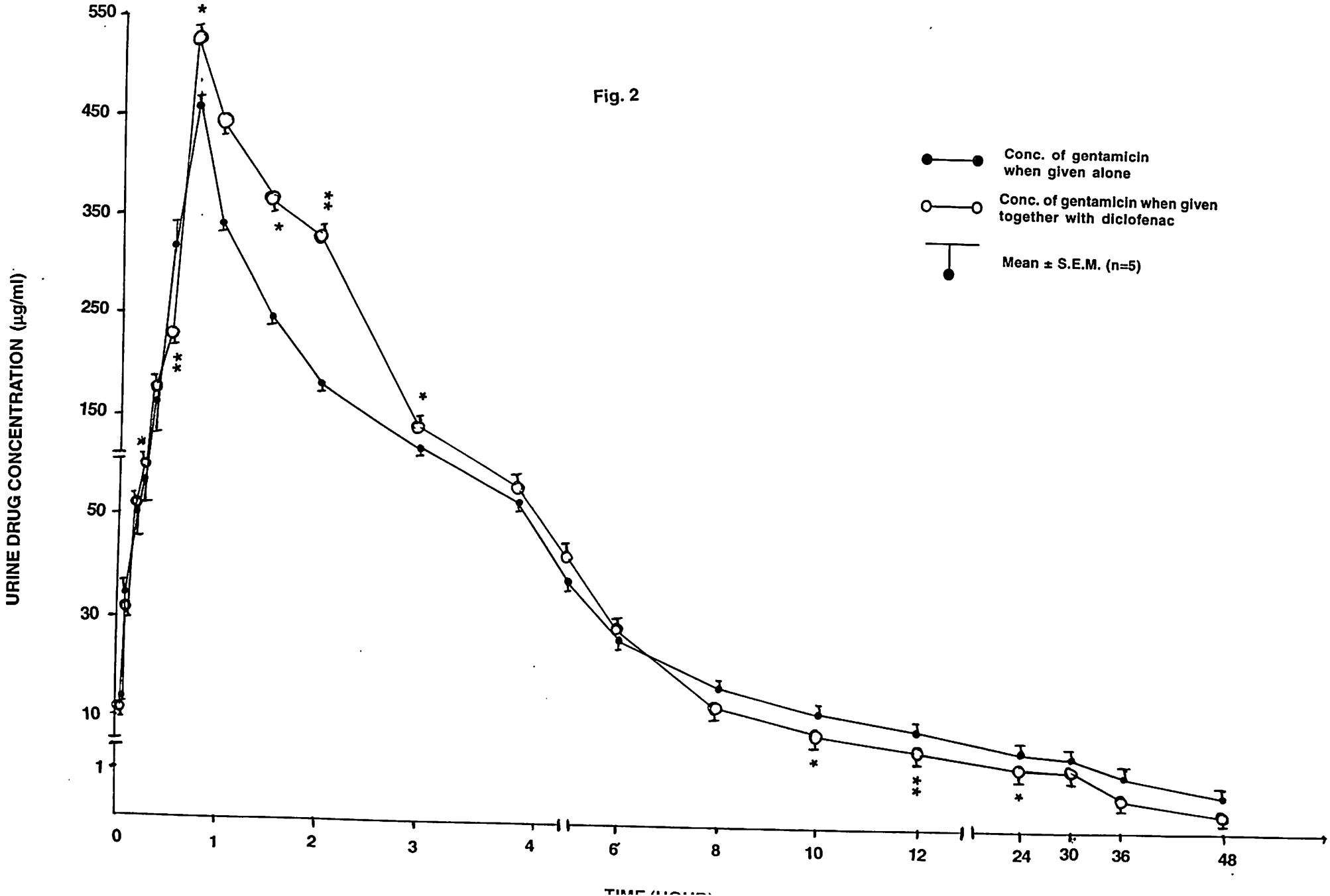
Table – 2

Urine concentrations (μg/ml) of gentamicin in healthy goats following single intravenous dose (7.5 mg/kg)

Time (h)	ANIMAL NUMBER					Mean ± S.E.M
	1	2	3	4	5	
0.042	15.22	12.62	18.56	10.28	11.94	13.72 ± 1.45
0.083	29.40	38.34	42.80	29.48	30.80	34.16 ± 2.72
0.167	56.10	60.58	61.34	58.16	75.28	62.29 ± 3.38
0.25	73.80	86.11	90.35	76.12	100.1	85.30 ± 4.80
0.333	167.7	198.1	200.1	180.2	185.3	186.3 ± 5.98
0.50	324.0	311.6	330.1	305.8	311.4	316.6 ± 4.50
0.75	442.3	496.2	410.4	448.2	480.1	455.43 ± 15.05
1	277.1	340.2	335.2	390.1	365.1	341.5 ± 18.85
1.5	195.1	221.6	281.1	291.3	235.5	244.9 ± 18.13
2	173.6	165.2	195.8	170.2	198.5	180.7 ± 6.88
3	122.5	132.8	108.3	102.4	110.6	115.3 ± 5.47
4	46.89	36.80	76.24	78.12	72.32	62.07 ± 8.46
5	30.64	28.92	39.28	55.10	33.55	37.50 ± 4.74
6	21.89	19.48	29.50	35.00	28.60	26.89 ± 2.79
8	18.68	15.63	20.00	21.56	11.25	17.42 ± 1.83
10	16.52	13.12	11.28	12.75	8.30	12.39 ± 1.33
12	12.00	10.11	9.56	6.28	6.54	8.90 ± 1.09
24	6.78	5.89	5.70	3.65	5.30	5.46 ± 0.51
30	4.24	3.12	2.58	1.28	3.56	2.96 ± 0.50
36	0.83	2.62	1.16	0.46	1.95	1.40 ± 0.39
48	N.D	0.83	0.46	N.D.	0.80	0.70 ± 0.12

N.D. = Non detectable

Fig. 2


URINE DRUG CONCENTRATION (µg/ml)
550
450
350
250
150
50
30
10
1
0
1
2
3
4
6
8
10
12
24
30
36
48
Conc. of gentamicin when given alone
Conc. of gentamicin when given together with diclofenac
Mean ± S.E.M. (n=5)

with a mean of 1.40 ± 0.39 μg/ml. The drug was detectable in 3 out of 5 animals at 48 h and the mean was noted to be 0.70 ± 0.12 μg/ml. The mean therapeutic concentration of ≥ 2 μg/ml was maintained upto 30 h.

3. Kinetic parameters

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

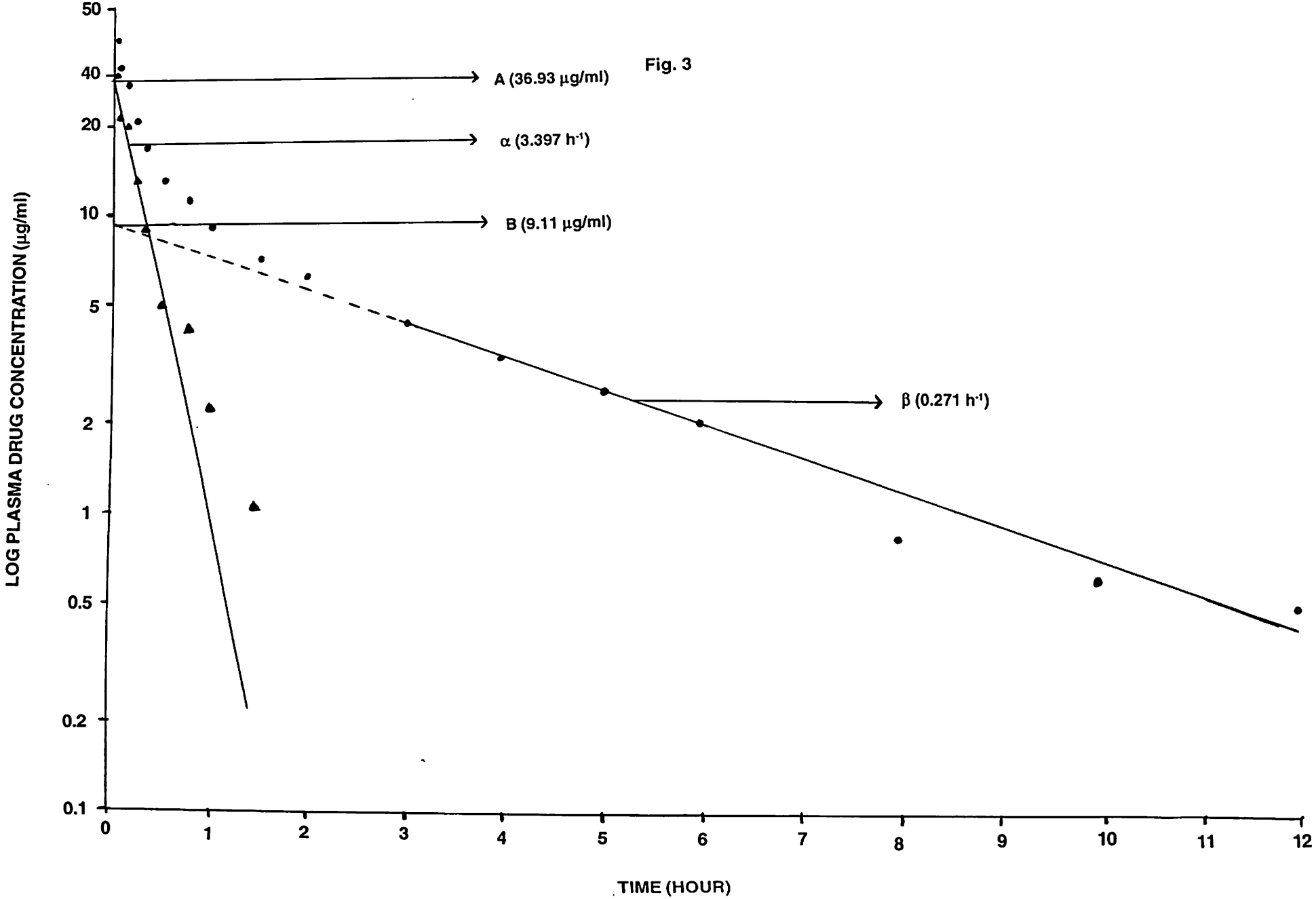
The mean extrapolated zero time concentration during distribution phase (A), elimination phase (B) and theoretical zero time concentration (C_p^o) were noted to be 23.05 ± 4.38, 5.43 ± 1.00 and 28.48 ± 5.21 μg/ml, respectively. The distribution rate constant (α) ranged from 1.196 to 3.397 h^{-1} with a mean of 1.944 ± 0.433 h^{-1} while its elimination rate constant (β) ranged from 0.126 to 0.271h^{-1} with a mean value of 0.185 ± 0.035 h^{-1}. The mean distribution half life ($t_{1/2}$ α) and elimination half life ($t_{1/2}$ β) were noted to be 0.42 ± 0.08 h and 4.28 ± 0.70 h, respectively. The mean area under curve (AUC) of 44.91 ± 7.27 $mg.L^{-1}.h$, area under first moment curve (AUMC) of 212.4 ± 55.58 $mg.L^{-1}h^2$ and mean residential time (MRT)

Table – 3

Kinetic parameters of gentamicin in healthy goats (calculated by 2-compartment open model) after a single intravenous dose (7.5 mg/kg)

Kinetic arameters	Unit	Animal Number					Mean ± S. E. M.
		1	2	3	4	5	
A	$\mu g.ml^{-1}$	10.22	24.77	18.56	36.93	24.76	23.05 ± 4.38
B	$\mu g.ml^{-1}$	4.63	5.82	3.30	9.11	4.31	5.43 ± 1.00
C_p^o	$\mu g.ml^{-1}$	14.85	30.59	21.86	46.04	29.07	28.48 ± 5.21
α	h^{-1}	2.485	1.358	1.284	3.397	1.196	1.944 ± 0.433
$t_{½\alpha}$	h	0.28	0.51	0.54	0.20	0.58	0.42 ± 0.08
β	h^{-1}	0.271	0.126	0.129	0.271	0.128	0.185 ± 0.035
$t_{½\beta}$	h	2.56	5.50	5.37	2.56	5.43	4.28 ± 0.70
AUC	$mg.L^{-1}.h$	21.20	64.43	40.04	44.49	54.37	44.91 ± 7.27
AUMC	$mg.L^{-1}h^2$	64.70	380.0	209.6	127.3	280.1	212.4 ± 55.58
MRT	h	3.05	5.90	5.23	2.86	5.16	4.53 ± 0.65
K_{12}	h^{-1}	1.095	0.649	0.563	1.743	0.503	0.911 ± 0.233
K_{21}	h^{-1}	0.961	0.360	0.303	0.889	0.286	0.560 ± 0.150
Kel	h^{-1}	0.700	0.475	0.547	1.036	0.535	0.659 ± 0.101
Fc	-	0.39	0.27	0.24	0.26	0.24	0.28 ± 0.03
$T \approx P$	-	1.59	2.77	3.24	2.82	3.18	2.72 ± 0.30
Vd_c	$L.kg^{-1}$	0.51	0.25	0.34	0.16	0.26	0.30 ± 0.06
Vd_B	$L.kg^{-1}$	1.62	1.29	2.27	0.82	1.74	1.55 ± 0.24
Vd_{area}	$L.kg^{-1}$	1.31	0.92	1.45	0.62	1.08	1.08 ± 0.15
Vd_{ss}	$L.kg^{-1}$	1.09	0.70	0.97	0.47	0.72	0.79 ± 0.11
Cl_B	$ml.kg^{-1}.min^{-1}$	5.91	1.93	3.12	2.80	2.30	3.21 ± 0.70

Fig. 3


A (36.93 µg/ml)
α (3.397 h⁻¹)
B (9.11 µg/ml)
β (0.271 h⁻¹)
LOG PLASMA DRUG CONCENTRATION (µg/ml)
50
40
20
10
5
2
1
0.5
0.2
0.1
0
1
2
3
4
5
6
7
8
9
10
11
12
TIME (HOUR)

of 4.53 ± 0.65 h were noted in the present study. The average rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central (Kel) compartment were calculated to be 0.911 ± 0.233, 0.560 ± 0.150 and 0.659 ± 0.101 h^{-1}, respectively. The fraction of drug available for elimination from central compartment (Fc) and approximate tissue to plasma concentration ratio (T≈P) were noted to be 0.28 ± 0.03 and 2.72 ± 0.030, respectively. Various values of volume distribution obtained by different methods are shown in Table 3. A mean Vd_{area} of 1.08 ± 0.15 $L.kg^{-1}$ was noted. The total body clearance (Cl_B) value ranged from 1.93 to 5.91 $ml.kg^{-1}.min^{-1}$ with a mean of 3.21 ± 0.70 $ml.kg^{-1}.min^{-1}$.

4. Dosage regimen

The dosage regimen required to maintain the different levels of therapeutic concentration (C_p^{∞} min =1,2 and 4 μg/ml) in plasma for i.v. route in buffalo calves at different selected dosage intervals (γ) of 8 and 12 h are presented in Table 4. For maintaining C_p^{∞} min of 1 μg/ml, the loading doses (D*s) were calculated to be 5.29 ± 1.62 and 13.18 ± 5.58 mg/kg, while maintenance doses (D_os) were calculated to be 4.22 ± 1.58 and 12.10 ± 5.56 mg/kg at the dosage intervals (γ) of 8 and 12 h, respectively. The D*s were calculated to be 10.58 ± 2.89 and 26.35 ± 11.17 mg/kg, while D_os were found to be

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Table - 4

Dosage regimen of gentamicin in healthy goats following after single intravenous dose (7.5 mg/kg).

in 1)	γ (h)	Dose (mg/kg)	Animal Number					Mean ± S. E. M.
			1	2	3	4	5	
	8	D*	11.45	2.52	4.07	5.42	3.00	5.29 ±1.62
		D_o	10.14	1.60	2.62	4.80	1.93	4.22 ± 1.58
	12	D*	33.85	4.17	6.82	16.02	5.02	13.18 ± 5.58
		D_o	32.54	3.25	5.37	15.40	3.94	12.10 ± 5.56
	8	D*	22.90	5.04	8.14	10.80	6.00	10.58 ± 2.89
		D_o	20.28	3.20	5.24	9.60	3.86	8.44 ± 3.16
	12	D*	67.70	8.34	13.64	32.04	10.04	26.35 ± 11.17
		D_o	65.08	6.50	10.74	30.80	7.88	24.20 ± 11.12
	8	D*	45.80	10.08	16.28	21.60	12.00	21.29 ± 6.41
		D_o	40.56	6.40	10.48	19.20	7.72	16.87 ± 6.33
	12	D*	135.40	16.68	27.28	64.08	20.08	52.70 ± 22.33
		D_o	130.16	13.00	21.48	61.60	15.76	48.40 ± 22.25

D* = Priming or loading dose

D_0 = Maintenance dose

γ = Dosage interval

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

8.44 ± 3.16 and 24.20 ± 11.12 mg/kg at γ of 8 and 12 h, respectively, for maintaining C_p^∞ min of 2 μg/ml. Likewise, to maintain C_p^∞ min of 4 μg/ml the D*s were calculated to be 21.29 ± 6.41 and 52.70 ± 22.33 mg/kg, while D_0s were found to be 16.87 ± 6.33 and 48.40 ± 22.25 mg/kg at γ of 8 and 12 h.

(B) DICLOFENAC

1. Plasma levels

Table 5 and Fig. 4 depict the concentration of diclofenac in plasma of goats at different time intervals following single i.v. dose of 2 mg/kg. The mean plasma concentration of the drug at 0.042h was found to be 56.35 ± 10.47 μg/ml and the value ranged from 38.80 to 95.10 μg/ml. The drug was detectable in 2 out of 5 animals at 12 h with the mean plasma concentration of 0.08 ± 0.03 μg/ml. The drug was not detectable in any of the animals at 24 h.

2. Urine levels

The drug concentration in urine following single i.v. administration of diclofenac (2 mg/kg) are presented in Table 6 and Fig. 5. The drug appeared at 0.042 h with a mean of 1.68 ± 0.24 μg/ml and was maintained upto 48 h in all animals with a mean value of 0.33 ± 0.07 μg/ml. The mean peak urine concentration of 95.64 ± 15.33 μg/ml was observed at 30 min.

Table – 5

Plasma concentration (μg/ml) of diclofenac in healthy goats following administration of diclofenac (2 mg/kg) after i.v. administration

Time (h)	ANIMAL NUMBER					Mean ± S.E.M
	1	2	3	4	5	
0.042	40.50	95.10	46.00	61.35	38.80	56.35 ± 10.47
0.083	25.80	64.51	25.84	50.05	22.00	37.64 ± 8.36
0.167	20.20	57.80	20.24	33.28	12.50	28.80 ± 7.98
0.25	11.80	22.98	19.50	10.36	9.00	14.73 ± 2.75
0.333	4.10	7.43	11.84	6.48	5.45	7.06 ± 1.32
0.50	2.25	6.06	8.33	6.29	3.48	5.28 ± 1.08
0.75	1.81	2.23	4.10	6.14	2.50	3.36 ± 0.80
1	1.30	1.65	2.11	5.19	1.78	2.41 ± 0.71
1.5	1.24	1.60	2.04	2.92	1.50	1.86 ± 0.29
2	0.84	1.30	1.81	2.28	1.20	1.49 ± 0.25
3	0.63	0.84	1.48	2.05	0.98	1.20 ± 0.26
4	0.56	0.78	1.32	1.76	0.64	1.01 ± 0.23
5	0.50	0.70	1.24	1.03	0.55	0.80 ± 0.14
6	0.40	0.42	0.87	0.25	0.50	0.49 ± 0.10
8	0.22	0.18	0.84	0.15	0.26	0.33 ± 0.13
10	0.14	0.10	0.53	0.12	0.18	0.21 ± 0.08
12	N.D.	0.05	N.D.	0.10	N.D.	0.08 ± 0.03
24	-	N.D.	-	N.D.	-	-

N.D. = Non detectable

Fig. 4

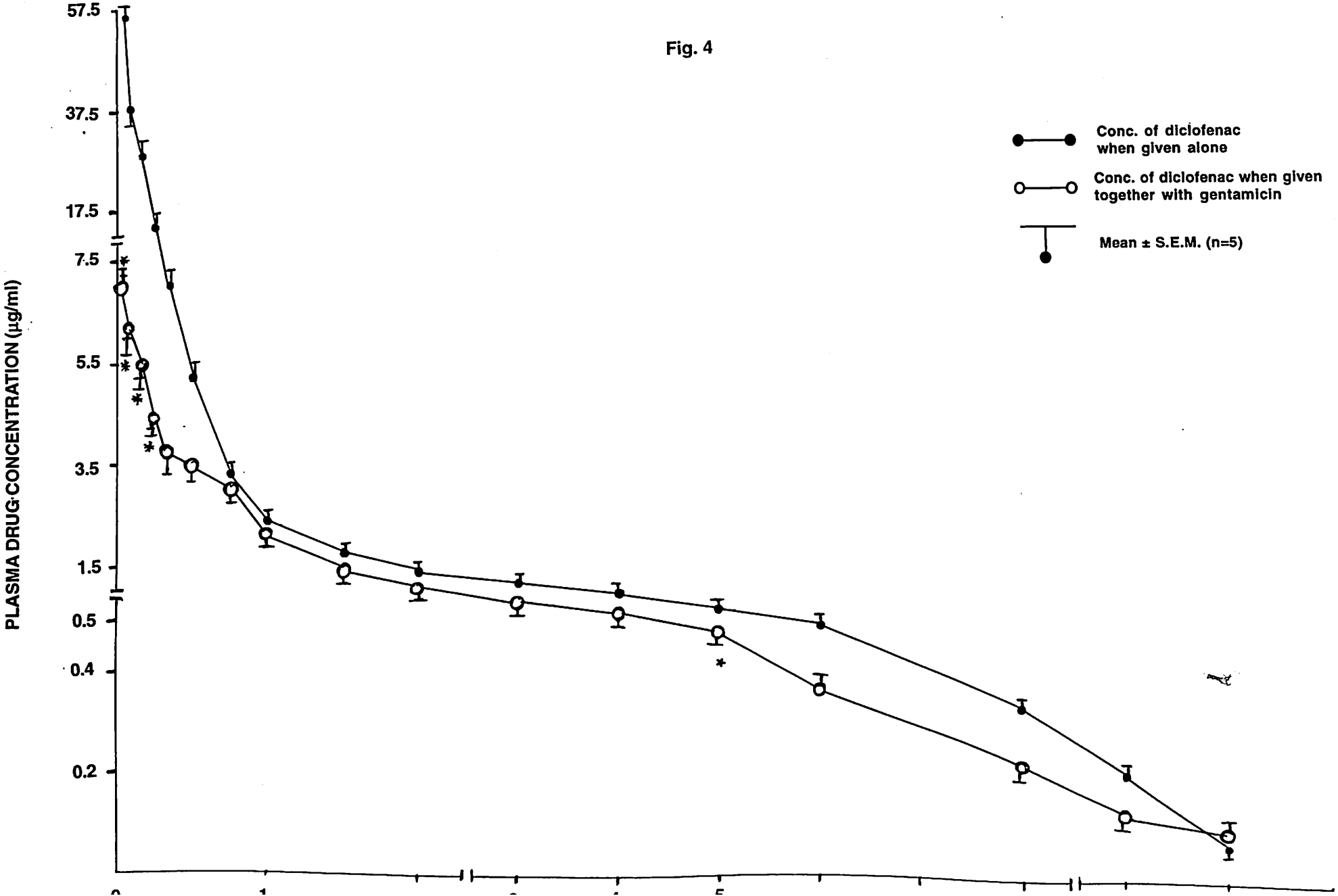
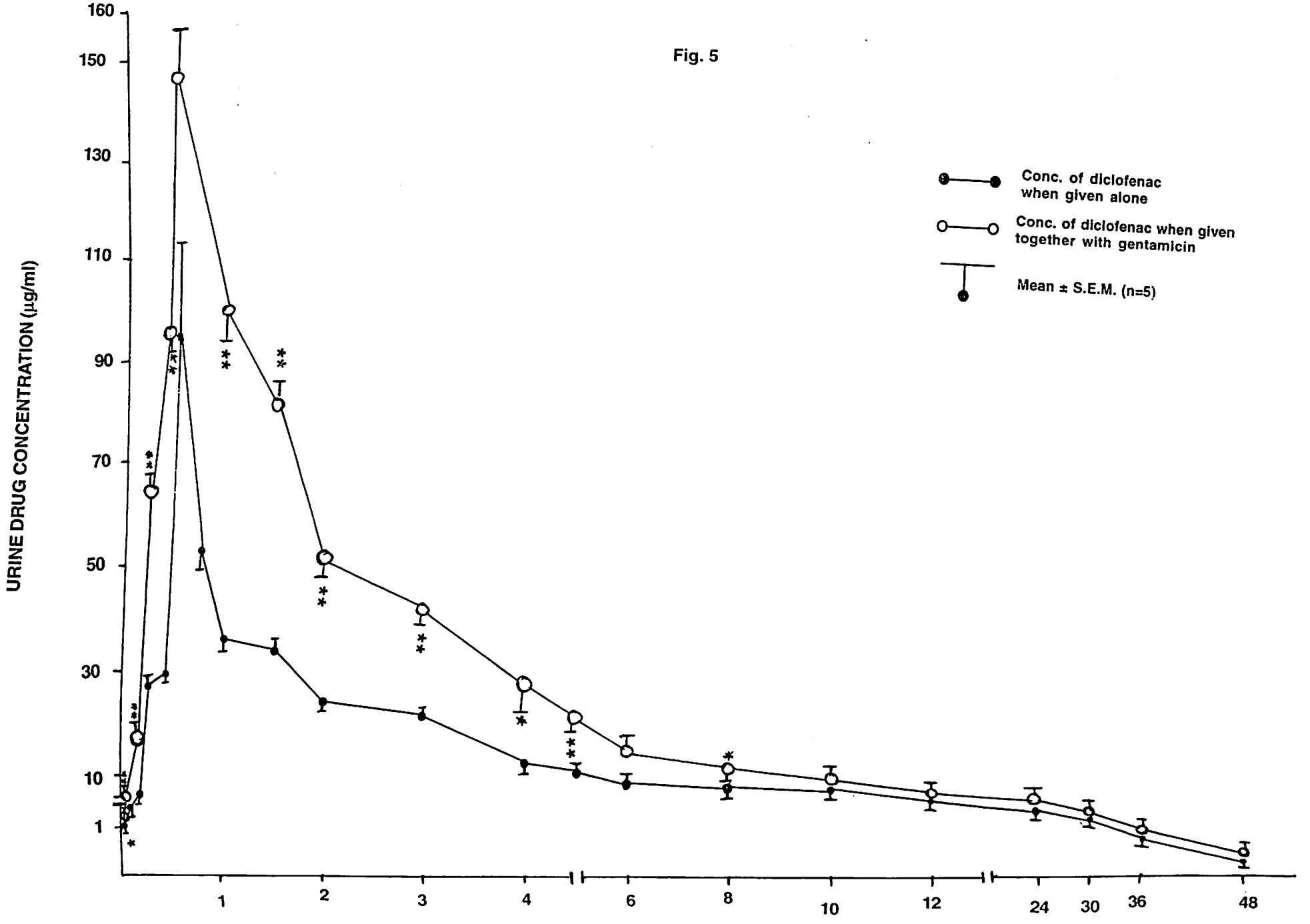

57.5
37.5
17.5
7.5
5.5
3.5
1.5
0.5
0.4
0.2
PLASMA DRUG CONCENTRATION (µg/ml)
Conc. of diclofenac when given alone
Conc. of diclofenac when given together with gentamicin
Mean ± S.E.M. (n=5)

Table – 6

Urine concentrations (μg/ml) of diclofenac in healthy goats following single intravenous dose of 2 mg/kg.

Time (h)	ANIMAL NUMBER					Mean ± S.E.M
	1	2	3	4	5	
0.042	2.26	0.95	1.68	2.10	1.42	1.68 ± 0.24
0.083	4.45	1.93	2.16	4.10	3.12	3.15 ± 0.50
0.167	7.75	4.88	5.61	7.68	6.23	6.43 ± 0.57
0.25	26.05	29.54	28.10	25.11	27.15	27.19 ± 0.77
0.333	28.00	32.02	29.10	27.92	31.18	29.64 ± 0.84
0.50	86.40	153.06	90.08	88.11	60.56	95.64 ± 15.33
0.75	50.40	50.04	61.56	59.10	46.18	53.46 ± 2.93
1	39.60	31.56	40.11	35.48	37.20	36.79 ± 1.55
1.5	37.70	29.04	38.24	33.11	34.18	34.45 ± 1.67
2	21.70	21.87	26.51	27.20	25.33	24.52 ± 1.16
3	19.54	20.47	23.11	21.68	22.16	21.39 ± 0.63
4	11.80	12.81	13.52	11.11	14.56	12.76 ± 0.61
5	9.16	11.36	10.12	9.81	10.65	10.22 ± 0.37
6	8.48	9.51	8.14	8.10	9.10	8.67 ± 0.28
8	7.20	7.80	7.10	7.13	6.54	7.15 ± 0.20
10	6.98	7.40	6.54	6.15	5.16	7.03 ± 0.14
12	6.10	5.14	5.86	5.86	4.21	5.43 ± 0.35
24	4.24	4.48	4.01	3.21	3.68	3.92 ± 0.22
30	1.56	3.06	2.58	1.58	1.50	2.06 ± 0.32
36	0.85	1.51	0.65	0.65	0.65	0.86 ± 0.17
48	0.36	0.58	0.32	0.11	0.28	0.33 ± 0.07

Fig. 5


URINE DRUG CONCENTRATION (µg/ml)
160
150
130
110
90
70
50
30
10
1
1
2
3
4
6
8
10
12
24
30
36
48
Conc. of diclofenac when given alone
Conc. of diclofenac when given together with gentamicin
Mean ± S.E.M. (n=5)

3. Kinetic parameters

Log plasma drug concentration versus time profile has confirmed the two-compartment open model. Table 7 shows the values of different kinetic parameters calculated by the above noted compartment model.

The mean extrapolated zero time concentration of the drug in plasma during distribution phase (A), elimination phase (B) and theoretical zero time concentration (C_p^o = A + B) were noted to be 45.95 ± 21.13, 2.69 ± 0.65 and 48.65 ± 21.24 μg/ml, respectively. The distribution rate constant (α) ranged from 3.561 to 7.773 h^{-1} with a mean value of 4.467 ± 0.827 h^{-1} while its elimination rate constant (β) ranged from 0.142 to 0.370 h^{-1} with a mean value of 0.262 ± 0.40 h^{-1}. The mean distribution half life ($t_{1/2}$ α) and elimination half life ($t_{1/2}$ β) value were observed to be 0.17 ± 0.02 and 2.97 ± 0.53 h, respectively. The value of area under curve in plasma (AUC) and area under first moment curve (AUMC) were found to 19.40±2.98 $mg.L^{-1}.h$ and 51.50 ± 17.56 $mg.L^{-1}.h^2$ with a mean residential time (MRT) of 2.62 ± 0.61 h. The average rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central (Kel) compartment were calculated to be 1.710 ± 0.141, 0.514 ± 0.041 and 2.509 ± 0.884 h^{-1}, respectively. The fraction of drug

Table - 7

Kinetic parameters of diclofenac in healthy goat (calculated by 2-compartment open model) after a single intravenous dose of 2 mg/kg

Kinetic Parameter	Unit	ANIMAL NUMBER					Mean ± S. E. M.
		1	2	3	4	5	
A	$\mu g.ml^{-1}$	20.70	130.00	30.25	29.46	19.34	45.95 ± 21.13
B	$\mu g.ml^{-1}$	1.35	2.91	2.32	5.08	1.82	2.69 ± 0.65
C_p^o	$\mu g.ml^{-1}$	22.05	132.91	32.57	34.54	21.16	48.65 ± 21.24
α	h^{-1}	3.749	7.773	3.561	3.642	3.630	4.467 ± 0.827
β	h	0.221	0.336	0.142	0.370	0.239	0.262 ± 0.04
$t_{1/2}\ \alpha$	h^{-1}	0.18	0.09	0.19	0.19	0.19	0.17 ± 0.02
$t_{1/2}\ \beta$	h	3.13	2.06	4.87	1.87	2.90	2.97 ± 0.53
AUC	$mg.L^{-1}.h$	11.66	25.55	25.07	21.82	12.91	19.40 ± 2.98
AUMC	$mg.L^{-1}.h^2$	29.36	28.88	120.75	45.20	33.06	51.50 ± 17.52
MRT	h	2.52	1.13	4.82	2.07	2.56	2.62 ± 0.61
K_{12}	h^{-1}	1.651	1.696	2.191	1.310	1.704	1.710 ± 0.141
K_{21}	h^{-1}	0.441	0.437	0.499	0.660	0.531	0.514 ± 0.041
Kel	h^{-1}	1.881	5.976	1.013	2.042	1.634	2.509 ± 0.884
Fc	-	0.12	0.06	0.14	0.18	0.15	0.13 ± 0.02
$T \approx P$	-	7.50	16.79	6.14	4.52	5.84	8.16 ± 2.21
Vd_c	$L.kg^{-1}$	0.09	0.02	0.06	0.06	0.09	0.06 ± 0.01
Vd_B	$L.kg^{-1}$	1.48	0.69	0.86	0.39	1.10	0.90 ± 0.18
Vd_{area}	$L.kg^{-1}$	0.78	0.23	0.56	0.25	0.65	0.49 ± 0.11
Vd_{SS}	$L.kg^{-1}$	0.43	0.09	0.32	0.18	0.38	0.28 ± 0.06
Cl_B	$ml.kg^{-1}.min^{-1}$	2.83	1.29	1.32	1.54	2.59	1.91 ± 0.33

available for elimination from central compartment (Fc) and approximate tissue to plasma concentration ratio (T≈P) were noted to be 0.13 ± 0.02 and 8.16 ± 2.21. The various values of value of distribution calculated by different method are shown in Table-7. The mean value of Vd_{area} was calculated to be 0.49 ± 0.11 $L.kg^{-1}$. The total body clearance (Cl_B) ranged from 1.29 to 2.83 with a mean value of 1.91 ± 0.33 $ml.kg^{-1}.min^{-1}$.

II. KINETIC STUDIES OF GENTAMICIN AND DICLOFENAC AFTER THEIR COMBINED ADMINISTRATION

(A) GENTAMICIN

1. Plasma levels

Plasma concentrations of gentamicin at different time intervals following combined i.v. administration of gentamicin (7.5 mg/kg) and diclofenac (2 mg/kg) have been shown in Table 8 and Fig. 1. The drug was present at 0.042 h with a mean of 36.91 ± 4.98 μg/ml and was detectable in plasma samples of all the goats upto 12h with a mean value of 0.27 ± 0.03 μg/ml. The drug was detectable in 3 out of 5 animals at 24 h. The mean therapeutic concentration (≥ 2 μg/ml) was maintained around 4 h.

Table – 8

Plasma concentrations (μg/ml) of gentamicin in healthy goats following combined administration of gentamicin (7.5 mg/kg) and diclofenac (2mg/kg) after i.v. administration

Time (h)	ANIMAL NUMBER					Mean ± S.E.M
	1	2	3	4	5	
0.042	21.11	48.11	38.12	31.10	46.12	36.91 ± 4.98
0.083	18.38	44.56	33.32	25.63	37.10	31.80 ± 4.54
0.167	13.30	37.10	29.54	23.10	32.33	27.07 ± 4.12
0.25	10.56	31.35	24.63	18.66	27.17	22.47 ± 3.62
0.333	8.38	26.33	20.11	15.32	22.15	18.46 ± 3.08
0.50	6.65	21.00	15.58	12.12	17.33	14.54 ± 2.44
0.75	5.28	15.20	11.56	9.82	11.12	10.60 ± 1.60
1	4.19	12.10	8.86	7.21	8.81	8.23 ± 1.29
1.5	3.10	8.38	5.93	4.62	6.66	5.74 ± 0.90
2	2.64	6.40	4.20	3.33	5.40	4.39 ± 0.68
3	1.66	4.30	3.81	2.21	3.51	3.10 ± 0.50
4	1.30	3.17	2.11	1.83	2.72	2.23 ± 0.33
5	1.05	2.16	1.56	1.46	1.98	1.64 ± 0.20
6	0.83	1.80	1.21	1.18	1.46	1.30 ± 0.16
8	0.52	1.00	0.68	0.73	0.81	0.75 ± 0.08
10	0.31	0.56	0.40	0.45	0.42	0.43 ± 0.04
12	0.22	0.31	0.23	0.36	0.22	0.27 ± 0.03
24	N.D.	0.10	N.D.	0.16	0.11	0.12 ± 0.02

N.D. = Non detectable

2. Urine levels

Urine concentrations of gentamicin at various time intervals following combined i.v. administration of gentamicin (7.5 mg/kg) and diclofenac (2 mg/kg) have been presented in Table 9 and Fig. 2. The drug appeared in all animals at 0.042 h with the mean of 12.29 ± 0.97 μg/ml, The drug attained its peak concentration of 525.9 ± 11.96 μg/ml at 45 min. The drug was detectable in all the animals upto 36 h with a mean value of 0.64 ± 0.14 μg/ml. The concentration of the drug was obtained only in 3 out of 5 animals at 48 h with a mean value of 0.30 ± 0.09 μg/ml. The therapeutic concentration of ≥ 2 μg/ml was maintained upto 24 h.

3. Kinetic parameters

Plasma drug concentration *versus* time profile has confirmed a two compartment open model and hence, the kinetic parameters were calculated by using the formula of the above noted compartment model.

Table 10 presents the values of different kinetic parameters of gentamicin after its combined i.v. administration with diclofenac. The mean extrapolated zero time concentration of drug in plasma during distribution phase (A), elimination phase (B) and the theoretical zero time concentration (C_p°) were noted to be 30.00 ± 5.17, 4.22 ± 0 .96 and 34.22 ± 6.03 μg/ml, respectively. The

Table – 9

Urine concentrations (μg/ml) of gentamicin in healthy goats following combined administration of gentamicin (7.5 mg/kg) and diclofenac (2 mg/kg) after intravenous administration.

Time (h)	ANIMAL NUMBER					Mean ± S.E.M
	1	2	3	4	5	
0.042	9.59	11.56	15.44	13.11	11.76	12.29 ± 0.97
0.083	29.60	35.12	32.12	30.10	36.28	32.64 ± 1.33
0.167	69.48	70.34	65.12	68.11	62.10	67.03 ± 1.52
0.25	90.68	100.12	98.12	95.45	98.54	96.58 ± 1.66
0.333	180.6	199.2	186.9	176.1	165.100	181.6 ± 5.66
0.50	221.0	230.5	228.5	252.1	235.1	233.4 ± 5.19
0.75	533.2	560.2	535.8	510.1	490.1	525.9 ± 11.96
1	496.5	460.1	4.35.7	410.3	405.2	441.6 ±16.88
1.5	408.3	380.5	375.4	360.1	320.6	369.0 ± 14.38
2	350.2	290.2	310.5	280.2	300.1	326.3 ± 19.65
3	123.2	150.5	130.5	140.2	135.6	136.0 ± 4.60
4	72.32	80.16	78.12	71.00	73.12	74.94 ± 1.77
5	33.56	50.42	38.61	45.13	46.31	42.80 ± 2.99
6	28.54	28.66	26.12	30.12	29.34	28.56 ± 0.67
8	11.12	15.43	14.34	12.13	12.31	13.07 ± 0.79
10	8.13	9.10	7.32	6.54	8.21	7.86 ± 0.43
12	5.40	5.68	5.12	4.13	4.38	4.94 ± 0.29
24	1.58	3.12	2.10	3.10	2.34	2.45 ± 0.29
30	0.86	1.18	1.10	2.10	0.88	1.22 ± 0.23
36	0.32	0.86	0.73	0.98	0.32	0.64 ± 0.14
48	N.D.	0.12	0.33	0.45	N.D.	0.30 ± 0.09

N.D. = Non detectable

Table – 10

Kinetic parameters of gentamicin in healthy goats following combined administration of gentamicin (7.5 mg/kg) and diclofenac (2 mg/kg) after intravenous administration.

Kinetic parameter	Unit	ANIMAL NUMBER					Mean ± S.E.M.
		1	2	3	4	5	
A	$\mu g.ml^{-1}$	13.03	37.80	42.19	24.47	32.50	30.00 ± 5.17
B	$\mu g.ml^{-1}$	2.31	5.00	7.53	2.43	3.83	4.22 ± 0.96
C_p^o	$\mu g.ml^{-1}$	15.34	42.80	49.72	26.90	36.33	34.22 ± 6.03
α	h^{-1}	1.498	1.392	3.093	1.483	1.427	1.777 ± 0.329
$t_{½\alpha}$	h	0.46	0.50	0.22	0.47	0.49	0.43 ± 0.05
β	h^{-1}	0.161	0.180	0.296	0.127	0.170	0.187 ± 0.029
$t_{½\beta}$	h	4.31	3.85	2.35	5.47	4.28	4.01 ± 0.50
AUC	$mg.L^{-1}.h$	23.05	54.93	39.08	35.63	45.30	39.60 ± 5.28
AUMC	$mg.L^{-1}.h^2$	94.92	173.8	90.35	161.8	148.5	133.9 ± 17.32
MRT	h	4.12	3.16	2.31	4.54	3.28	3.48 ± 0.39
K_{12}	h^{-1}	0.631	0.472	1.397	0.605	0.492	0.519 ± 0.043
K_{21}	h^{-1}	0.362	0.322	0.719	0.249	0.302	0.391 ± 0.084
Kel	h^{-1}	0.666	0.778	1.273	0.756	0.803	0.855 ± 0.107
Fc	-	0.24	0.23	0.23	0.17	0.21	0.22 ± 0.01
$T \approx P$	-	3.14	3.11	3.30	4.96	3.73	3.65 ± 0.35
Vd_c	$L.kg^{-1}$	0.49	0.18	0.15	0.28	0.21	0.26 ± 0.06
Vd_B	$L.kg^{-1}$	3.25	1.50	0.99	3.09	1.96	2.16 ± 0.44
Vd_{area}	$L.kg^{-1}$	2.02	0.76	0.65	1.66	0.97	1.21 ± 0.27
Vd_{ss}	$L.kg^{-1}$	1.34	0.44	0.44	0.96	0.55	0.75 ± 0.18
Cl_B	$ml.kg^{-1}.min^{-1}$	5.42	2.28	3.21	3.51	2.75	3.43 ± 0.54

distribution rate constant (α) ranged from 1.392 to 3.093 h^{-1} with the mean value of 1.777 ± 0.329 h^{-1} whereas its elimination rate constant ranged from 0.161 to 0.296 h^{-1} with a mean of 0.187 ± 0.029 h^{-1}. The mean distribution half life ($t_{1/2}\ \alpha$) and elimination half life ($t_{1/2}\ \beta$) were observed to be 0.43 ± 0.05 and 4.01 ± 0.50 h. The value of area under curve in plasma (AUC), area under first moment curve (AUMC) and mean residential time (MRT) were found to be 39.60 ± 5.28 $mg.L^{-1}h$, 133.98 ± 17.32 $mg.L^{-1}.h^2$ and 3.48 ± 0.39 h, respectively. The average rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central (Kel) compartment were calculated to be 0.519 ± 0.043, 0.391 ± 0.084 and 0.855 ± 0.107 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 0.22 ± 0.01 and 3.65 ± 0.35. The various values of volume distribution calculated by different methods are shown in Table 10. The mean value of Vd_{area} was calculated to be 1.21 ± 0.27 $L.kg^{-1}$. The total body clearance (Cl_B) ranged from 2.28 to 5.42 with an average of 3.43 ± 0.54 $ml.kg^{-1}.min^{-1}$.

4. Dosage regimen

Table 11 presents the calculated dosage regimen of gentamicin following combined administration of this drug with diclofenac in goats. For maintaining C_p^{∞} min of 1 μg/ml, the loading

Table – 11

Dosage regimen of gentamicin in healthy goats following administration of diclofenac (2 mg/kg) and gentamicin (7.5 mg/kg) after i.v. administration

C_p° min (μg/ml)	γ (h)	Dose (mg/kg)	Animal Number					Mean ± S. E. M.
			1	2	3	4	5	
1	8	D*	7.32	3.21	6.94	4.59	3.78	5.17 ± 0.83
		D_o	5.30	2.45	6.29	2.92	2.81	3.95 ± 0.77
	12	D*	13.94	6.59	22.67	7.62	7.46	11.66 ± 3.05
		D_o	11.92	5.83	22.02	5.96	6.49	10.44 ± 3.11
2	8	D*	14.64	6.42	13.88	9.18	7.56	10.34 ± 1.66
		D_o	10.60	4.90	12.58	5.84	5.62	7.91 ± 1.54
	12	D*	27.88	13.18	45.34	15.24	14.92	23.31 ± 6.10
		D_o	23.84	11.66	44.04	11.92	12.98	20.89 ± 6.22
4	8	D*	29.28	12.84	27.76	18.36	15.12	20.67 ± 3.33
		D_o	21.20	9.80	25.16	11.68	11.24	15.82 ± 3.09
	12	D*	55.76	26.36	136.02	30.48	29.84	55.69 ± 20.76
		D_o	47.68	23.32	88.08	23.84	25.96	11.78 ± 12.43

D* = Priming or loading dose

D_0 = Maintenance dose

γ = Dosage interval

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

doses (D*s) were calculated to be 5.17 ± 0.83 and 11.66 ± 3.05 mg/kg, while maintenance doses (D_0s) were calculated to be 3.95 ± 0.77 and 10.44 ± 3.11 mg/kg at selected dosage intervals (γ) of 8 and 12 h, respectively. Similarly for maintaining C_p^{∞} min of 2 μg/ml, the D*s were noted be 10.34 ± 1.66 and 23.31 ± 6.10 mg/kg, while D_0s were noted to be 7.91 ± 1.54 and 20.89 ± 6.22 mg/kg at γ of 8 and 12 h, respectively. For maintaining C_p^{∞} min of 4 μg/ml, the calculated D*s and D_0s were noted to be 20.67 ± 3.33 and 55.69 ± 20.76 and 15.82 ± 3.09 and 41.78 ± 12.43 mg/kg, respectively at γ of 8 and 12 h.

(B) DICLOFENAC

1. Plasma levels

Concentrations of diclofenac in plasma after combined i.v. administration of gentamicin (7.5 mg/kg) and diclofenac (2 mg/kg) are presented in Table 12 and Fig. 4. The drug appeared with a mean concentration of 7.04 ± 0.48 μg/ml at 0.042 h. The drug was present in all animals upto 10 h with a mean of 0.14 ± 0.04 μg/ml. The concentration of the drug was obtained in 4 out of 5 animals at 12 h with a mean of 0.10 ± 0.03 μg/ml. The drug was not detectable in any animal at 24 h.

Table – 12

Plasma concentrations (μg/ml) of diclofenac in goats following combined administration of gentamicin (7.5 mg/kg) and diclofenac (2 mg/kg) after i.v. administration.

Time (h)	ANIMAL NUMBER					Mean ± S.E.M
	1	2	3	4	5	
0.042	7.70	5.43	7.50	8.10	6.48	7.04 ± 0.48
0.083	7.40	5.39	6.10	6.20	6.12	6.24 ± 0.32
0.167	7.10	4.86	5.02	5.15	5.50	5.53 ± 0.41
0.25	4.65	4.10	4.60	4.70	4.28	4.47 ± 0.12
0.333	3.45	3.77	4.15	4.24	3.55	3.83 ± 0.16
0.50	3.30	3.60	3.85	3.90	3.20	3.57 ± 0.14
0.75	3.20	2.86	3.05	3.32	2.65	3.02 ± 0.12
1	1.98	2.41	2.48	2.60	1.85	2.26 ± 0.15
1.5	1.22	1.46	1.54	2.02	1.20	1.49 ± 0.15
2	0.82	1.13	1.35	1.50	1.00	1.16 ± 0.12
3	0.53	0.90	1.05	1.05	0.74	0.85 ± 0.09
4	0.40	0.74	0.82	0.83	0.48	0.65 ± 0.09
5	0.18	0.52	0.74	0.54	0.40	0.48 ± 0.09
6	0.15	0.46	0.56	0.45	0.25	0.37 ± 0.08
8	0.08	0.24	0.42	0.24	0.14	0.22 ± 0.06
10	0.06	0.17	0.25	0.14	0.06	0.14 ± 0.04
12	0.05	0.09	0.18	0.07	N.D.	0.10 ± 0.03
24	N.D.	N.D.	N.D.	N.D.	N.D.	-

N.D. = Non detectable

2. Urine levels

Table 13 and Fig. 5 depict the urine concentrations of diclofenac in goats following combined i.v. and administration of gentamicin (7.5 mg/kg) and diclofenac (2 mg/kg) The drug was detectable in all the animals at 0.042 h and it ranged from 1.81 to 2.80 μg/ml with a mean of 2.34 ± 0.19 μg/ml. The drug reached it peak urine concentration of 147.96 ± 9.02 μg/ml at 30 min. Thereafter, concentration of the drug declined with time and was present in all animals upto 48 h with a mean of 0.48 ± 0.13 μg/ml.

3. Kinetic parameters

Plasma diclofenac concentrations *versus* time profile had shown biphasic pattern following combined i.v. administration of gentamicin and diclofenac and hence, kinetic parameters were derived by using 2-compartment open model.

Table 14 presents the different values of kinetic parameters calculated by the above noted compartment model. The extrapolated zero time concentration during distribution phase (A), elimination phase (B) and theoretical zero time concentration (C_p^o) were noted to be 4.90 ± 0.39, 1.91 ± 0.26 and 6.82 ± 0.16 $\mu g.ml^{-1}$. The distribution rate constant (α) and elimination rate constant (β) were noted to be 1.892 ± 0.143 and 0.285 ± 0.027 h^{-1}, respectively.

Table – 13

Urine concentrations (μg/ml) of diclofenac in healthy goats following combined administration of gentamicin (7.5 mg/kg) and diclofenac (2 mg/kg) after intravenous administrations

Time (h)	ANIMAL NUMBER					Mean ± S.E.M
	1	2	3	4	5	
0.042	2.80	2.48	1.81	2.62	1.98	2.34 ± 0.19
0.083	6.15	5.40	7.10	6.83	6.54	6.40 ± 0.30
0.167	15.50	14.36	16.11	19.12	20.11	17.04 ± 1.10
0.25	50.16	48.52	53.12	59.16	60.23	64.24 ± 2.35
0.333	90.23	88.67	96.12	100.18	102.34	95.51 ± 2.67
0.50	131.3	128.2	140.1	170.1	168.1	147.6 ± 9.02
0.75	122.8	115.1	128.3	145.2	140.2	130.3 ± 5.53
1	98.4	88.11	100.1	106.9	105.1	99.74 ± 3.30
1.5	77.60	76.10	86.54	81.12	86.54	81.58 ± 2.18
2	41.28	38.19	65.12	50.12	64.12	51.77 ± 1.60
3	39.60	37.11	46.11	38.63	45.13	41.31 ± 1.80
4	24.20	20.20	32.57	20.18	38.75	27.18 ± 3.67
5	22.60	18.11	19.18	18.11	22.06	20.01 ± 0.97
6	16.58	13.12	15.68	10.28	16.85	14.50 ± 1.24
8	12.18	10.16	11.35	8.16	12.81	10.93 ± 0.82
10	10.11	8.11	9.10	6.23	9.63	8.63 ± 0.69
12	8.31	7.26	6.54	4.16	6.54	6.56 ± 0.68
24	7.37	6.58	5.13	2.11	4.32	5.10 ± 0.92
30	4.48	3.12	2.18	0.92	1.68	2.48 ± 0.43
36	1.01	1.11	0.98	0.63	0.74	0.89 ± 0.09
48	0.63	0.89	0.32	0.12	0.42	0.48 ± 0.13

Table – 14

Kinetic parameters of diclofenac in healthy goats following combined administration of gentamicin (7.5 mg/kg) and diclofenac (2 mg/kg) after intravenous administration

Kinetic Parameter	Unit	ANIMAL NUMBER					Mean ± S.E.M.
		1	2	3	4	5	
A	$\mu g.ml^{-1}$	6.30	4.38	5.04	4.01	4.79	4.90 ± 0.39
B	$\mu g.ml^{-1}$	1.02	1.96	1.89	2.65	2.04	1.91 ± 0.26
C_p^o	$\mu g.ml^{-1}$	7.32	6.34	6.93	6.66	6.83	6.82 ± 0.16
α	h^{-1}	1.570	2.08	1.720	1.731	2.357	1.892 ± 0.143
β	h	0.330	0.250	0.200	0.301	0.346	0.285 ± 0.027
$t_{1/2}\,\alpha$	h^{-1}	0.44	0.33	0.40	0.40	0.29	0.37 ± 0.03
$t_{1/2}\,\beta$	h	2.12	2.73	3.53	2.31	2.01	2.53 ± 0.27
AUC	$mg.L^{-1}h$	7.10	9.95	12.38	11.12	7.93	9.69 ± 0.98
AUMC	$mg.L^{-1}h^2$	11.92	32.52	48.95	30.59	17.90	28.38 ± 6.42
MRT	h	1.68	3.27	3.95	2.75	2.26	2.78 ± 0.39
K_{12}	h^{-1}	0.371	0.880	0.750	0.560	0.891	0.69 ± 0.010
K_{21}	h^{-1}	0.501	0.820	0.610	0.870	0.951	0.750 ± 0.084
Kel	h^{-1}	1.030	0.632	0.561	0.601	0.860	0.737 ± 0.090
Fc	-	0.32	0.39	0.36	0.50	0.40	0.39 ± 0.03
$T \approx P$	-	2.18	1.54	1.83	0.98	1.47	1.59 ± 0.20
Vd_c	$L.kg^{-1}$	0.27	0.32	0.29	0.30	0.29	0.29 ± 0.08
Vd_B	$L.kg^{-1}$	1.96	1.02	1.06	0.76	0.98	1.16 ± 0.21
Vd_{area}	$L.kg^{-1}$	0.85	0.80	0.81	0.60	0.73	0.76 ± 0.04
Vd_{SS}	$L.kg^{-1}$	0.47	0.66	0.65	0.49	0.56	0.57 ± 0.04
Cl_B	$ml.kg^{-1}.min^{-1}$	4.67	3.32	2.70	3.01	4.21	3.58 ± 0.37

Distribution half life ($t_{1/2}\ \alpha$) ranged from 0.29 to 0.44 h with a mean of 0.37 ± 0.03 h whereas the elimination half life ($t_{1/2}\ \beta$) ranged from 2.01 to 3.53 h with an average of 2.53 ± 0.27 h. Area under curve (AUC) area under first moment curve (AUMC) and mean residential time (MRT) were calculated to be 9.69 ± 0.98 $mg.L^{-1}.h$, 28.38 ± 6.42 $mg.L^{-1}h^2$ and 2.78 ± 0.39 h, respectively. The average rate constant of drug transfer from central to peripheral (K_{12}), peripheral to central (K_{21}) and elimination from central (Kel) compartment were observed to be 0.690 ± 0.010, 0.750 ± 0.084 and 0.737 ± 0.090 h^{-1}, respectively. The mean value of fraction of drug available for elimination from central compartment (Fc) and approximate tissue to plasma concentration ratio (T ≈ P) were observed to be 0.39 ± 0.03 and 1.59 ± 0.20, respectively. The different values of volume distribution calculated by different methods are shown in Table 14. The mean Vd_{area} of 0.76 ± 0.04 $L.kg^{-1}$ was observed. The total body clearance (Cl_B) ranged from 2.70 ± 4.67 $ml.kg^{-1}.min^{-1}$ with the mean value of 3.58 ± 0.37 $ml.kg^{-1}.min^{-1}$.

III. COMPARISON OF KINETICS OF GENTAMICIN WHEN GIVEN ALONE AND WHEN GIVEN TOGETHER WITH DICLOFENAC BY I.V. ADMINISTRATION.

1. Plasma levels

Comparison of plasma concentrations of gentamicin (7.5 mg/kg) when given alone and when given together with

diclofenac (2 mg/kg) after i.v. administration are presented in Table 15 and Fig.1. Concentration of gentamicin were found to differ non-significantly when it was given along with diclofenac as compared to its single administration from 0.042 to 3 h and at 8, 12 and 24 h. Significant difference was observed from 4 to 6 h and at 10 h. The therapeutic concentration (≥ 2 μg/ml) was maintained upto 5 h when gentamicin was given alone and upto 4 h when gentamicin was administered along with diclofenac. Concentrations of gentamicin were detected upto 24 h in both the groups.

2. Urine levels

Table 15 and Fig. 2 reveal urine concentrations of gentamicin when given alone and when given together with diclofenac. Concentrations of the drug is urine were significantly higher ($p < 0.01$) at 30 min. and 12 h in case of single administration. The drug was present in significantly higher concentrations ($p<0.05$) at 15 min, 45 min, 1.5 to 3 h, in case of combined administration. The drug was detected upto 48 h in both the groups. The drug attained its peak level at 45 min in both the groups with a concentration of 455.4 ± 15.05 μg/ml when given alone as compared to the concentration of 526.0 ± 11.96 μg/ml when given in combination with diclofenac. The mean therapeutic concentration in urine (≥ 2 μg/ml) was maintained upto 30 h in case of single administration and 24 h in case of combined administration.

Table – 15

Comparison of plasma and urine concentrations (μg/ml) of gentamicin (7.5 mg/kg) when given alone and when given together with diclofenac (2mg/kg) in healthy goats following intravenous administration

Time (h)	Gentamicin given alone		Gentamicin + Diclofenac given together	
	Plasma	Urine	Plasma	Urine
0.042	34.39 ± 3.62	13.72 ± 1.45	36.91± 4.98$^{N.S}$	12.29 ± 0.97 $^{N.S}$
0.083	26.17 ± 3.34	34.16 ± 2.72	31.80 ± 4.54 $^{N.S}$	32.64 ± 1.33 $^{N.S}$
0.167	22.67 ± 3.35	62.29 ± 3.38	27.07 ± 4.12 $^{N.S}$	67.03 ± 1.52 $^{N.S}$
0.25	17.41 ± 2.35	85.30 ± 4.80	22.47 ± 3.62 $^{N.S}$	96.58 ± 1.66 *
0.333	15.38 ± 2.29	186.3 ± 5.98	18.46 ± 3.08 $^{N.S}$	181.6 ± 5.66 $^{N.S}$
0.50	12.44 ± 1.98	316.6 ± 4.50	14.54 ± 2.44 $^{N.S}$	233.4 ± 5.19 **
0.75	9.97 ± 1.38	455.4 ± 5.05	10.60 ± 1.60 $^{N.S}$	526.0 ± 11.96 *
1	7.78 ± 0.97	341.5 ± 18.85	8.23 ± 1.29 $^{N.S}$	441.6 ± 16.88 $^{N.S}$
1.5	6.49 ± 0.98	244.9 ± 18.13	5.74 ± 0.90 $^{N.S}$	369.0 ± 14.38 *
2	5.10 ± 0.66	180.7 ± 6.88	4.39 ± 0.68 $^{N.S}$	327.0 ± 19.65 **
3	3.83 ± 0.55	115.3 ± 5.47	3.10 ± 0.50 $^{N.S}$	136.0 ± 4.60 *
4	3.07 ± 0.51	62.07 ± 8.46	2.23 ± 0.33 *	74.94 ± 1.77 $^{N.S}$
5	2.32 ± 0.40	37.50 ± 4.74	1.64 ± 0.20 *	42.8 ± 2.99 $^{N.S}$
6	1.82 ± 0.33	26.89 ± 2.79	1.30 ± 0.16 *	28.56 ± 0.67 $^{N.S}$
8	1.09 ± 0.20	17.42 ± 1.83	0.75 ± 0.08 $^{N.S}$	13.07 ± 0.79 $^{N.S}$
10	0.70 ± 0.13	12.39 ± 1.33	0.43 ± 0.04 *	7.86 ± 0.43 *
12	0.46 ± 0.10	8.90 ± 1.09	0.27 ± 0.03 $^{N.S}$	4.94 ± 0.29 **
24	0.31 ± 0.05	5.46 ± 0.51	0.12 ± 0.02 $^{N.S}$	2.45 ± 0.29 *
30	N.D.	2.96 ± 0.50	N.D.	1.22 ± 0.23 $^{N.S}$
36	N.D.	1.40 ± 0.39	N.D.	0.64 ± 0.14 $^{N.S}$
48	N.D.	0.70 ± 0.12	N.D.	0.30 ± 0.09 $^{N.S}$

N.D. = Non detectable,

$^{N.S}$ = Non significant, * $p < 0.05$, ** $p < 0.01$

3. Kinetic parameters

Table 16 presents the kinetic parameters of gentamicin when it was given alone (7.5 mg/kg) and when given together with diclofenac (2 mg/kg) following i.v. administration. The values of extrapolated zero time concentration during distribution phase (A), elimination phase (B), theoretical zero time concentration (C_p^o), elimination rate constant (β), distribution rate constant (α), elimination half life ($t_{1/2}\,\beta$), distribution half life ($t_{1/2}\,\alpha$), mean area under curve (AUC), area under first moment curve (AUMC), mean residential time (MRT), rate of transfer of drug from central to peripheral (K_{12}), peripheral to central (K_{21}), elimination from central (Kel) compartment, fraction of drug available for elimination from central compartment (Fc), approximate tissue to plasma concentration ratio ($T \approx P$), various volume of distribution (Vd_c, Vd_B, Vd_{area}, Vd_{SS}) and total body clearance (Cl_B) did not differ significantly between both the groups.

4. Dosage regimen

The comparison of calculated dosage regimen of gentamicin when given alone and when given together with diclofenac in goats following i.v. administration are shown in Table 17. No significant difference was observed for loading doses (D*s) and

Table – 16

Comparison of kinetic parameters of gentamicin when given alone (7.5 mg/kg) and when given together with diclofenac (2 mg/kg) in healthy goats following i.v. administration

Kinetic Parameter	Unit	Gentamicin given alone	Gentamicin + Diclofenac given together
A	$\mu g.ml^{-1}$	23.05 ± 4.38	30.00 ± 5.17
B	$\mu g.ml^{-1}$	5.43 ± 1.00	4.22 ± 0.96
C_p^o	$\mu g.ml^{-1}$	28.48 ± 5.21	34.22 ± 6.03
α	h^{-1}	1.944 ± 0.433	1.777 ± 0.329
$t_{1/2}\ \alpha$	h	0.42 ± 0.08	0.43 ± 0.05
β	h^{-1}	0.185 ± 0.035	0.187 ± 0.029
$t_{1/2}\ \beta$	h	4.28 ± 0.70	4.01 ± 0.50
AUC	$mg.L^{-1}h$	44.91 ± 7.27	39.60 ± 5.28
AUMC	$mg.L^{-1}h^2$	212.4 ± 55.58	133.9 ± 17.32
MRT	h	4.53 ± 0.65	3.48 ± 0.39
K_{12}	h^{-1}	0.911 ± 0.233	0.519 ± 0.043
K_{21}	h^{-1}	0.566 ± 0.150	0.391 ± 0.084
Kcl	h^{-1}	0.659 ± 0.101	0.855 ± 0.107
Fc	-	0.28 ± 0.03	0.22 ± 0.01
$T \approx P$	-	2.72 ± 0.30	3.65 ± 0.35
Vd_c	$L.kg^{-1}$	0.30 ± 0.06	0.26 ± 0.06
Vd_B	$L.kg^{-1}$	1.55 ± 0.24	2.16 ± 0.44
Vd_{area}	$L.kg^{-1}$	1.08 ± 0.15	1.21 ± 0.27
Vd_{SS}	$L.kg^{-1}$	0.79 ± 0.11	0.75 ± 0.18
Cl_B	$ml.kg^{-1}.min^{-1}$	3.21 ± 0.70	3.43 ± 0.54

All data are non-significant

Table – 17

Comparison of calculated dosage regimen of gentamicin when given alone and when together with diclofenac in healthy goats following i.v. administration

min g/ml)	γ (h)	Dose (mg/kg)	Gentamicin given alone	Gentamicin + Diclofenac
	8	D*	5.29 ± 1.62	5.17 ± 0.83
		D_o	4.22 ± 1.58	3.95 ± 0.77
	12	D*	13.18 ± 5.58	11.66 ± 3.05
		D_o	12.10 ± 5.56	10.44 ± 3.11
2	8	D*	10.58 ± 2.89	10.34 ± 1.66
		D_o	8.44 ± 3.16	7.91 ± 1.54
	12	D*	26.35 ± 11.77	23.31 ± 6.10
		D_o	24.20 ± 11.12	20.89 ± 6.22
4	8	D*	21.29 ± 6.41	20.67 ± 3.33
		D_o	16.87 ± 6.33	15.82 ± 3.09
	12	D*	52.70 ± 2.33	55.64 ± 20.76
		D_o	48.40 ± 22.25	41.78 ± 12.43

All data are non-significant

D* = Priming or loading dose

D_0 = Maintenance dose

γ = Dosage interval

C_p^r min = Minimum therapeutic concentration in plasma (MIC)

maintenance doses (D_0s) for maintaining (C_p° min) of 1, 2 and 4 μg/ml at the selected dosage interval (γ) of 8 and 12 h for both the groups.

IV. COMPARISON OF KINETICS OF DICLOFENAC WHEN GIVEN ALONE AND WHEN GIVEN TOGETHER WITH GENTAMICIN

1. Plasma levels

Plasma concentrations of diclofenac when given alone (2 mg/kg) and when given together with gentamicin (7.5 mg/kg) after i.v. administration in goats are presented in Table 18 and Fig. 5. Concentrations of diclofenac in plasma were noted to be significantly higher ($p<0.05$) from 0.042 h to 0.25 h and at 5 h when given alone as compared to its combined administration in the gentamicin.

2. Urine levels

Table 18 and Fig. 6 depict the comparison of urine concentrations of diclofenac when given alone and when together with gentamicin. Concentrations of diclofenac were observed to be significantly higher from 0.042 to 8 h (except at 0.5 and 6 h) in case of combined administration as compared to its single i.v. administration. No significant difference was observed at 0.5 and 6 h and from 10 to 48 h between both the groups.

Table – 18

Comparison of plasma and urine concentration of diclofenac when given alone (2 mg/kg) and when given together with gentamicin (7.5 mg/kg) in healthy goats following intravenous administration

Time (h)	Diclofenac given alone		Diclofenac + Gentamicin given together	
	Plasma	Urine	Plasma	Urine
0.042	56.35 ± 10.47	1.68 ± 0.24	7.04 ± 0.48*	2.34 ±0.19*
0.083	37.64 ± 8.36	3.15 ± 0.50	6.24 ± 0.32 *	6.40±0.30 **
0.167	28.80 ± 7.98	6.43 ± 0.57	5.53 ± 0.41 *	17.04±1.10 **
0.25	14.73 ± 2.75	27.19 ± 0.77	4.47 ± 0.12 *	64.24±2.35 **
0.333	7.06 ± 1.32	29.64 ± 0.84	3.83 ± 0.16 N.S.	95.51±2.67 **
0.50	5.28 ± 1.08	95.64 ± 15.33	3.57 ± 0.14 N.S.	147.6±9.02 N.S
0.75	3.36 ± 0.80	53.46 ± 2.93	3.02 ± 0.12 N.S.	130.3±5.53 **
1	2.41 ± 0.71	36.79 ± 1.55	2.26 ± 0.15 N.S.	99.74±3.30 **
1.5	1.86 ± 0.29	34.45 ± 1.67	1.49 ± 0.15 N.S.	81.58±2.18 **
2	1.49 ± 0.25	24.52 ± 1.16	1.16 ± 0.12 N.S.	51.77±1.60 **
3	1.20 ± 0.26	21.39 ± 0.63	0.85 ± 0.09 N.S.	41.31±1.80 **
4	1.01 ± 0.23	12.76 ± 0.61	0.65 ± 0.09 N.S.	27.18±3.67 *
5	0.80 ± 0.14	10.22 ± 0.37	0.48 ± 0.09 *	20.01±0.97 **
6	0.49 ± 0.10	8.67 ± 0.28	0.37 ± 0.08 N.S.	14.50±1.24 N.S
8	0.33 ± 0.13	7.15 ± 0.20	0.22 ± 0.06 N.S.	10.93±0.82 *
10	0.21 ± 0.08	7.03 ± 0.14	0.14 ± 0.04 N.S.	8.63±0.69 N.S
12	0.08 ± 0.03	5.43 ± 0.35	0.10 ± 0.03 N.S.	6.56±0.68 N.S
24	N.D.	3.92 ± 0.22	N.D.	5.10±0.92 N.S
30	N.D.	2.06 ± 0.32	N.D.	2.48±0.43 N.S
36	N.D.	0.86 ± 0.17	N.D.	0.89±0.09 N.S
48	N.D.	0.33 ± 0.07	N.D.	0.48±0.13 N.S

N.D. = Non detectable

N.S. = Non significant, * $p < 0.05$ ** $p < 0.01$

Table – 19

Comparison of kinetic parameters of diclofenac (2mg/kg) when it was given alone and when given together with gentamicin (7.5 mg/kg) in healthy goats following i.v. administration

Kinetic Parameter	Unit	Diclofenac alone	Diclofenac + gentamicin
A	$\mu g.ml^{-1}$	45.95 ± 21.13	4.90 ± 0.39 N.S.
B	$\mu g.ml^{-1}$	2.69 ± 0.65	1.91 ± 0.26 N.S.
C_p^o	$\mu g.ml^{-1}$	48.65 ± 21.24	6.82 ± 0.16 N.S.
α	h^{-1}	4.46 ± 0.827	1.892 ± 0.143 *
$t_{1/2}\ \alpha$	h	0.17 ± 0.02	0.37 ± 0.03 **
β	h^{-1}	0.262 ± 0.040	0.285 ± 0.27 N.S.
$t_{1/2}\ \beta$	h	2.97 ± 0.53	2.53 ± 0.27 N.S.
AUC	$mg.L^{-1}.h$	19.40 ± 2.98	9.69 ± 0.98 *
AUMC	$mg.L^{-1}.h^2$	51.50 ± 17.56	28.38 ± 6.42 N.S.
MRT	h	2.62 ± 0.61	2.78 ± 0.39 N.S.
K_{12}	h^{-1}	1.710 ± 0.141	0.690 ± 0.010 **
K_{21}	h^{-1}	0.514 ± 0.041	0.750 ± 0.084 *
Kcl	h^{-1}	2.509 ± 0.884	0.737 ± 0.090 N.S.
Fc	-	0.13 ± 0.02	0.39 ± 0.03 **
$T \approx P$	-	8.16 ± 2.21	1.59 ± 0.20 *
Vd_c	$L.kg^{-1}$	0.06 ± 0.01	0.29 ± 0.008 **
Vd_B	$L.kg^{-1}$	0.90 ± 0.18	1.16 ± 0.21 N.S.
Vd_{area}	$L.kg^{-1}$	0.49 ± 0.11	0.76 ± 0.04 *
Vd_{SS}	$L.kg^{-1}$	0.28 ± 0.06	0.57 ± 0.04 *
Cl_B	$ml.kg^{-1}.min^{-1}$	1.91 ± 0.33	3.58 ± 0.37 **

N.S. = Non significant , * $p<0.05$, ** $p<0.01$

3. Kinetic parameters

Kinetic parameters of diclofenac when given alone and when given together with gentamicin after single i.v. administration are presented in Table-19. The values of distribution rate constant (α), average rate of transfer of drug from central to peripheral (K_{12}), area under curve in plasma (AUC) and approximate tissue to plasma concentration rate ($T \approx P$) were found to be significantly lower in case of combined administration of the diclofenac as compared to its alone administration. Similarly distribution half life ($t_{1/2}\ \alpha$), average rate of transfer of drug from peripheral to central compartment (K_{21}), fraction of drug available for elimination from central compartment (Fc), various values of volume of distribution i.e. Vdc, Vd_{area} and Vd_{SS} and total body clearance (Cl_B) were noted to be significantly higher when given in combined administration as compared to single i.v. administration of diclofenac. Rest of the kinetic parameters did not differ significantly between both the groups.

Chapter - 5

Discussion

DISCUSSION

Gentamicin, an important member of aminoglycoside group of antibiotics, is clinically used because of its many advantage such as bactericidal effects on aerobic gram negative bacillary organisms, easily available, economical and available in all forms *viz.*, parental, oral and local applications. It is widely used in veterinary and human medicine to treat various systemic and local infections. Its best actions are seen in urinary tract infections, mammary gland infections and eye infections. Pharmacokinetic studies of gentamicin have been conducted in many species of animals including goats but so far, it seems little work has been done on kinetic interaction of gentamicin with diclofenac in goats.

Diclofenac, a potent NSAID having analgesic and antipyretic properties is frequently employed in treating inflammatory conditions associated with pyrexia and inflammatory conditions in animals. Antimicrobial agents are concurrently used along with diclofenac for treating microbial infections as well as to treat inflammatory and febrile conditions. Though pharmacokinetic interactions between antimicrobials and NSAIDs were studied in

different species of animals but interaction between gentamicin and diclofenac was so far not carried out in goats. Therefore, the present study was undertaken to know the kinetic interactions of gentamicin with diclofenac in goats.

I. KINETIC STUDY OF GENTAMICIN

(a) Distribution is plasma

Concentrations of gentamicin at different time intervals post i.v. injection of gentamicin (7.5 mg/kg) when given along with diclofenac (2 mg/kg) was significantly lower as compared to its single administration from 4 h to 6 h and at 10 h; rest of time (0.042 to 3 h and at 8, 12 & 24 h) the concentrations of the drug did not differ significantly (Table 15 and Fig. 1). The therapeutic concentration (≥ 2 μg/ml) was maintained upto 5 h in single administration and upto 4 h in case of combined administration with diclofenac. Serum concentrations of gentamicin were detected upto 24 h in both the groups of goats in the present study, whereas it was detectable upto 6 h in febrile and afebrile condition, when gentamicin was given at a lower dose (5 mg/kg) in goats (Ahmad *et al.*, 1994).

(b) Urinary excretion

Concentrations of gentamicin in urine were significantly higher at 0.25, 0.75, 1.5, 2 and 3 h when it was given in combination

with diclofenac as compared to its alone administration. Due to significantly higher amount of excretion of gentamicin when given concurrently with diclofenac, concentrations of gentamicin in plasma were found to be significantly lower from 4 to 6 h and 10 h when given along with diclofenac as compared to its single administration. The drug was detected upto 48 h in both the groups. The drug attained its peak level at the same time interval (0.75 h) in both the groups with a concentration of 455.4 ± 15.05 $\mu g.ml^{-1}$ when gentamicin was given alone as compared to 526.0 ± 11.96 $\mu g.ml^{-1}$ when given in combination with diclofenac. The mean therapeutic concentration in urine (≥ 2 μg/ml) was maintained upto 30 h in alone administration as compared to a shorter period of around 24 h when it was given in combination with diclofenac.

(c) Kinetic parameters

The distribution of half-life ($t_{1/2}$ α) was noted to be more or less similar (0.42 ± 0.08 h) in case of single administration of gentamicin as compared to its combined administration (0.43 ± 0.05 h). This clearly indicates that diclofenac doesn't influence the rate of distribution of gentamicin. On the other hand, Baxla (2004) noted quicker distribution of gentamicin (low $t_{1/2}$ α) in tissues and body fluids when given along with paracetamol. In contrast, Sudha Kumari

(1998) noted no influence of paracetamol on the rate of distribution of enrofloxacin. Similarly, Nitesh Kumar *et al.* (2003) noted no influence of diclofenac in the rate of distribution of enrofloxacin. Distribution half life ($t_{1/2}\,\alpha$) of 0.05 ± 0.01 h in cow (Satish *et al.*, 1989), 0.38 ± 0.07 h in rabbit (Uppal *et al.*, 1992) and 17.36 ± 4.64 min in chicken (Garg *et al.*, 1989) were found to be lower as compared to the $t_{1/2}\,\alpha$ value noted in the present study (0.42 ± 0.08 h) in goats. This shows that gentamicin may be distributed comparatively slowly in goats as compared to other species noted above.

The elimination rate constant of (β) was noted to be 0.185 ± 0.035 h^{-1}, while the elimination half life ($t_{1/2}\,\beta$) was noted to be 4.28 ± 0.70 h following single i.v. administration of gentamicin (7.5 mg/kg) These values did not differ significantly in goats when gentamicin was given along with diclofenac (β = 0.187 ± 0.029 h^{-1}; $t_{1/2}\,\beta$ = 4.01 ± 0.56 h). This denotes that similar rate of elimination occurred in both the groups and diclofenac has no influence in the elimination of gentamicin. Due to this, the values of mean residential time (MRT), area under curve (AUC) and area under first moment curve (AUMC) between gentamicin when given alone and when given together did not differ significantly (Table 16) Similarly Nitesh Kumar *et al.* (2003) noted non significant influence of diclofenac in $t_{1/2}\,\beta$ of

enrofloxacin and Baxla (2004) noted non significant change in t½β of when gentamicin was given along with paracetamol as compared to alone administration of antimicribials. In contrast, Sudha Kumari (1998) noted faster elimination of enrofloxacin (low $t_{1/2}$ β) when given along with paracetamol as compared to its alone administration. Lower $t_{1/2}$ β of 1.12 ± 0.25 h in cow (Satish *et al.*, 1989), 3.43 ± 0.84 in horse (Zurich *et al.*, 1995), 2.92 ± 0.12 h in camel (Wasfi *et al.*, 1992), 3.50 ± 0.23 h in swine (Giroux *et al.*, 1995) and 2.98 ± 0.65 h in chicken (Garg *et al.*, 1989) were noted. The above data showed that gentamicin is comparatively removed slower in goats as compared to other species.

The values of rate constant of drug transfer from central to peripheral compartment (K_{12}), peripheral to central compartment (K_{21}) and elimination rate constant of the drug from central compartment (Kel) did not differ significantly when gentamicin was given alone and when given together with diclofenac. This indicates movement of drug from central (plasma) to peripheral (tissue) or vice-versa is similar in both the cases. This has lead to non significant changes in fraction of drug available for elimination from central compartment (Fc) and approximate tissue to plasma concentration (T ≈ P) value when gentamicin was administered along with diclofenac.

Notri (1980) stated that for a two compartment open model, the value of $Vd_B > Vd_{area} > Vd_{SS}$ and Vd_S. He further mentioned that among these values of volume distribution, only Vd_{area} correctly predicts the amount of drug in the body during elimination phase, whereas Vd_B overestimate and Vd_{SS} and Vdc underestimate the amount of drug in the body. Vd_{area} of 1.08 ± 0.15 and 1.21 ± .27 $L.kg^{-1}$ were obtained when gentamicin was given alone and when given together with diclofenac. These values do not differ significantly. Vd_{area} of 0.37 ± 0.13 $L.kg^{-1}$ in cow (Satish *et al.*, 1983), 0.80 ± 0.04 $L.kg^{-1}$ in buffalo calf (Baxla, 2004), 0.13 ± 0.08 $L.kg^{-1}$ in horse (Zurich *et al.*, 1995), 0.45 ± 0.11 $L.kg^{-1}$ in rabbit (Uppal *et al.*, 1992) and 0.32 ± 0.05 $L.kg^{-1}$ in chicken (Garg *et al.*, 1989) were noted to be lower as compared to be present value obtained in goats. This may indicate that gentamicin may be distributed to a greater amount in body of goats as compared to the above noted species.

(d) Dosage regimen

In the present study, calculation of dosage regimen of gentamicin when given alone and when given together with diclofenac was carried out at three different therapeutic levels (C_p^{∞} min = 1, 2 and 4 $\mu g.ml^{-1}$) in order to combat mild, moderate and

severe infections, respectively, at convenient dosage interval (γ) of 8 and 12 h. Gentamicin differ non significantly at loading doses (D*s) and maintenance doses (D_0s), at all dosage intervals when given with diclofenac as compared to its alone administration. Gentamicin can be administered either alone or along with diclofenac for treating mild or moderate infections at the calculated loading and maintenance dose at γ of 8 h, where as is case of γ of 12 h the calculated D* and Do are very high, which may cause higher plasma concentration of gentamicin leading to toxicity. In case of severe infections (C_p^{∞} min = 4 $\mu g/ml^{-1}$) the calculated D*s and D_0s at γ of 8 and 12 h are very high, which may cause higher plasma levels of gentamicin leading to toxicity. Plasma levels of gentamicin in the range of 7 to 10 $\mu g.ml^{-1}$ were non toxic (Gyselynek *et al.*, 1971). From the study, it seems that gentamicin may be effectively combined with diclofenac for treating susceptible bacterial infections accompanied by pyrexia and other inflammatory conditions.

II. KINETIC STUDY OF DICLOFENAC

Kinetic studies of diclofenac in animals are very little and studies in man (Willis *et al.*, 1979; Kurowski, 1988), pig (Oberle *et al.*, 1994), rat (Peris-Ribera *et al.*, 1991) and buffalo calf (Nitesh Kumar *et al.*, 2003) were reported.

(a) Distribution in plasma

Concentrations of diclofenac in plasma were found to be significantly lower initially from 0.042 to 0.25 h and then differ non significantly later from 0.333 to 12 h in goats when administered with gentamicin as compared to single administration of diclofenac by iv. route (Table 18 and Fig. 4). Plasma concentrations of diclofenac were detected upto 12 h in both the groups of goats, whereas it was detectable upto 24 h in buffalo calf when diclofenac (1 $mg.kg^{-1}$) was given i.v. (Nitesh Kumar *et al.*, 2003).

(b) Urinary excretion

Concentrations of diclofenac in urine were noted to be significantly higher initially from 0.042 to 8 h and differed non significantly later from 10 to 48 h in goats when diclofenac was given together with gentamicin as compared to its single i.v. administration. This may probably led to significantly lower concentrations of diclofenac initially (0.042 to 0.25 h) when given along with diclofenac as compared to its alone administration (Table 18 and Fig 5). Peak concentrations in urine were noted at 0.50 h in both case. Diclofenac was detected in urine till 48 h in both the groups of goats. Similarly, diclofenac was detected upto 48 h in buffalo calf when diclofenac was

given alone and when given together with enrofloxacin (Nitesh Kumar *et al.*, 2003).

(c) Kinetic parameters

The extrapolated zero time concentration during distribution phase (A), during elimination phase (B) and theoretical zero time concentration (C_p^o) differed only non significantly for diclofenac when given alone as compared to its combined administration with gentamicin (Table 19).

The distribution rate constant (α) was significantly ($p<0.05$) lower (1.892 ± 0.143) when diclofenac was administered with gentamicin as compared to its single administration (4.467 ± 0.827 h^{-1}). The distribution half life ($t_{1/2}\,\alpha$) was found be significantly ($p < 0.01$) higher (0.37 ± 0.03 h) when diclofenac was administered with gentamicin as compared to its single administration (0.17 ± 0.02 h). This indicates that the drug may be distributed faster when diclofenac was given alone as compared to its combined administration with gentamicin. Distribution half life ($t_{1/2}\,\alpha$) of 0.34 ± 0.08 h noted for diclofenac in buffalo calf (Nitesh Kumar *et al.*, 2003) was found to be higher as compared to the $t_{1/2}\,\alpha$ value noted in the present study (0.017 ± 0.02 h) in goats. This shows that gentamicin

may be distributed comparatively faster in goats as compared to buffalo calf.

The elimination rate constant (β) and elimination half life ($t_{1/2}$ β) of diclofenac differed non significantly when it was administered alone (β = 0.262 ± 0.040 h^{-1}, $t_{1/2}$ β = 2.97 ± 0.53 h) as compared to its combined administration with gentamicin (β = 0.285 ± 0.027 h^{-1}; $t_{1/2}$ β = 2.53 ± 0.27 h). This denotes that similar rate of elimination occurred in both the groups. Due to this, the values of mean residential time (MRT) and area under first moment curve (AUMC) does not differs significantly when diclofenac was given alone and when given together with gentamicin. Higher elimination half life of 4.06 ± 0.59 h was noted in buffalo calf (Niesh Kumar *et al.*, 2003). The $t_{1/2}$ β value of 1.1 h and 1.8 in man after i.v. administration of diclofenac (Willis *et al.*, 1979; Oberle *et al.*, 1994) were found to be very low than the value obtained in goats in present study. In contrast, the terminal half life ($t_{1/2}$ β) of diclofenac noted in pigs (2.4 h) is similar.

The value of area under plasma concentrations time curve (AUC) was significantly lower (9.69 ± 0.98 $mg.L^{-1}.h$) when diclofenac was administered along with gentamicin than when

administered alone (19.40 ± 2.98 $mg.L^{-1}.h$). The value of total area under the first moment curve (AUMC) in goats after i.v. administration was noted to differ non significantly when diclofenac was administered alone (51.50 ± 17.56 $mg.L^{-1}.h^2$) as compared to its combined administration with gentamicin (28.38 ± 6.42 $mg.L^{-1}.h^2$). The value of mean residential time (MRT) in goats after i.v. administration was noted to be 2.62 ± 0.61 h when given alone which does not differ significantly than that obtained after combined administration with gentamicin (2.78 ± 0.39 h). In contrast Nitesh Kumar *et al.* (2003) observed significantly higher ($p < 0.01$) MRT value of 18.07 ± 9.12 h for diclofenac when it was given with enrofloxacin as compared to 4.72 ± 0.85 h when it was given alone.

The value of rate constant of drug transfer from central to peripheral (K_{12}) compartment was noted to be significantly low ($p<0.01$) when diclofenac was given along with gentamicin (0.690 ± 0.010 h^{-1}) as compared to its alone administration (1.710 ± 0.141 h^{-1}). This indicates slower movement of drug from central (plasma) to peripheral (tissue) compartment. The value of rate constant of drug transfer from peripheral to central compartment (K_{21}) was noted to be significantly higher ($p <0.05$) when diclofenac was administered along with gentamicin (0.750 ± 0.084 h) as compared to its alone

administration (0.514 ± 0.041 h^{-1}). This indicates faster movements of drug from peripheral (tissue) to central (plasma) compartment. Significantly higher ($p < 0.01$) fraction of drug available for elimination from central compartment (Fc) was obtained for diclofenac when it was given with gentamicin (0.39 ± 0.03) as compared to its alone administration (0.13 ± 0.02). This value along with significantly higher (K_{21}) value obtained when diclofenac was given along with gentamicin led to significantly low approximately tissue to plasma concentration (T≈P) value of 1.59 ± 0.20 when diclofenac was given along with gentamicin as compared to the value of 8.16 ± 2.21 when diclofenac was given alone. This shows that diclofenac may be distributed to a lesser amount in peripheral tissues and fluids when given in combination with gentamicin as compared to its alone administration.

The various values of volume of distribution except Vd_B were significantly higher in goats when diclofenac was given together with gentamicin as compared to single administration of diclofenac after i.v. administration (Table 19). Vd_{area} of 0.49 ± 0.11 $L.kg^{-1}$ was noted for single administration of diclofenac which was significantly ($p<0.05$) low as compared to its combined administration with gentamicin (0.76 ± 0.04 $L.kg^{-1}$). Similar to the present study Nitesh Kumar *et al.*, (2003) noted highly significantly ($p < 0.01$) increase in

Vd_{area} (1.34 ± 0.04 $L.kg^{-1}$) when it was given in combination with enrofloxacin as compared to alone (0.54 ± 0.10 $L.kg^{-1}$) administration in buffalo calves. A very low value of Vd was noted in man by Willis *et al.*, (1979) i.e. 0.049 ± 0.11 $L.kg^{-1}$ as compared to present study.

The total body clearance (Cl_B) value of 1.91 ± 0.83 and 3.58 ± 0.37 $ml.kg^{-1}.min^{-1}$ in goats when given together with gentamicin, respectively, were noted in present study and the values differed highly significantly ($p < 0.01$). A high Cl_B value of 42±0.9 $ml.kg^{-1}.min^{-1}$ in man (Willis *et al.*, 1979) and 1.52 ± 0.07 $ml.kg^{-1}.min^{-1}$ in buffalo calf (Kumar *et al.*, 2003) was observed. The total plasma clearance (Cl_B) in minipigs was five fold slower than in man (57±17 $ml.kg^{-1}h^{-1}$ or 0.95 ± 0.28 $ml.kg^{-1}min^{-1}$ vs 252 ± 54 $ml.kg^{-1}.h^{-1}$ or 4.2 ± 0.9 $ml.kg^{-1}.min^{-1}$) as noted by Oberle *et al.* (1994).

III. KINETIC INTERACTIONS BETWEEN GENTAMICIN AND DICLOFENAC

Distribution of gentamicin and diclofenac in plasma and urine as well as various kinetic parameters have been described above when given alone or in combination following i.v. administration. Definite kinetic interactions between the drugs occurred in goats and the salient features are descried below.

The results of the present study clearly establish that diclofenac does not have any influence over kinetics of gentamicin which results in similar calculated loading (D*) and maintenance (Do) doses when gentamicin was given alone or when administered together with diclofenac. The above statement leads to the inference that gentamicin can be used effectively along with diclofenac in clinical cases of drug sensitive microbial infections accompanied by pyrexia and any other inflammatory conditions.

In contrast, gentamicin may influence over diclofenac as noted by significant changes in plasma and urine levels as well as on various kinetic parameters (Table 18 and 19). Since gentamicin has increased $t_{1/2}$ α, Fc, Vdc Vd_{area} and Cl_B which may be beneficial under inflammatory conditions since the drug may be distributed in greater amount in body tissues and remain for longer time when diclofenac was administered together with gentamicin as compared to its alone administration.

Chapter - 6

Summary

SUMMARY

A detailed pharmacokinetic study of gentamicin and diclofenac when given alone and their interactions when given concurrently was carried out in goats weighing between 20-22 kg following intravenous administrations. Concentrations of the drugs in plasma and urine as well as various kinetic parameters were calculated by using two compartment open model when given alone or when given concurrently. Attempts were made to calculate the rational dosage regimen of gentamicin on the basis of kinetic data and maintenance of therapeutic concentrations (MICs) in plasma. The following salient findings were obtained :

1. The results of the present investigation clearly established that after combined i.v. administration of gentamicin (7.5 mg/kg) with diclofenac (2 mg/kg), plasma concentrations of gentamicin were significantly lower when it was given along with diclofenac as compared to its single administration from 4 to 6 and at 10 h. No significant differences was observed from 0.042 to 3 h and 8, 12 and 24 h. The therapeutic concentration (≥ 2 μg/ml) was maintained upto 4 h when gentamicin was administered along with diclofenac and upto 5 h when gentamicin was administered alone. In case of urine, concentrations of gentamicin were

significantly higher at 0.25, 0.75, 1.5 2 and 3 h following combined administration with diclofenac as compared to its alone administration. No significant differences was observed at 0.042, 0.083, 0.167, 0.333, 1, 4, 5, 6, 8, 30, 36 and 48 h. The drug attained its peak level in urine at the same time interval of 0.75 h in both the groups with a concentration of 455.43 ± 15.05 $\mu g.ml^{-1}$ when given alone as compared to the concentration of 525.88 ± 11.96 $\mu g.ml^{-1}$ in combination with diclofenac. The mean therapeutic concentration in urine (≥ 2 $\mu g.ml^{-1}$) was maintained upto 30 h in case of single administration and 24 h in case of combined administration.

2. Various kinetic parameters of gentamicin did not differ significantly when gentamicin was administered alone or in combination with diclofenac (Table 16). The above noted results show that diclofenac may not have any influence over kinetics of gentamicin and thereby does not affect its distribution and elimination in goats. This may be the reason that the calculated dosage regimen of gentamicin did not differ significantly when given alone or in combination with diclofenac (Table 17).

3. For treating mild systemic infections [C_p^{∞} min (MIC) = 1 μg/ml] a mean loading dose (D*) and maintenance dose (D_0) of around 13 and 12 mg/kg at the dosage interval of (γ) of 8 and 12 h can be

effectively used. In case of moderate systemic interactions [C_p^{∞} min (MIC) = 2 μg/ml], the calculated D* and D_0 of 11 and 8 mg/kg, respectively at γ of 8 h can be used. in case of severe systemic interactions (C_p^{∞} min (MIC) = 4 μg/ml] the calculated D* and D_0 of 21 and 17 mg/Kg at γ of 8 h can be used with caution since the doses are high, which may cause toxicity if used for a longer period. It is observed had plasma levels of gentamicin in the range of 7 to 10 $\mu g.ml^{-1}$ were non-toxic (Gyselynek *et al.*, 1971) and above this level may cause toxicity.

4. Concentrations of diclofenac in plasma were found to be significantly lower from 0.042 to 0.25 h and at 5 h when diclofenac was given in combination with gentamicin as compared to its alone administration. In case of urine, concentrations of diclofenac wee found to be significantly higher from 0.042 to 8 h (except at 0.50 and 6 h) when diclofenac was administered along with gentamicin ad compared to its alone administration (Table 6).

5. The extrapolated zero time concentration during distribution phase (A), extrapolated zero time concentration during elimination (B) phase, theoretical zero time concentration (C_p^0) and elimination half life were ($t_{1/2}\beta$) found to differ non significantly when diclofenac was administered along with gentamicin as compared to its alone administration. The value of distribution

half-life ($t_{1/2}\alpha$) was noted to be significantly ($P<0.01$) higher for diclofenac when it was given in combination with gentamicin as compared to its alone administration, which indicates that the drug is expected to be distributed slowly when given along with gentamicin.

6. Area under curve (AUC), rate constant of drug transfer from central to peripheral compartment (K_{12}) and approximate tissue to plasma concentration ration ($T \approx P$) were significantly lower. On the other hand, the values of rate constant of drug transfer form peripheral to central compartment (K_{21}) and fraction of drug available for elimination from central compartment (Fc) were noted to significantly higher for diclofenac when it was given together with gentamicin as compared to its single administration. There was no significant changes in area under first moment curve (AUMC), and mean residual time (MRT) when diclofenac was given alone or in combination with gentamicin.

7. Various volume of distribution (Vd_c, Vd_{area} and Vd_{SS}) and total body clearance (Cl_B) were significantly higher for diclofenac when it was given together with gentamicin as compared to its alone administration.

The present study clearly establishes that diclofenac does not have much influence over kinetics of gentamicin, which results in

similar calculated loading (D*) and maintenance (D_0) dose when gentamicin was given alone or when administered together with diclofenac. The above statements lead to the inference that gentamicin can be used effectively along with diclofenac in clinical cases of drug sensitive microbial infections accompanied by other inflammatory conditions.

In contrast, gentamicin may influence over kinetics of diclofenac as noted by significant changes in plasma and urine levels as well as on various kinetic parameters. The values of $t_{1/2}\,\alpha$, $t_{1/2}\,\beta$, K_{21}, Fc and Vd_{area} are significantly higher when diclofenac was administered along with gentamicin as compared to its alone administration.

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✪✪✪✪✪

Appendix

APPENDIX-I

CALCULATION OF KINETIC PARAMETERS

Kinetic parameters were calculated from log plasma drug concentration versus time profile. An example is noted below from the data of goat no. 3 obtained after a single i.v. injection of gentamicin (7.5 mg/kg). This data showed a biphasic curve and hence, fits well into a two compartment open model. Here, elimination phase (β) starts from 3 h.

Sl. No.	Time (h) X	X^2	Plasma drug concentration (Y) (μg/ml)	Log Y	XY
1	3	9	3.12	0.4942	1.4826
2	4	16	2.50	0.3979	1.5916
3	5	25	1.99	0.2989	1.4945
4	6	36	1.56	0.1931	1.1586
5	8	64	1.00	0.0000	0.0000
6	10	100	0.64	-0.1938	-1.9380
7	12	144	0.38	-0.4202	-5.0424
8	24	576	0.22	-0.6576	-15.7824
$\Sigma n = 8$	$\Sigma x = 72$ $\overline{X} = 9.0$	$\Sigma x^2 = 970$		$\Sigma \log Y = 0.1125$ $\overline{Y} = 0.0141$	$\Sigma XY = -17.0355$

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

$$= \frac{8 \times (-)17.0355 - 72 \times 0.1125}{8 \times 970 - (72)^2}$$

$$= \frac{-136.284 - 8.1}{7760 - 5184}$$

$$= \frac{144.384}{2576} = -0.05604$$

β, elimination rate constant = b × (-2.303)

= -0.056 × -2.303

= 0.129 h^{-1}

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

$\overline{Y}$ = mean drug concentration

$\overline{X}$ = mean time

b = slope of line

a = zero time concentration.

Therefore,

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

= 0.0141 – (-0.056) × 9

Log 0.5181

Zero time concentration (B) = antilog of 0.5181

= 3.296 or 3.30 μg/ml

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

Substracting the theoretical value from observed values, a series of residual concentrations were obtained and slope of line in natural log (distribution rate constant, α) and zero time intercept (zero time concentration during distribution phase, A) can be calculated as per method adopted for calculation of B and β. The value of A is 18.56 μg/ml and α is 1.284 h^{-1}.

The theoretical plasma concentration at zero-time

$C_p^0 = A + B = 18.56 + 3.30 = 21.86$ μg/ml.

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

$$t_{1/2}\alpha = \frac{0.693}{\alpha} = \frac{0.693}{1.284} = 0.54h$$

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

$$t_{1/2}\beta = \frac{0.693}{\beta} = \frac{0.693}{0.129} = 5.37h$$

Area under curve, AUC

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

$$= \frac{18.56}{1.284} + \frac{3.30}{0.129}$$

$$= 14.4548 + 25.5814$$

$$= 40.04 \text{ mg.L}^{-1}\text{.h}$$

Area under first moment curve. Plasma drug concentration time curve, AUMC

$$AUMC = \frac{A}{\alpha^2} + \frac{B}{\beta^2} = \frac{18.56}{(1.284)^2} + \frac{3.30}{(0.129)^2}$$

$$= \frac{18.56}{1.649} + \frac{3.30}{0.017}$$

$$= 11.26 + 198.31$$

$$= 209.56 \text{ mg.L}^{-1}.\text{h}^2$$

Mean residential time, MRT

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

$$= \frac{209.56}{40.04} = 5.23 \text{ h}$$

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

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

$$= \frac{18.56 \times 0.129 + 3.30 \times 1.284}{21.86}$$

$$= \frac{2.394 + 4.237}{21.86} = \frac{6.6312}{21.86}$$

$$= 0.303 \text{ h}^{-1}$$

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

$$Kel = \frac{\alpha \times \beta}{K_{21}} = \frac{1.284 \times 0.129}{0.303}$$

$$= 0.547\ h^{-1}$$

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

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

$$= 1.284 + 0.129 - 0.303 - 0.547$$

$$= 0.563\ h^{-1}$$

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

$$Fc = \frac{\beta}{Kel} = \frac{0.129}{0.547} = 0.24$$

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

$$T \approx P = \frac{K_{12}}{K_{21} - \beta} = \frac{0.563}{0.303 - 0.129}$$

$$= \frac{0.563}{0.174} = 3.24$$

Volume of distribution based on both distribution and elimination, d_C

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

$$= \frac{7.5}{21.86} = 0.34 L.kg^{-1}$$

Volume of distribution based on elimination, Vd_B

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

$$= \frac{7.5}{3.30} = 2.27\ L.kg^{-1}$$

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

$$Vd_{area} = \frac{D}{AUC.\beta} = \frac{7.5}{40.04 \times 0.129} = \frac{7.5}{5.165} = 1.45 L.kg^{-1}$$

Volume of distribution at steady state, Vd_{SS}

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

$$= \frac{0.563 + 0.303}{0.303} \times 0.34 = 0.97 L.kg^{-1}$$

Total body clearance, Cl_B

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

$$= 1.45 \times 0.129$$

$$= 0.187\ L/kg/h$$

$$= 3.12\ ml/kg/min$$

APPENDIX-II

CALCULATION OF DOSAGE REGMIEN

Dosage regimen for antimicrobial agents are generally calculated to maintain the minimum inhibitory concentration (MIC) in plasma at desired dosage interval (γ) using formulae noted by Baggot (1977) and described by Saini and Srivastava (1997).

The data of animal no. 3 obtained after a single i.v. injection of gentamicin in healthy goats has been used as an example for calculation of dosage regimen for maintaining C_p^o min (MIC) of 1 µg/ml at the dosage interval of 8 and 12 h.

Calculation of loading (D*) and maitenance (D_o) dose: -

The loading dose (D*) is the initial dose that may be given the onset to reach the target concentration rapidly. The maintenance (D_o) dose is the dose given at particular dosage interval for maintaining C_p^∞ min (MIC) during the course of treatment. The loading (D*) and maintenance (D_o) doses of gentamicin can be calculated by the formula given below:

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

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

Where,

D^* = Loading dose

D_0 = Maintenance dose

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

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

β = Elimination rate constant.

γ =Dosage interval

e = Base of natural logarithm.

The loading and maintenance doses of gentamicin are repeated at different time intervals (8 and 12 h) to maintain the minimum plasma concentration of 1 μg/ml. Hence by considering 1 μg/ml as the minimum therapeutic concentration (C_p^{∞} min = MIC) at dosage interval (γ) of 8 h in animal no. 3 after i.v. administration of the drug. and D_0 were calculated as shown below: -

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

$$= 1 \times 1.45 \times e^{0.129 \times 8}$$

$$= 1 \times 1.45 \times 2.81$$

$$= 4.07 \text{ mg.kg}^{-1}$$

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

$$= 1 \times 1.45 \times (e^{0.129 \times 8} - 1)$$

$$= 1.45 \times 1.81$$

$$= 2.62 \text{ mg.kg}^{-1}$$

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

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

CERTIFICATE - I

This is to certify that the thesis entitled "PHARMACOKINETICS OF GENTAMICIN AND ITS INTERACTION WITH DICLOFENAC 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. DEEPAK KUMAR PRASAD**, under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

Jayachandran 22/5/04

(C. Jayachandran)
Major Advisor

Endorsed :

22/5/04

(Chairman / Head of the Department)