PHARMACOKINETIC STUDY OF GENTAMICIN AND ITS INTERACTION WITH PARACETAMOL IN BUFFALO CALVES

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

PUSA (SAMASTIPUR) BIHAR

(FACULTY OF POST-GRADUATE STUDIES)

In partial fulfilment of the requirement

FOR THE DEGREE OF

Master of Veterinary Science

IN

VETERINARY PHARMACOLOGY AND TOXICOLOGY

By

Sushma Lalita Baxla

Registration No. - M/VPT/69/2000-2001

Department of Veterinary Pharmacology and Toxicology

BIHAR VETERINARY COLLEGE

PATNA (BIHAR)

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2004

Dedicated to

my benevolent
and
adorable
PARENTS

DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY
Bihar Veterinary College, Patna-800014
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CERTIFICATE - I

This is to certify that the thesis entitled **"PHARMACOKINETIC STUDY OF GENTAMICIN AND ITS INTERACTION WITH PARACETAMOL IN BUFFALO CALVES"** 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. SUSHMA LALITA BAXLA**, under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

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We, the undersigned, members of the Advisory Committee of **DR. SUSHMA LALITA BAXLA**, a candidate for the degree of Master of Veterinary Science with Major in **Veterinary Pharmacology & Toxicology**, have gone through the manuscript of the thesis and agree that the thesis entitled "**PHARMACOKINETIC STUDY OF GENTAMICIN AND ITS INTERACTION WITH PARACETAMOL IN BUFFALO CALVES**" may be submitted by **DR. SUSHMA LALITA BAXLA** in partial fulfillment of the requirements for the degree.

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This is to certify that the thesis entitled "**PHARMACOKINETIC STUDY OF GENTAMICIN AND ITS INTERACTION WITH PARACETAMOL IN BUFFALO CALVES**" submitted by **DR. SUSHMA LALITA BAXLA**, 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 22-03-04.

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Place - Patna

Date - 22/03/2004

Sushma Lalita Baxla

(Sushma Lalita Baxla)

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Veterinarian's Oath

Being admitted to the profession of veterinary medicine, I solemnly swear to use my scientific knowledge and skills for the benefit of society through the protection of animal health, the relief of animal suffering, the conservation of livestock resources, the promotion of public health, and the advancement of medical knowledge.

I will practice my profession conscientiously, with dignity, and in keeping with the principles of veterinary medical ethics.

I accept as a lifelong obligation the continual improvement of my professional knowledge and competence.

The best
Doctor in the world
is a
VETERINARIAN
He can't ask his patients
what is the matter
He has got to just know.

— Will Rogers

Chapter - 1

Introduction

INTRODUCTION

In recent years, the main focus of attention is to make antibacterial drug therapy effective, safe and affordable to human and animal practices. For the last decade, it has been observed that the effects of many drugs when given concurrently, are not necessarily predictable on the basis of knowledge of their effects when given alone. Thus, the subject of drug interactions interests not only Pharmacologists but also it is now highly important to clinical practitioners. 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 therapeutic advantages in animals and human to enable a physician for effective treatment as well as to minimize or prevent drug toxicity by adjustment of the dosage schedule.

Gentamicin, a broad specturm aminoglycoside antibiotic was derived from *Micromonospora purpurea*. Gentamicin was first studied and described by Weinstein and co-workers in 1963. It has bactericidal effects and commonly used in veterinary (Conzelman 1980) and human medicine to treat various infections caused by aerobic gram negative bacteria such as *Escherichia coli, Salmonella,*

Shigella, Klebsiella, Proteus, Haemophillus, Pasteurella, Enterobacter Campylobacter, Pseudomonas and Serratia. Gentamicin is a mixture of 3 closely related antibactrial agents and available for both parenteral and topical use. It is very effective for treating systemic as well as local infections Gentamicin has been successfully used for the treatment of urinary tract infections, respiratory tract infections, skin, burn and soft tissue infections. It is also highly effective for treating various poultry diseases like colibacillosis, staphylococosis, necrotic dermatitis and *Paracolobactrum arizonae* infected turkey poults. The disposition kinetic data of gentamicin in cattle, equine, sheep, dogs, cats, rabbits and some of the avian species were conducted by different workers.

Nonsteroidal antiinflammatory drugs (NSAIDs) are generally administered along with atimicrobials in cases of bacterial diseases associated with fever, pain and inflammation. Paracetamol also called acetaminophen, is a paramino phenol derivative classified under NSAIDs. It possesses powerful antipyretic action with analgesic and antiinflammatory properties. It is commonly employed for treating febrile condition of animals. The advantages of paracetamol are that it doesn't affect the function of platelets and clotting factors and is less gastrointestinal irritant. Paracetamol is one of most

commonly used "over the counter" antipyretic and analgesic for headache, musculoskeletal pain and dysmenorrhoea.

Antimicrobials and NSAIDs are frequently used concomitantly and pharmacokinetic interactions between them have been described (Kampmann *et al.*, 1972; Carbon *et al.*, 1981, 1984; Sudha Kumari, 1998;. Nitesh Kumar, 2002 ; Mukta, 2002). In experimental staphylococcal osteomyelitis, ibuprofen given concomitantly with oxacillin significantly increased antibiotic efficacy but the mechanism of interaction was not fully studied (Khurana and Deddish, 1986). Joly *et al.* (1988) showed enhancement of the therapeutic effects of cephalosporins (cefotiam, cefmonoxime and ceftriaxone) in experimental endocarditis by altering their pharmacokinetics when simultaneously used with the NSAIDs, diclofenac. Concurrent administration of anti inflammatory drugs with antimicrobials may change their disposition characteristics (Joly *et al*, 1988; Nitesh Kumar 2002; Mukta, 2002) and thereby changing their dosage regimen. Sudha Kumari (1998) established definite kinetic interactions between enrofloxacin and paracetamol as noted by significant variations in drug concentrations in body fluids and kinetic parameters of both the drugs. The kinetic interactions between these drugs led to change in dosage regimen of enrofloxacin,

particularly for treating systemic infections including septicemia as well as local infections such as mammary gland infections.

Buffalo (*Bubalus bubalis*) is commonly known as 'Asian animal'. It is the chief milk yielding species of Indian subcontinent and recognized as the 'milk machine'. Buffalo plays an important role in small hold farmer's economy where it forms an integral part of agricultural system. It is one of the most important sources of milk and draught power, which contributes to the upliftment of poor farmers in this country. By considering the huge contribution of buffalo in national economy, its proper health coverage is essential by using combined therapy of gentamicin and paracetamol.

Before using a drug in therapy, it is essential to study its pharmacokinetic behaviour in detail and on the basis of pharmacokinetic parameters, suitable dosage regimen can be derived. Concurrent administration of antiinflammatory drugs with antimicrobials may alter the kinetic parameters of drugs and thereby changing their dosage regimen. Although pharmacokinetic studies of gentamicin have been conducted in many species of animals, it seems little work has been done on kinetic interaction of gentamicin with NSAIDs in buffalo calves particularly on the interaction of gentamicin with paracetamol.

Keeping in view of the above mentioned facts, the present study was undertaken with the following specific aims and objectives: -

1. Estimation of concentrations of gentamicin and paracetamol at different time intervals in body fluids following i.v. administration when given alone in buffalo calf.
2. Determination of kinetic parameters of gentamicin and paracetamol when administered alone.
3. Calculation of dosage regimen of gentamicin when administered alone.
4. Estimation of drug(s) concentrations in biological fluids, as well as calculation of kinetic parameters of gentamicin and paracetamol and calculation of dosage regimen of gentamicin when the drugs are given alone and given together to know the interaction of the drugs.

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Chapter - 2

Review of Literature

REVIEW OF LITERATURE

Aminoglycosides are generally used in clinical practice in veterinary and human medicine to treat infections caused by aerobic gram-negative bacteria. Members of aminoglycosides are streptomycin, gentamicin, neomycin, kanamycin, tobramycin, amikacin, netilmicin etc. Gentamicin, one of the most popular aminoglycoside antibiotics having bactericidal effect, is commonly employed for treating various systemic infections as well as local infections in animals.

GENTAMICIN

Gentamicin, a broad spectrum aminoglycoside is widely used to treat various infections caused by aerobic gram-negative bacteria such as *Escherichia coli, Salmonella, Klebsiella, Proteus, Haemophilus, Pasteurella, Campylobactor 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, specially those due to *Pseudomonas aeruginosa, Enterobacter, Klebsiella, Serratia* and other species resistant to other antibiotics. 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.

History

The geneology of aminoglycoside group of antimicrobials began in 1944 with the production of streptomycin from *Streptomyces griseus* by Schatz, Bugie and Waksman for treatment of infections caused by aerobic gram-negative organisms but its clinical usefulness was limited because of emergence of streptomycin resistant gram-negative bacilli. Later on, neomycin was produced by Waksman for topical applications and local effects in bowel since it leads to severe nephro and oto-toxicity on systemic administration. Umezewa and co-workers produced another agent kanamycin, from *Streptomyces kanamyceticus* in 1957 but its use is restricted owing to its toxicity and emergence of resistant microorganisms as well. Now a days, it has largely been replaced by the three newer aminoglycosides viz., gentamicin, tobramycin and amikacin. Gentamincin was first studied and described by Weinstein & co-workers in 1963. It was isolated purified and characterized by Roselot and co-workers (1964).

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. The gentamicin family, which includes gentamicin C1, C1a and C2 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. I.

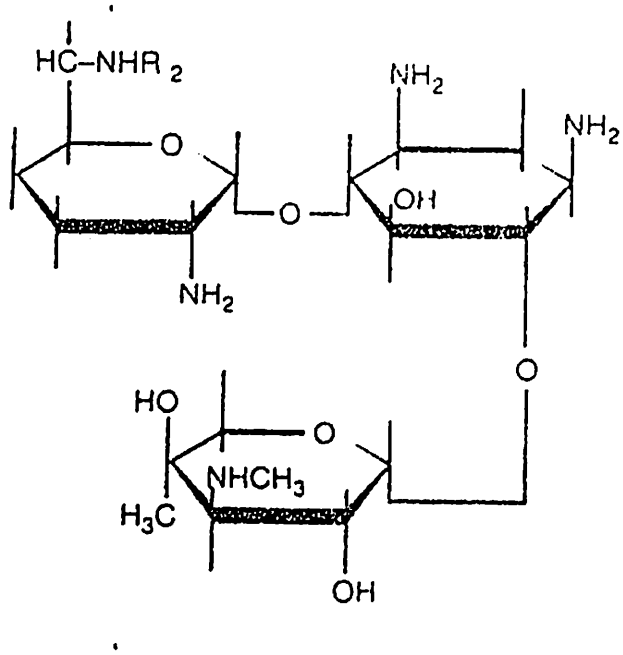


Fig. I. *Chemical structure of Gentamicin.*

Mechanism of action

The aminoglycoside antibiotics are rapidly acting bactericidals. Bacterial killing is concentration dependent; the higher the concentration, the greater the rate at which bacteria are killed (Kapushik *et al.*, 1988; Blaser, 1991).

The primary intracellular site of action of the gentamicin is the 30S ribosomal subunit, which consists of 21 proteins and a single 16S molecule of RNA (Mitssuhashi, 1975). However, it also appears to bind to several sites on the 50S ribosomal subunit as well (Davies, 1988). Gentamicin has the capacity to induce misreading of the mRNA template, and incorrect amino acids are incorporated into the growing polypeptide chain (Tai *et al.*, 1978). It remains to be established that this is the primary mechanism of aminoglycoside-induced cell death.

Antibacterial activity

The antibacterial activity of getamicin is primarily against aerobic gram-negative bacilli such as *Escherichia coli, Salmonella, Klebsiella, Proteus, Haemophilus, Enterobacter, Campylobacter* and *Pseudomonas.* It has little 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.

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 patients 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 drug into inflammed tissues and the associated conditions of low oxygen tension and low pH, both of which interfere with aminoglycoside antibacterial activity. Aminoglycoside in combination with a β-lactum antibiotic is recommended for treatment of pneumonia caused by *Pseudomonas 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.

Species	**(MIC_{90}) µg/ml Gentamicin**
Citrobacter freundii	0.5
Enterobacter spp.	0.5
Escherichia coli	0.5
Klebsiella pneumoniae	0.5
Proteus mirabilis	4
Providencia stuartii	8
Pseudomonas aeruginosa	8
Serratia spp.	4
Enterococcus faecalis	32
Staphylococcus aureus	0.5

KINETIC STUDY OF GENTAMICIN IN ANIMALS

1. *Cow*

Kinetic disposition and dosage regimen of gentamicin in pregnant cows were investigated after single i.v. administration (5 mg/kg). The distribution half life ($t_{1/2}$ α) and elimination half life ($t_{1/2}$ β) were calculated to be 0.05 ± 0.01 h 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 and 213.7 ± 28.4 ml/kg/h, respectively. To maintain the therapeutic plasma concentration (1 μg/ml) in pregnant cows, the dosage regimen of gentamicin would be 5 mg/kg body weight repeated at 4 h interval (Satish *et al.*, 1989).

Six healthy cows were injected i.v. or i.m. with gentamicin at 5 mg/kg body weight. Half-life ($t_{1/2}$ β) was calculated as 1.83 ± 0.18 h, Vd_C at 0.1 ± 0.02 L/kg and Vd_{SS} at 0.16 ± 0.03 L/kg. Absorption after i.m. injection was rapid with a half life ($t_{1/2}$ Ka) of 0.63 ± 0.28 h with an extent of absorption of 0.92 ± 0.15. After intrauterine administration of 2500 mg gentamicin once a day for 3 days, extent of absorption into the systemic circulation ranged from 18% to 96%. Mean peak serum and milk gentamicin concentrations were 4.98 ± 2.7 and 0.63 ± 0.77 μg/ml, respectively, 3 h after the last infusion. Endometrial gentamicin concentration at 24 h after the first infusion was 9.64 ± 3.55 μg/g tissue, increasing to 15.36 ± 5.48 μg/g at 48h and decreasing to 0.86 ± 0.43 μg/g at 120 h after the first infusion. Skeleted muscle concentrations were 0.82 and 0.24 μg/g at 25 and 73 h, respectively. The cows were then injected i.m. with gentamicin every 8 h for 10 days. Analysis on day 10 indicated a distribution phase half life of 2.59 ± 0.53 h and a terminal phase half life of 44.91 ± 9.38 h. Concentration throughout treatment were low in milk but high in uterine tissue. Renal function was not affected by treatment (Haddad, 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.

3. *Horse*

Serum levels and pharmacokinetic parameters were studied in 14 horses after i.v. administration of 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, 20, 30, 45, 60, 120, 240, 360 and 720 min after antibiotic administration. The results best fitted in two compartment open model with central volume of distribution (Vd_C) of 0.13 ± 0.08 and 0.19 ± 0.07 L/kg, elimination half life of 3.43 ± 0.84 and 3.78 ± 0.84 and total body clearance of 0.65 ± 0.45 and 0.99 ± 0.55 L/min/kg 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 perisist long enough to allow drug therapy in the horse. (Zurich *et al.*, 1995).

Swan *et al.* (1995) studied single and multiple dose pharmacokinetics of gentamicin administered i.v. and i.m. in adult thoroughbred mares. Gentamicin was administered to 6 horses at a dosage of 3.3 mg/kg body weight every 12 h for 5 i.v. or i.m. consecutive treatments. Equal number of horses was treated by either route during each phase. During each phase, serial blood samples were collected from each mare immediately before treatment and at 16 intervals following the 1st and 5th administration. Blood samples were also collected immediately before treatment and at 30 and 60 min following doses 2 through to 4. A distribution half life ($t_{1/2}\ \alpha$) of 0.1 ± 0.1 h, elimination half life ($t_{1/2}\ \beta$) of 1.2 ± 0.2 h, MRT of 1.4 ± 0.1 h and total body clearance (Cl_B) of 1.4 ± 0.2 ml/kg/min were observed following i.v. administration. The volume distribution at steady state (Vd_{SS}) was 117.6 ± 10.8 ml/kg. No significant difference was observed for any of the parameters between single or multiple doses for either route of administration. Except for AUC, significant differences were observed between multiple i.v. and i.m. treatments for all pharmacokinetic parameters determined.

Haddad *et al.* (1985) studied 6 healthy mature mares, which were given two doses of 5 mg/kg i.v. and i.m. 8 days apart. Venous blood samples collected regularly up to 48 h after i.v. injection

and up to 30 h after i.m. injection. Serum concentrations were determined by a liquid phase radioimmunoassay. The distribution phase half life was 0.12 ± 0.02 h and post distribution phase half life was 1.82 ± 0.22 h. The volume of the central compartment was 115.8 ± 6.0 ml/kg, Vd_{SS} was 188 ± 9.9 ml/kg and total body clearance was 1.27 ± 0.18 ml/kg/min. Intramuscular absorption was rapid with an absorption half life 0.64 ± 0.14 h. The extent of absorption was 0.87 ± 0.14. Kinetic calculations predicted that i.m. injections of 5 mg/kg every 8 hours would provide average steady state serum concentration of 7.0 μg/ml, with maximum and minimum steady state concentrations of 16.8 and 1.1 μg/ml, respectively.

4. *Camel*

Kinetics of gentamicin (3 mg/kg body weight) were determined in 6 camels (*Camelus dromedarius*) after i.v. and i.m. administration (Swan *et al.*, 1995). After i.v. administration, the overall elimination rate constant (β) was 0.24 ± 0.01 h and the half life was 2.92 ± 0.12 h. The mean residential time (MRT) was 1.20 ± 0.15 h. The volume of distribution at steady state (Vd_{SS}) was 260.6 ± 12.8 ml/kg and the total body clearance (Cl_B) was 62.7 ± 5.0 ml/kg/h. Following i.m. administration, gentamicin reached a peak serum concentration of 9.36 ± 0.5 μg/ml 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. Vd_{SS} was 254.1 ± 17.0 ml/kg but and Cl_B (total body clearance) was 62.9 ± 5 ml/kg/h; 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 bioavailability (F) was 89.1 ± 6.68.

5. *Llama*

Lackey *et al.* (1996) studied single intravenous and multiple dose pharmacokinetics of gentamicin in healthy llamas. 19 healthy male llamas were given gentamicin (5 mg/kg i.v.) as a signle bolus, and gentamicin concentrations were monitored over the next 48 h. Two months later, 10 of these llamas were given gentamicin (2.5 mg/kg i.v.) for the first day and then i.m. every 8 h for 7 days. Serum gentamicin concentrations and indices of renal function and damage were monitored during the 7 days. There were no significant dose or time-related differences 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 17.7 ± 6.59 min and $t_{1/2}$ β of 165 ± 40.3 min. Peak serum gentamicin concentration averaged 10.10 μg/ml in the multiple-dose trial and trough concentration averaged 1.50 μg/ml. There was no evidence of

renal impairment in the llamas. It is concluded that gentamicin pharmacokinetic variable in llamas resemble those in other ruminant species.

6. *Goats*

Garg *et al.* (1995) studied the disposition kinetics of parentrally administered gentamicin (5 mg/kg) in Gaddi goats. The serum concentration time profile was described by bi-exponential and mono-exponential equations 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. The bioavailability was higher following i.m. administration (96.3%) as compared to s.c. (76.9%). 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 intervals.

Pharmacokinetics of gentamicin following single i.v. dose in normal and febrile goats was studied by Ahmad and Sharma (1994). Gentamicin (Primicin, 5 mg/kg i.v.) was given first in clinically healthy female goats and then in the same goats after induction of fever by *Escherichia coli* endotoxin (0.2 μg/ml i.v.) Rectal temperature increased by 1-1.5^{0}C in febrile goats. Differences in the blood serum concentrations of gentamicin were not observed at any time between

febrile and normal goats. Median values for the half-lives of gentamicin were 103.6 min in normal and 136.0 min in febrile goats. The apparent volume of distribution (Vd) was 263.3 ml/kg in the febrile goats, which was not different from that in the normal goats (240.6 ml/kg). The volume of the central compartment (Vc) was almost indentical in normal and febrile goats. The total body clearance was 1.7 and 1.6 ml/kg/min. in normal and febrile goats, respectively. Dosage regimen for gentamicin was calculated on the basis of median kinetic data.

7. *Sheep*

Brown *et al.* (1986) studied dose dependent pharmacokinetics of gentamicin in sheep. To determine to what extent the pharmcokinetics of a single dose reflected conditions in practice when several doses may be given in sheep gentamicin was injected intravenously in single doses at 3, 10 and 20 mg/kg and multiple doses at 3 mg/kg at 8-hour intervals for 7 days. In the later case, the pharmacokinetics was complex and it was difficult to draw a withdrawal time between treatment and slaughter.

Wilson *et al.* (1984) studied the influence of endotoxin induced fever on the pharmacokinetics of gentamicin in ewes. The pharmacokinetics of gentamicin (3 mg/kg i.v. bolus) was evaluated in 6 adult ewes before and after fever was induced with *Escherichia coli*

endotoxin (1 μg/kg). In ewes with endotoxin-induced fever increased gentamicin concentrations that 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 and the over all biological half life or body clearance. Significant reductions 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.

8. *Swine*

Hypothyroidism or hyperthyroidism was induced in 10 pigs (5 pigs/ group) and each pig was administered gentamicin (6 mg/kg). Low thyroxine and triiodothyronine resulted in a decrease in creatinine clearance, an increase in serum creatinine concentration and a decrease in gentamicin systemic clearance as compared with the findings in five control pigs. These effects were probably secondary to a decreased glomerular filtration rate associated with hypothyroidism. A strong correlation among the 3 treatment groups was found between gentamicin systemic clearance and serum

creatinine clearance and between gentamicin systemic clearance and serum creatinine concentration. Hypothyroidism induced a slight but significant decrease in protein binding. However, the significant changes in the hypothyroid pigs and the hyperthyroid pigs were not of a magnitude sufficient to alter gentamicin elimination half-life. Gentamicin disposition was best described with a 4-compartment open model (Riond *et al.*, 1986).

Clawischnig *et al.* (1985) studied the pharmacokinetics of gentamicin in cattle and swine. Gentamicin was administered to 6 cows and 6 pigs in single intramuscular doses of 5 mg/kg. Serum concentrations were 50 to 100 μg/ml by 30 minutes. From the second hour after administration the serum concentrations fell considerably until the 12th hour. In milk, 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 and 75 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.

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 6, 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 concentrations, time data were 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 residential time was similarly longer in younger piglets (6.62±0.57 h) than in older animals (2.86 ±0.11 h). The steady-state volume of distribution (Vd_{SS}) was significantly larger for younger pigs (0.785 ± 0.036 L/kg) than in 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 piglets with a mean urinary half-life of 232.01 ±14.55 h. Percentages 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/kg) than in the 42-day old group (0.166 ± 0.10 L/h/kg). It is suggested that in the newborn piglets, the increase of Vd_{SS} could be explained by a higher proportion clearance could be attributed to a reduced glomerular filtration capacity (Giroux *et al.*, 1995).

9. *Rabbit*

Pharmacokinetics and dosage regimen of gentamicin were investigated in rabbits following a single intramuscular

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, respectively. Therapeutic plasma levels of 1 μg/ml were maintained upto 2 h. A satisfactory intramuscular dosage regimen would be 3.48 mg/kg and 3.10 mg/kg as priming and maintenance doses, 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 following i.v. administration of 4 mg/kg (Uppal *et al.*, 1992). 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 loading and maintenance doses, respectively, to be repeated at 8 h intervals.

Ogden *et al.* (1995) studied pharmacokinetics of gentamicin in rabbits. The bioavailability 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 a random sequence of administration i.v., i.m. and s.c. Gentamicin analysis was performed

on serial blood samples using fluorescence polarization immunoassay. The elimination half-life, mean residential time and serum gentamicin concentrations were not significantly different between the route of injection. It is concluded that in this study, the bioavailability of gentamicin slightly exceeded 100% and that the i.v. dose appeared to be less bioavailable than i.m and s.c. doses.

10. *Bull Calf*

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. Elimination half-life of gentamicin decreased from day 1 (149 minutes) to day 5 (119 minutes) but did not change between day 5 and 15 (111 min). Compared with the half-life in calves, that in cows was shorter (76 minutes). 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/ kg) was lower than that seen in calves on day 1 (1.92 ml/min/kg) and day 15 (2.10 ml/min/kg). 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. Age related changes in total body water were not found as measured by antipyrine space. The percentage protein binding of gentamicin was < 30% (Clarke *et al.*, 1985).

11. *Chicken*

Garg *et al.* (1989) studied disposition kinetics of gentamicin after repeated parenteral administration in the domestic fowl (*Gallus domesticus*). The pharmacokinetics of gentamicin following repeated i.m. or i.v. administration (4mg/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 lives were 24.15 ± 9.47 and 17.36 ± 4.64 min, respectively. The elimination half lives following repeated i.m. or i.v. administration were found to be 179.2 ± 39.18 and 97.41 ± 10.54 min, respectively. The values for apparent volume of distribution and total body clearance following repeated i.v. injection were 0.32 ± 0.05 L/kg and 2.27 ± 0.17 ml/kg/min., respectively. Statistical comparison of the values of disposition kinetics 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.m. or i.v.), the kinetic behaviour of gentamicin was changed significantly.

Garg *et al.* (1989) studied the kinetic disposition and bio-transformation of gentamicin sulfate in *Gallus domesticus* (White Leghorn chickens). Following intravenous administration of

gentamicin sulfate (4 mg/kg) in white Leghorn chickens, the disposition kinetics was best described by a two compartment open model. The distribution and elimination half lives of gentamicin were 10.25 ± 1.42 and 131.60 ± 15.14 min, respectively. The values for apparent volume of distribution and the values for apparent volume distribution and the total body clearance were 0.97 ± 0.18 L/kg and 5.01 ± 1.08 ml/kg/min, respectively. The concentration of gentamicin was highest in kidneys both at 15 min and 90 min after single administration (4 mg/kg) while 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.

12. *Pigeons*

Kosters *et al.* (1984) studied the pharmacokinetics of gentamicin in healthy pigeons and pigeons with salmonellosis. In healthy pigeons, a single i.m. injection of 5 mg gentamicin per kg body weight provided a therapeutic blood concentration of 4 μg/ml for 3 h. Treatment would have to be repeated at intervals of 4h to maintain high concentrations. There was no advantage in increasing the dosage. Blood concentrations were much lower in pigeons injected with gentamicin after infection with *Salmonella typhimurium* var. Copenhagen, which explains erratic results, from the treatment of salmonellosis.

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	-	-	4.16±0.37	-	-	5	i.v.	Haddad *et al.*, (1966)
Equine Horse	-	0.1±0.1	1.2±02	Vd_{SS}117.6 ± 10.8	1.4±0.2	3.3	i.v. & i.m.	Swan *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)
Camel	-	-	2.80±0.09	Vd_{ss}254.1±17.0	62.9±5.0	3	i.m.	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)

PARACETAMOL

Paracetamol, also known as acetaminophen, is the active metabolite of phenacetin, a so-called coal tar analgesic. Because paracetamol is well tolerated, lacks many of the side effects of aspirin, and is available without prescription. It has earned an important place as a common household analgesic. It is a potent antipyretic having analgesic and weak antiinflammatory property. It is currently widely used in veterinary and human medicine for the treatment of febrile conditions.

History

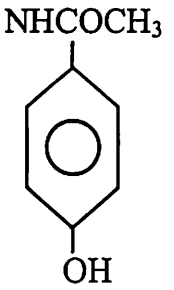
Acetanilide is the parent member of the group of drugs classified under para-aminophenol derivatives. It was introduced into medicine in 1886 under the trade name of antifebrin by Cahn and Hepp, who had accidentally discovered its antipyretic action. However, this compound proved to be excessively toxic. Later on, a number of chemical derivatives of para-aminophenol were then tested. One of the more satisfactory of these was phenacetin. It was introduced therapy in 1887 and was extensively employed in analgesic-mixtures until it was implicated in analgesic-abuse nephropathy.

Paracetamol (acetaminophen) was first used in medicine by von Mering in 1893. However, it has gained popularity only since

1949, after it was recognized as the major active metabolite of both acetanilide and phenacetin.

Chemistry

Chemically, paracetamol (actaminophen) is N-actyl-p-aminophenol. The chemical structure is as follows:



Emperical formula = $C_8H_{15}O_2N$.

Approx. molecular weight = 157.

Therapeutic activity

Paracetamol is a suitable substitute for aspirin for its antipyretic or analgesic action. It does not affect the acid-base balance, cardiovascular system, platelets and clotting factors. It is particularly valuable for patients in whom aspirin is contraindicated (eg. peptic ulcer) or whom the prolongation of bleeding time caused by aspirin would be a disadvantage. Paracetamol is an effective antipyretic, analgesic and least anti-inflammatory agent that is widely used in man and animals for treating febrile conditions.

KINETIC STUDIES OF PARACETAMOL IN ANIMALS

Pharmacokinetic studies on paracetamol were conducted in different species. They are described below: -

1. *Buffalo calf*

The disposition kinetics and dosage regimen of paracetamol in buffalo calves were carried out after i.m. administration (50 mg/kg) by Sidhu *et al.* (1993). The study showed absorption rate constant (Ka) of 1.73 ± 0.28 h^{-1}, and elimination rate constant (β) of 0.083 ± 0.008 h^{-1}. The absorption half-life ($t_{1/2}$ Ka) and elimination half life ($t_{1/2}$ β) of 0.47 ± 0.04 and 8.69 ± 0.83 h were noted. Vd_{area} of 1.22 ± 0.23 L/kg and the total body clearance (Cl_B) of 113.1 ± 39.8 ml/kg/h were calculated. They suggested a loading dose of 47.0 mg/kg followed by a maintenance dose of 23 mg/kg at 8 h interval.

2. *Cross-bred calf*

Sharma *et al.* (1995) conducted pharmacokinetic study of paracetamol following single i.m. administration (50 mg/kg) in cross bred calves. They noted Ka of 2.91 ± 0.36 h^{-1} and $t_{1/2}$ Ka of 0.251 ± 0.035 h. They further noted β of 0.167 ± 0.03 h^{-1} and $t_{1/2}$ β of 4.84 ± 1.26 h. They also noted Vd_{area} of 0.48 ± 0.11 L/kg and Cl_B of 79.6 ± 22.7 ml/kg/h.

3. *Goat*

Manna *et al.* (1994) studied the modification of the disposition kinetics of paracetamol by oxytetracyline and endotoxin induced fever in goats. The theoretical zero time concentration (C_p^o = A+B) of paracetamol alone (163.3 ± 9.9 μg/ml) was significantly ($p<0.01$) higher as compared to combined administration with oxytetracycline (56.0 ± 2.6 μg/ml) post i.v. dose of 50 mg/kg. The distribution rate constant (α) of 6.93 h^{-1} & distribution half life $t_{1/2}$ α of 0.10 h and elimination rate constant (β) of 1.30 h^{-1} & elimination half life ($t_{1/2}$ β) of 0.53 h were obtained when paracetamol (50 mg/kg) was administered alone in goat.

Sudha Kumari (1998) studied pharmacokinetic study of enrofloxacin and its interaction with paracetamol in goats. The distribution half life ($t_{1/2}$ α) of paracetamol 0.24 ± 0.04 h and elimination half life ($t_{1/2}$ β) of 3.56 ± 0.13 h were noted. Volume distribution and total body clearance were observed to be 5.48 ±1.40 L/kg and 17.37 ± 4.24 mg/kg/min, respectively. Significantly higher plasma drug concentrations were maintained from 5 to 45 min and also at 8 h in goats of combined administration of enrofloxacin and paracetamol as compared to single administration of enrofloxacin. Vd_{area} of 5.48 ± 1.40 and 1.57 ± 0.17 L/kg were noted for paracetamol when given alone by i.v. and s.c. routes, respectively. The higher value of Vd_{area} denotes good distribution of paracetamol in the body of goats as evidenced by high concentrations of the drug obtained in milk.

Table II : IMPORTANT KINETIC PARAMETERS OF PARACETAMOL IN DIFFERENT SPECIES

Species	Absorption half-life, $t_{1/2}$ Ka (h)	Distribution half-life, $t_{1/2}\alpha$,(h)	Elimination half-life, $t_{1/2}\beta$ (h)	Volume of distribution (L/kg)	Total body clearance (ml/kg/min)	Dose (mg/kg)	Route of administration	Reference
Buffalo calf	0.47±0.04	-	8.69±.0.83	1.22±0.23	113.1 ± 39.8	50	i.m.	Sidhu *et al.* (1993)
Cross bred calf	0.251±0.03 5	-	4.84±1.26	0.48±0.11	79.6 ± 22.7	50	i.m.	Sharma *et.al.* (1995)
Goat	--	0.10	0.53	--	--	50	i.v.	Manna *et al.* (1994)
	0.32 ± 0.04	0.24±0.04	3.56±0.13	5.48±1.40	17.37 ± 1.46	50	i.v	Sudha Kumari (1998)

KINETIC INTERACTIONS OF ANTIMICROBIALS WITH NON-STEROIDAL ANTIINFLAMMATORY DRUGS

Antimicrobials and nonsteroidal antiinflammory drugs (NSAIDs) are frequently used concomitantly and pharmacokinetic interactions between them have been described. (Kampmann *et al.*, 1972; Carbon *et al.*, 1981, 1984; Joly *et al.*, 1988; Mueller *et al.*, 1993; Manna *et al.*, 1994; Nergelius *et al.*, 1997; Sudha Kuamri, 1998; Tang *et al.*, 1999; Verma *et al.*, 2000; Choudhary *et al.*, 2002; Kumar Nitesh, 2002; Mukta, 2002).

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 ($t_{1/2}\beta$) of ceftriaxone and non significantly that of cefotiam but not of cefmenoxime.

Modification of the disposition kinetics of paracetamol by oxytetracycline in goats was carried out by Manna *et al.* (1994). They observed that the C_{max} value of paracetamol alone (128 ± 8.0 μg/ml) was significantly ($p < 0.01$) higher as compared to the combined therapy with oxytetracycline (46.8 ± 3.4 μg/ml) at 0.03 h post i.v.

drug administration. Paracetamol persisted in the blood till 2 h and 4 h for alone and combined therapy, respectively. The C_p^o value of paracetamol alone (163.3 ± 9.9 μg/ml) was significantly ($p < 0.01$) higher as compared to combined therapy (56.0 ± 2.6 μg/ml). The α and $t_{1/2}$ α values of paracetamol alone were higher and lower, respectively, as compared to combined administration. On the other hand $t_{1/2}$ β, Vd, Vd_B, Vd_{area} and Vd_{SS} values of combined therapy was significantly higher ($p < 0.02$) from the corresponding values of paracetamol alone.

No effect of diclofenac on the pharmacokinetics of cloxacillin was shown in man by Nergelius *et al.*, (1997). Total plasma clearance of cloxacillin was with placebo 219 ± 51 (mean ± S.D) and with diclofenac 212 ± 39 ml/min/ 1.73 m^2 (ns); renal clearance 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 ± 0.37 h with diclofenac (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^o), 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 ± 0.96 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 diclofenac metabolism by interaction with quinidine was studied in monkeys by Tang *et al.*, (1999). After a dose of diclofenac via portal vein infusion at 0.055 mg/kg/h, steady state systemic plasma drug concentrations 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 concentrations were 50, 59 and

18 ng/ml, representing 57, 56 and 56% of control values, respectively. In contrast, steady state systemic diclofenac concentration 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 remained unchanged in the presence of quinidine. Therefore, the decreases 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 increase 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 metabolism is enhanced by quinidine.

The mean pharmacokinetic characteristic of cyclosporine were unchanged during co-administration with diclofenac was studied in man by Mueller *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 48 h after each cyclosporine dose and over a dosing interval for diclofenac on day 7 (diclofenac alone) and day 8 (co-administration of diclofenac with cyclosporine). Based on area under the curve (AUC) comparison, lack of a pharmacokinetic interaction was conclusively demonstrated for the extent of cyclosporine absorption. The diclofenac 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.

Pharmacokinetic 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_{aera} (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 given alone (5.9 h, 7.1 L/kg, 0.4 h, 6.8 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.h and 0.82 μg/ml, respectively). Diclofenac sodium significantly ($p<0.1$) reduced the plasma concentration of ciprofloxacin (as metabolite of enrofloxaxin). 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 a therapeutic concentration of 0.1 μg/ml is recommended in cattle (Verma *et al.*, 2000).

Chaudhary *et al.* (2002) studied the pharmacokinetics of cefuroxime administered with paracetamol in buffalo calves. Cefuroxime was administered by single i.v. dose (10 mg/kg) and paracetamol was given by single intramuscular dose (50 mg/kg). Pharmacokinetics of cefuroxime was described by two-compartment open model. The highest concentration of cefuroxime was 80.9 ± 6.40 μg/ml at 1 min, which decreased to 0.97 ± 0.07 μg/ml at 8 h after its administration. The values of distribution half life, elimination half life and Vd_{area} were 0.101 ± 0.009 h, 1.91 ± 0.07 h and 0.55 ± 0.12

L/kg, respectively. The dosage regimen of cefuroxime when administered with paracetamol was calculated to be 10 mg/kg body wt. repeated at 8 h intervals.

Nitesh Kumar (2002) showed no effect of diclofenac in the pharmacokinetics of enrofloxacin and its active metabolite ciprofloxacin. In contrast, enrofloxacin significantly influenced the pharmacokinetics of diclofenac. Significantly higher $t_{1/2}$ β, AUMC, MRT, Vd_{area} values of 12.84 ± 1.29 h, 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 pharmacokinetics of amikacin and its interaction with diclofenac in buffalo calves. Concentrations of amikacin in plasma at all time intervals in case of its (7.5 mg/kg, i.v.) combined administration with diclofenac (1 mg/kg, i.v.) were found to be significantly lower as compared to its single administration. This has led to significant changes in various kinetic parameters when amikacin was administered concurrently with diclofenac. The values of various kinetic parameters like $t_{1/2}$ α (0.75 ± 0.23 h), $t_{1/2}$ β (4.67 ± 0.45 h) MRT (5.16 ± 0.30 h), Kel (1.006 ± 0.262 h^{-1}), Vd_{aera} (1.06 ± 0.06 L/kg) and Cl_B (2.78 ± 1.45 ml/kg/min) were obtained.

GENERAL PHARMACOKINETICS

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

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

One compartment open model

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

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

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

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

Where,

C_p = Concentration of drug plasma

B = Extrapolated zero time intercept of mono-exponential curve

β = Over all elimination rate constant

t = Time elapsed after drug administration

e = Base of natural logarithm

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

Two compartment open model

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

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

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

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

Where,

C_p = Plasma concentration of the drug,

A = Zero time intercept of distribution phase.

B = Zero time intercept of elimination phase.

α = Distribution rate constant.

β = Elimination rate constant

e = Base of natural logarithm

t = Time elapsed after drug administration

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

Three or multi compartment open model

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

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

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

PHARMACOKINETICS OF CLINICAL IMPORTANCE

Clinically, the pharmacokinetic study consists of: -

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

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

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

SOME IMPORTANT PHARMACOKINETIC PARAMETERS

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

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

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

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

3. Elimination rate constant (β)

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

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

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

iii. The total body clearance (Cl_B)

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

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

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

5. Volume of distribution

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

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

6. Total body clearance (Cl_B)

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

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

DOSAGE REGIMEN

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

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Chapter - 3

Materials and Methods

MATERIALS AND METHODS

In the present study, five clinically healthy female buffalo calves of non-descript breed between 12 to 18 months of age and 102 to 180 kg body weight were used. The buffalo calves 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 paracetamol were administered separately in each of five healthy female buffalo calves 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 buffalo calves.

DRUGS USED

Gentamicin and paracetamol were used in the present experiment. Progenta® — an injectable commercial preparation containing gentamicin in concentration of 40 mg/ml marketed by Vetsfarma and Paracetol-vet® — an injectable commercial preparation containing paracetamol in concentration of 150 mg/ml marketed by Sarabhai Zydus, India, was used.

COLLECTION OF BIOLOGICAL FLUIDS AND THEIR TIMINGS

The samples of various biological fluids were collected after i.v. administration of drugs in healthy buffalo calves. 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 buffalo calf 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 (Progenta®) containing 40 mg of gentamicin per ml was injected at the dose rate of 5 mg/kg body weight by i.v. route in each healthy female buffalo calf. Paracetamol (Paracetol®) injection containing 150 mg of paracetamol per ml was administered at the dose rate of 40 mg/kg body weight by i.v. route in each healthy buffalo calf. After conducting kinetic study of gentamicin and paracetamol 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. 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^{0}C 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 PARACETAMOL BY SPECTRO PHOTOMETRIC METHOD

Concentrations of paracetamol in plasma and urine were estimated by spectrophotometric method. The details of the procedure are as follows: -

Reagents

1. Hydrochloric acid 6 M.
2. Sodium nitrite 10% (Freshly prepared).
3. Trichloroacetic acid 10%.
4. Ammonium sulfamate 15%.
5. Sodium hydroxide 25%.

Preparation of standards of paracetamol in biological fluids

Paracetamol (Paracetol-vet®) — an injectable commercial preparation containing paracetamol in concentration of 150 mg/ml was used. Paracetamol was diluted in glass distilled water to different strength *viz.*, 1000, 500, 250, 100, 50 and 20 µg/ml. From each standard solution, 0.1 ml was added to a sterile vials containing 0.9

ml of plasma or urine collected prior to drug administration. This yielded standards of 100, 50, 25, 10, 5 and 2.0 and 1.0 μg/ml in the above noted biological fluids. These standards were simultaneously used along with test samples for the determination of drug concentrations in biological fluids.

Procedure

1. In a glass test tube, 1 ml of biological fluid was taken and 2 ml of trichloroacetic acid was added and thoroughly mixed for 30 sec. The content was then filtered through Whatmann filter paper No.1
2. Whole filterate was taken into a 25 ml test tube containing 1 ml of 6 M HCl and 2 ml of sodium nitrite solution. The contents were mixed properly and allowed to stand exactly for 2 min at room temperature.
3. Then 2 ml of ammonium sulfamate (15%) solution was added drop by drop into the tubes and thoroughly mixed for 1 min.
4. Again 2.5 ml of sodium hydroxide (25%) solution was added and mixed for 15 sec and allow to stand for 2 min at room temperature.
5. The optical density of the resultant colour developed was measured at 430 nm in a spectrophotometer against blank.

6. The exact concentration of paracetamol was calculated with the help of standard curve simultaneously prepared in biological fluids collected prior to drug administration.

7. Blank was simultaneously prepared in the same way using 1 ml of biological fluid taken prior to drug administration in place of test sample.

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 (1975) and Notari (1980). For a two compartment model, the concentration of the drug in plasma at any time is obtained from the formula: -

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

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

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

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

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

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

(d) Distribution half life ($t_{1/2}$ α) and elimination half life ($t_{1/2}$ β) 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.

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Chapter - 4

Results

RESULTS

I. PHARMACOKINETIC STUDY AFTER SINGLE INTRAVENOUS 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 5 mg/kg have been shown in Table 1 and Fig. 1. The mean peak plasma concentration of 19.80 ± 1.36 μg/ml was attained at 0.042h. The drug was detectable upto 24 h in all animals with the mean of 0.23 ± 0.03 μg/ml. The mean therapeutic concentration (≥ 2 μg/ml) was maintained upto 6 h.

2. Urine levels: -

Concentrations of gentamicin in urine at different time intervals after its single i.v. administration at the dose rate of 5 mg/kg are presented in Table 2 and Fig 2. The drug appeared in urine of all the animals with a mean of 7.66 ± 0.82 μg/ml at 0.042 h. The mean peak urine drug concentration of 83.42 ± 3.17 μg/ml was achieved at

TABLE – 1

Plasma concentrations (μg/ml) of gentamicin in healthy female buffalo calves following single intravenous dose (5 mg/kg)

Time (h)	ANIMAL NUMBER					Mean ± S.E.M.
	1	2	3	4	5	
0.042	16.21	24.45	18.25	20.35	19.75	19.80±1.36
0.083	13.59	20.50	14.56	17.28	17.25	16.64±1.21
0.167	11.38	17.85	12.12	16.50	15.62	14.69±1.26
0.25	11.00	14.12	10.14	14.85	13.02	12.63±0.90
0.333	9.54	10.65	9.42	12.92	11.85	10.88±0.67
0.50	9.14	9.42	8.65	10.66	10.25	9.62±0..37
0.75	7.99	8.45	7.92	9.80	9.85	8.80±0.43
1	6.70	7.68	7.00	8.40	8.82	7.72±0.40
1.5	4.70	6.00	6.42	7.62	7.62	6.47±0.55
2	3.94	5.42	4.85	5.20	7.00	5.28±0.50
3	3.30	3.75	3.92	3.95	3.80	3.74±0.12
4	2.76	2.85	3.26	3.70	3.10	3.13±0.17
5	2.32	2.62	2.70	2.62	2.25	2.50±0.09
6	1.94	2.00	2.40	2.50	1.92	2.15±0.12
8	0.80	1.58	1.50	1.60	1.45	1.39±0.15
10	0.47	0.98	1.12	1.15	1.30	1.00±0.14
12	0.28	0.80	0.82	0.68	0.65	0.65±0.10
24	0.14	0.32	0.28	0.20	0.22	0.23±0.03

Fig. 1

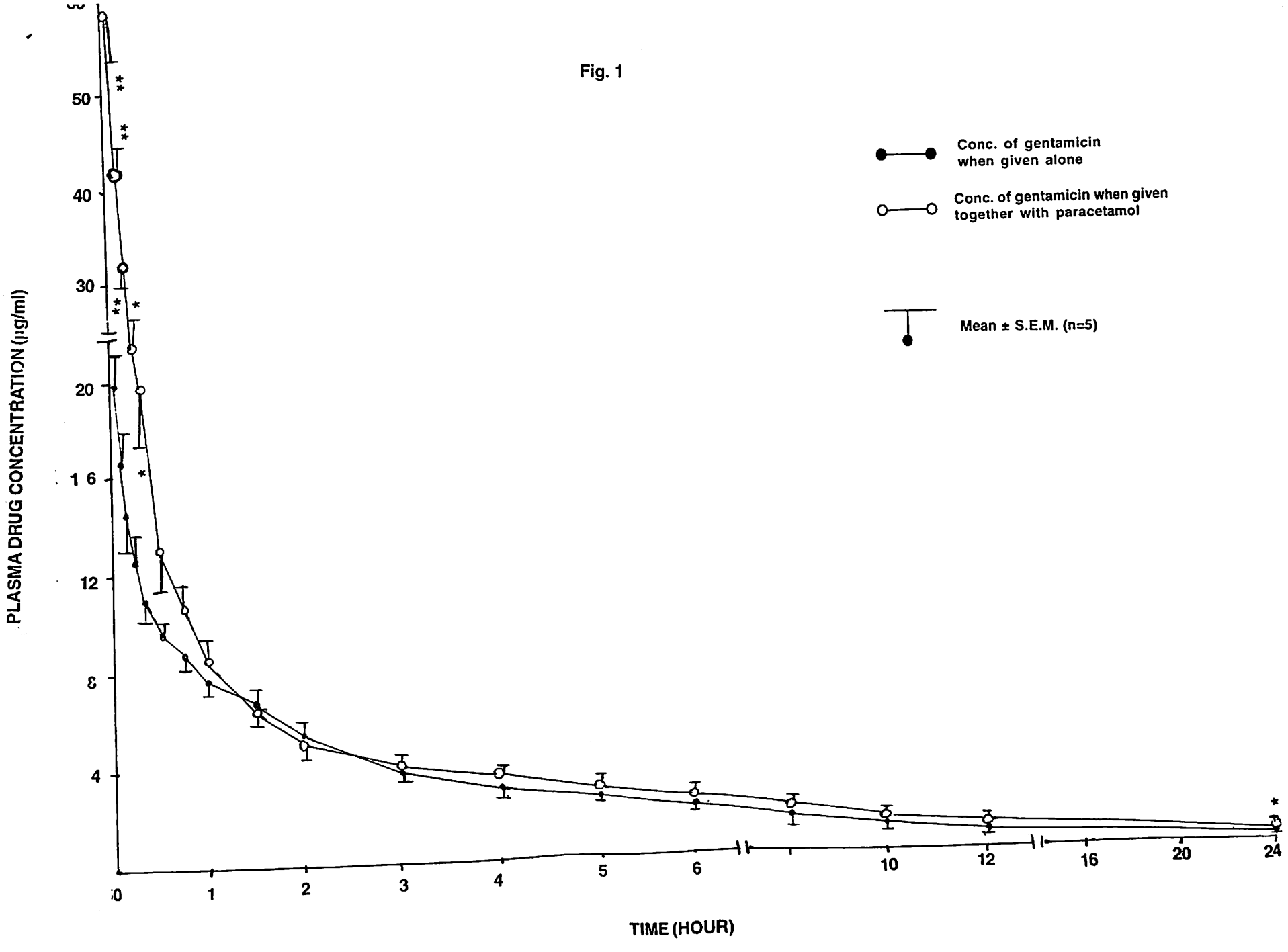

Conc. of gentamicin when given alone
Conc. of gentamicin when given together with paracetamol
Mean ± S.E.M. (n=5)
PLASMA DRUG CONCENTRATION (µg/ml)
50
40
30
20
16
12
8
4
0
1
2
3
4
5
6
10
12
16
20
24
TIME (HOUR)

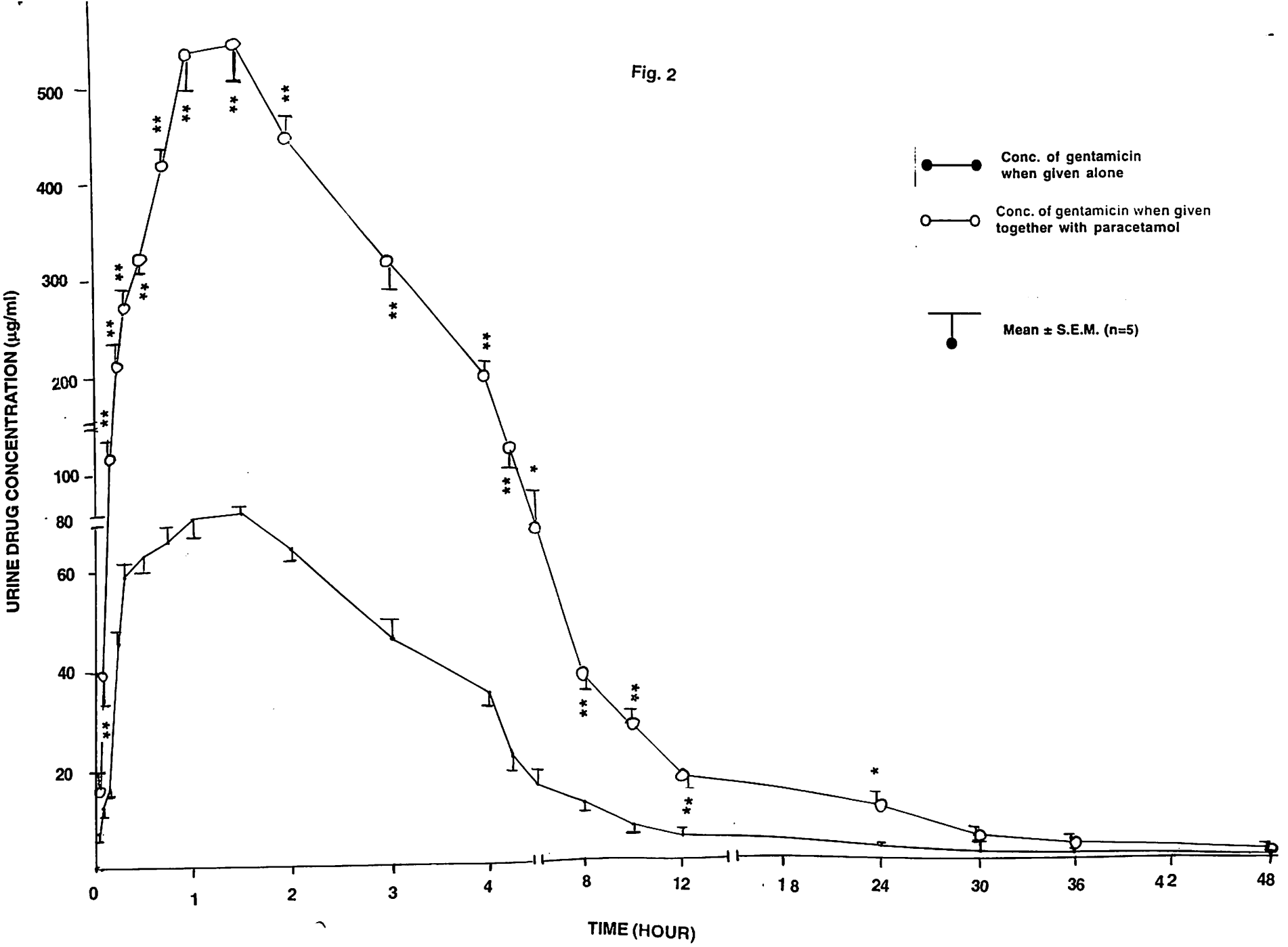
TABLE – 2

Urine concentrations (μg/ml) of gentamicin in healthy female buffalo calves following single intravenous dose (5 mg/kg)

Time (h)	ANIMAL NUMBER					Mean ± S.E.M.
	1	2	3	4	5	
0.042	5.60	10.12	7.25	8.90	6.45	7.66±0.82
0.083	11.76	13.50	11.92	12.76	12.90	12.57±0.32
0.167	12.30	18.60	15.14	18.40	16.00	16.09±1.16
0.25	42.50	49.30	36.28	50.18	49.20	45.49±2.68
0.333	51.10	65.80	55.10	62.10	61.95	59.21±2.66
0.50	53.94	78.25	60.32	70.26	69.30	66.41±4.22
0.75	56.82	81.36	66.16	76.91	78.40	71.93±4.57
1	61.50	92.18	70.15	82.40	95.45	80.34±6.45
1.5	74.00	88.41	78.41	91.16	85.12	83.42±3.17
2	61.50	63.20	69.10	76.25	70.46	68.10±2.65
3	38.20	48.62	36.95	50.30	55.10	45.83±3.54
4	29.40	35.91	30.22	34.60	42.60	34.55±2.37
5	14.40	29.70	18.14	20.42	25.45	21.62±2.70
6	12.00	20.00	14.00	16.55	18.32	16.17±1.44
8	5.60	14.32	12.96	12.38	14.96	12.04±1.68
10	3.88	8.90	8.40	8.95	7.60	7.55±0.95
12	3.24	5.33	5.60	4.30	5.20	4.73±0.43
24	2.68	2.74	3.10	2.70	2.81	2.81±0.08
30	1.12	1.50	1.62	0.95	0.90	1.22±0.15
36	0.92	0.86	0.78	0.50	0.68	0.75±0.07
48	0.16	0.18	0.15	N.D	N.D	0.10±0.04

N. D. = Non detectable

Fig. 2


URINE DRUG CONCENTRATION (µg/ml)
500
400
300
200
100
80
60
40
20
0
1
2
3
4
8
12
18
24
30
36
42
48
TIME (HOUR)
Conc. of gentamicin when given alone
Conc. of gentamicin when given together with paracetamol
Mean ± S.E.M. (n=5)

1.5 h. The drug was detectable upto 36 h in all animals with a mean of 0.75 ± 0.07 μg/ml. The drug was detectable in 3 out of 5 animals at 48 h. The mean therapeutic concentration of ≥ 2 μg/ml was maintained upto 24 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^0) were noted to be 11.08 ± 0.86, 4.81 ± 0.31 and 15.89 ± 0.85 μg/ml, respectively. The distribution rate constant (α) ranged from 0.700 to 1.305 h^{-1} with a mean of 1.058 ± 0.100 h^{-1}, while its elimination rate constant (β) ranged from 0.116 to 0.165 h^{-1} with a mean value of 0.139 ± 0.008 h^{-1}. The mean distribution half life ($t_{1/2}\,\alpha$) and elimination half life ($t_{1/2}\,\beta$) were noted to be 0.69 ± 0.08 and 5.05 ± 0.30 h, respectively. The mean area under curve (AUC) of 45.90 ± 3.10 mg/L.h, area under first moment curve (AUMC) of 268.2 ± 27.86 mg/L.h^2 and mean residential time (MRT) of 5.79 ± 0.34 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 0.425 ± 0.054, 0.421 ± 0.042 and 0.351 ± 0.023 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 0.40 ± 0.02 and 1.54 ± 0.16. Various values of volume distribution obtained by different methods are shown in Table 3. A mean Vd_{area} of 0.80 ± 0.04 L/kg was noted. The total body clearance (Cl_B) value ranged from 1.65 to 2.48 ml/kg/min with a mean of 1.86 ± 0.16 ml/kg/min.

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 2.47 ± 0.23 and 4.37 ± 0.54 mg/kg, while maintenance doses (D_os) were calculated to be 1.67 ± 0.20 and 3.57 ± 0.53 mg/kg at the dosage intervals of 8 and 12 h, respectively.

Fig. 3

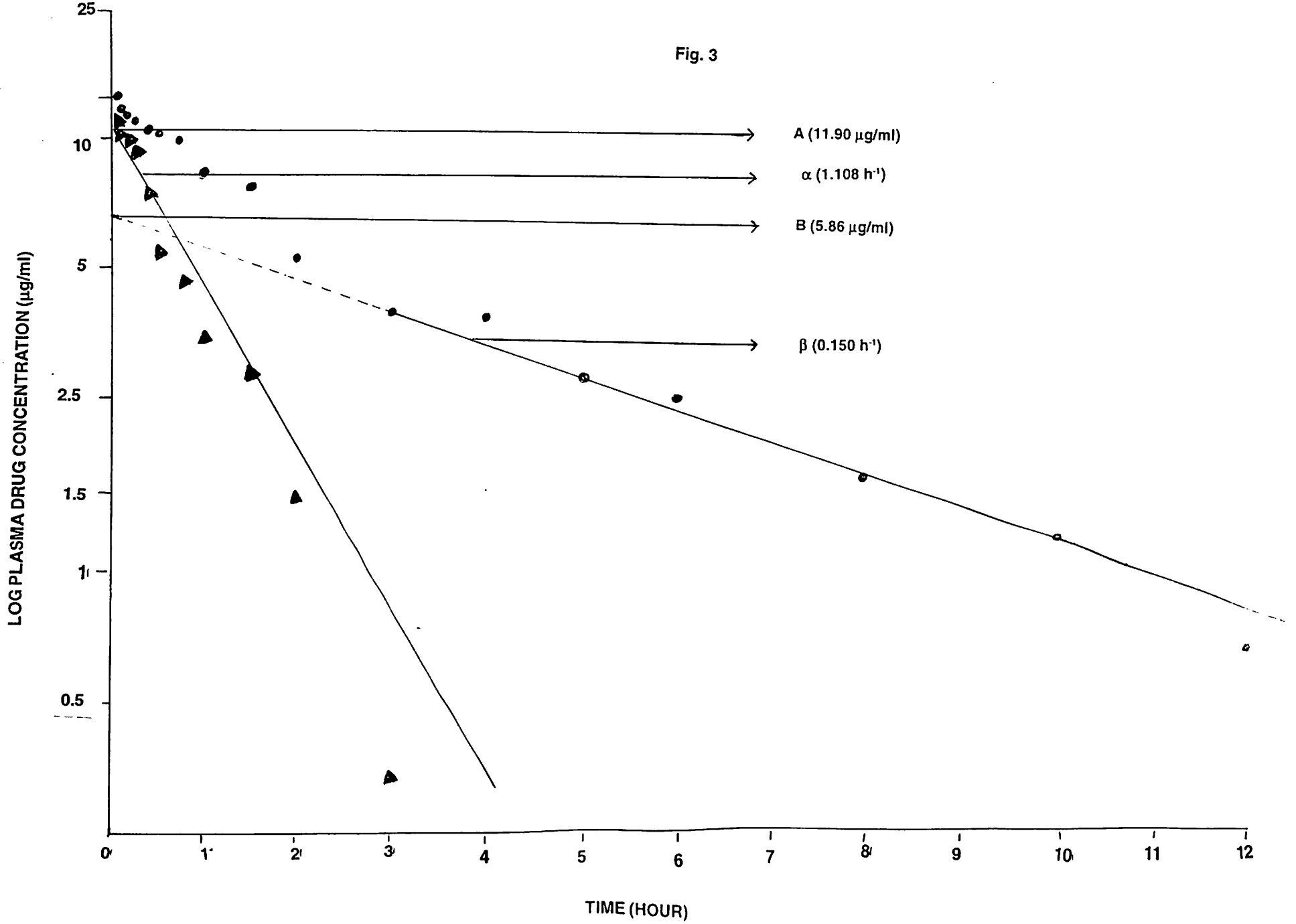

25
10
5
2.5
1.5
1
0.5
LOG PLASMA DRUG CONCENTRATION (µg/ml)
A (11.90 µg/ml)
α (1.108 h⁻¹)
B (5.86 µg/ml)
β (0.150 h⁻¹)
0
1
2
3
4
5
6
7
8
9
10
11
12
TIME (HOUR)

TABLE – 3

Kinetic parameters of gentamicin in healthy female buffalo calves (calculated by 2-compartment open model) after a single intravenous dose (5 mg/kg)

Kinetic parameter (Unit)	ANIMAL NUMBER					Mean ± S.E.M.
	1	2	3	4	5	
A (μg/ml)	10.17	13.65	8.50	11.90	11.17	11.08±0.86
B (μg/ml)	4.28	4.18	5.13	5.86	4.61	4.81±0.31
C_p^0 (μg/ml)	14.45	17.83	13.63	17.76	15.78	15.89±0.85
α (h^{-1})	1.305	1.074	1.101	1.108	0.700	1.058±0.10
$t_{1/2}$ α (h)	0.53	0.65	0.63	0.63	0.99	0.69±0.08
β (h^{-1})	0.165	0.116	0.131	0.150	0.134	0.139±0.008
$t_{1/2}$ β (h)	4.20	5.97	5.29	4.62	5.17	5.05±0.30
AUC (mg./L.h)	33.73	48.74	46.88	49.81	50.36	45.90±3.10
AUMC (mg/L.h^2)	163.2	322.5	305.9	270.1	279.5	268.2±27.86
MRT (h)	4.84	6.62	6.53	5.42	5.55	5.79±0.34
K_{12} (h^{-1})	0.539	0.484	0.445	0.435	0.221	0.425±0.054
K_{21} (h^{-1})	0.503	0.341	0.496	0.466	0.299	0.421±0.042
Kel (h^{-1})	0.428	0.365	0.291	2.357	0.314	0.351±0.023
Fc	0.39	0.32	0.45	2.42	0.43	0.40±0.02
T ≈ P	1.59	2.15	1.22	1.38	1.34	1.54±0.16
Vd_c (L/kg)	0.35	0.28	0.37	0.28	0.32	0.32±.0.02
Vd_B (L/kg)	1.17	1.20	0.97	0.85	1.08	1.05±0.06
Vd_{area} (L/kg)	0.90	0.88	0.81	0.67	0.74	0.80±0.04
Vd_{SS} (L/kg)	0.73	0.68	0.70	0.54	0.56	0.64±0.04
Cl_B (ml/kg/min)	2.48	1.70	1.77	1.68	1.65	1.86±0.16

TABLE – 4

Dosage regimen of gentamicin in healthy female buffalo calf

C_p^{∞} min (µg/ml)	γ (h)	Dose (mg/kg)	ANIMAL NUMBER					Mean ± S.E.M.
			1	2	3	4	5	
1	8	D*	3.37	2.23	2.31	2.22	2.21	2.47±0.23
		D_o	.2.47	1.35	1.50	1.55	1.47	1.67±0.20
	12	D*	6.52	3.54	3.90	4.05	3.83	4.37±0.54
		D_o	5.62	2.66	3.09	3.38	3.09	3.57±0.53
2	8	D*	6.74	4.45	4.62	4.45	4.43	4.94±0.45
		D_o	4.94	2.69	3.00	3.11	2.95	3.34±0.41
	12	D*	13.04	7.08	7.80	8.11	7.66	8.74±1.09
		D_o	11.24	5.32	6.18	6.77	6.18	7.14±1.05
4	8	D*	13.48	8.90	9.24	8.90	8.86	9.88±0.90
		D_o	9.88	5.38	6.00	6.22	5.90	6.68±0.81
	12	D*	26.08	14.16	15.60	16.22	15.32	17.48±2.17
		D_o	22.48	10.64	12.36	13.54	12.36	14.28±2.10

D* = Priming or loading dose

D_o = Maintenance dose

γ = Dosage interval

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

The D*s were calculated to be 4.94 ± 0.45 and 8.74 ± 1.09 mg/kg, while D_os were found to be 3.34 ± 0.41 and 7.14 ± 1.05 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 9.88 ± 0.90 and 17.48 ± 2.17 mg/kg, while D_os were found to be 6.68 ± 0.81 and 14.28 ± 2.10 mg/kg at γ of 8 and 12 h.

(B) PARACETAMOL

1. Plasma levels: -

Table 5 and Fig. 4 depict the concentrations of paracetamol in plasma of buffalo calves at different time intervals following single i.v. dose of 40 mg/kg. The mean plasma concentration of the drug at 0.042 h was found to be 20.23 ± 0.84 μg/ml and the value ranged from 17.95 to 22.50 μg/ml. The drug was detectable in 4 out of 5 animals at 2 h and the mean plasma concentration was observed to be 1.87 ± 0.48 μg/ml.

2. Urine levels: -

The drug concentrations in urine following single i.v. administration of paracetamol (40 mg/kg) are presented in Table 6 and Fig. 5. The drug appeared at 0.042 h with a mean of 12.01 ± 1.38 μg/ml and was maintained upto 48 h in all animals with a mean value of 10.90 ± 1.14 μg/ml. The mean peak urine concentration of 2022 ± 118.8 μg/ml was observed at 1.5 h.

Fig. 4

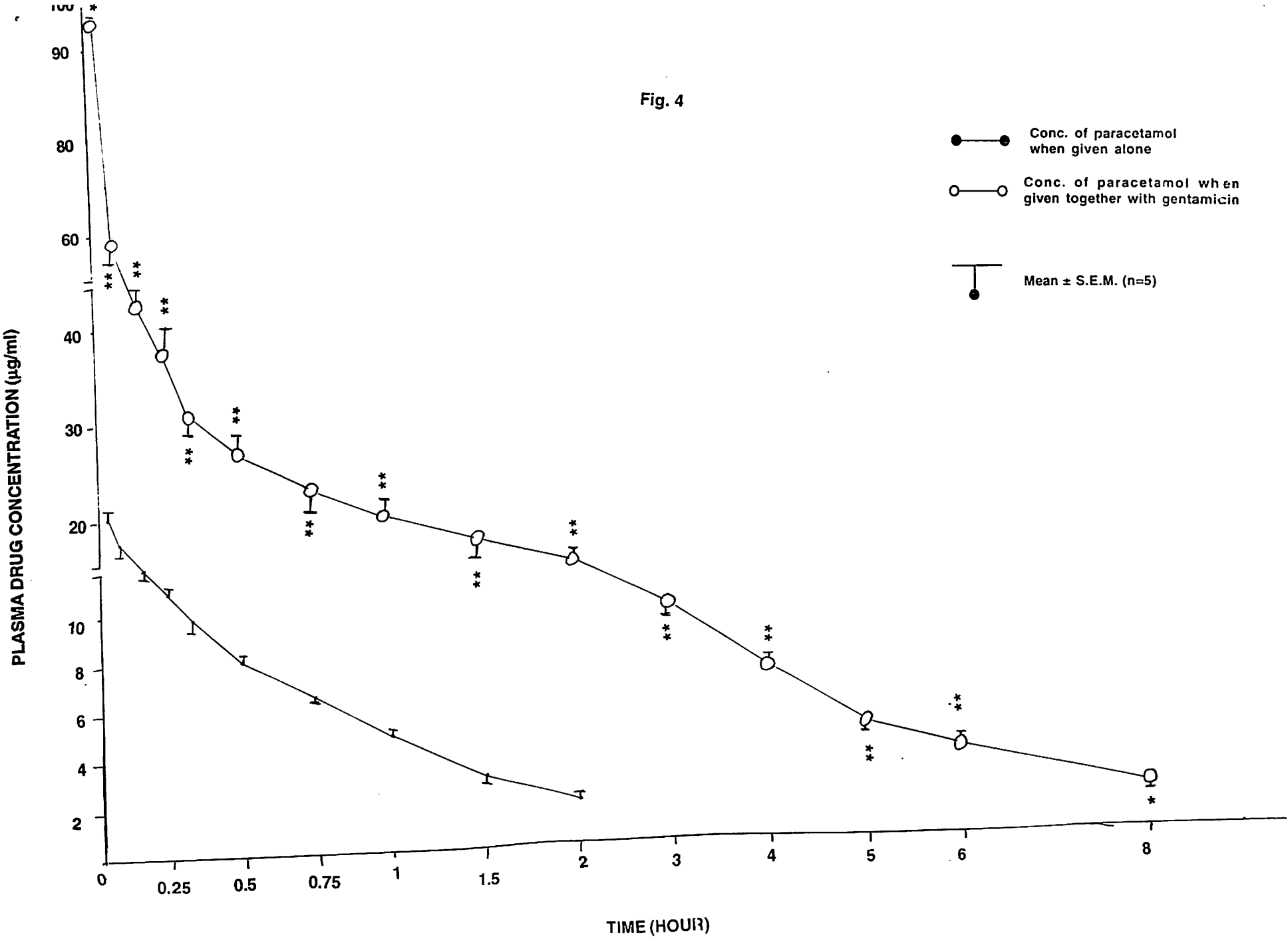

Conc. of paracetamol when given alone
Conc. of paracetamol when given together with gentamicin
Mean ± S.E.M. (n=5)
PLASMA DRUG CONCENTRATION (µg/ml)
TIME (HOUR)
100
90
80
60
40
30
20
10
8
6
4
2
0
0.25
0.5
0.75
1
1.5
2
3
4
5
6
8

TABLE – 5

Plasma concentrations (μg/ml) of paracetamol in female healthy buffalo calves following single intravenous dose of 40 mg/kg

Time (h)	ANIMAL NUMBER					Mean ± S.E.M.
	1	2	3	4	5	
0.042	17.95	22.50	20.25	21.62	18.82	20.23 ±0.84
0.083	15.93	18.20	18.68	19.00	15.85	17.53 ±0.68
0.167	13.92	15.00	15.04	15.85	14.12	14.79 ±0.35
0.25	12.02	12.65	12.00	12.88	11.85	12.28 ±0.20
0.333	9.88	11.20	8.88	9.02	10.65	9.93 ±0.45
0.50	7.86	8.25	7.80	7.45	9.00	8.07 ±0.26
0.75	6.84	6.55	6.66	6.12	6.60	6.55 ±0.12
1	3.82	4.38	5.15	5.25	5.42	4.80 ±0.30
1.5	1.80	2.76	2.80	4.02	4.12	3.10 ±0.43
2	N.D.	2.05	2.10	2.54	2.65	1.87 ±0.48
3	N.D.	N.D.	N.D.	N.D.	N.D.	---

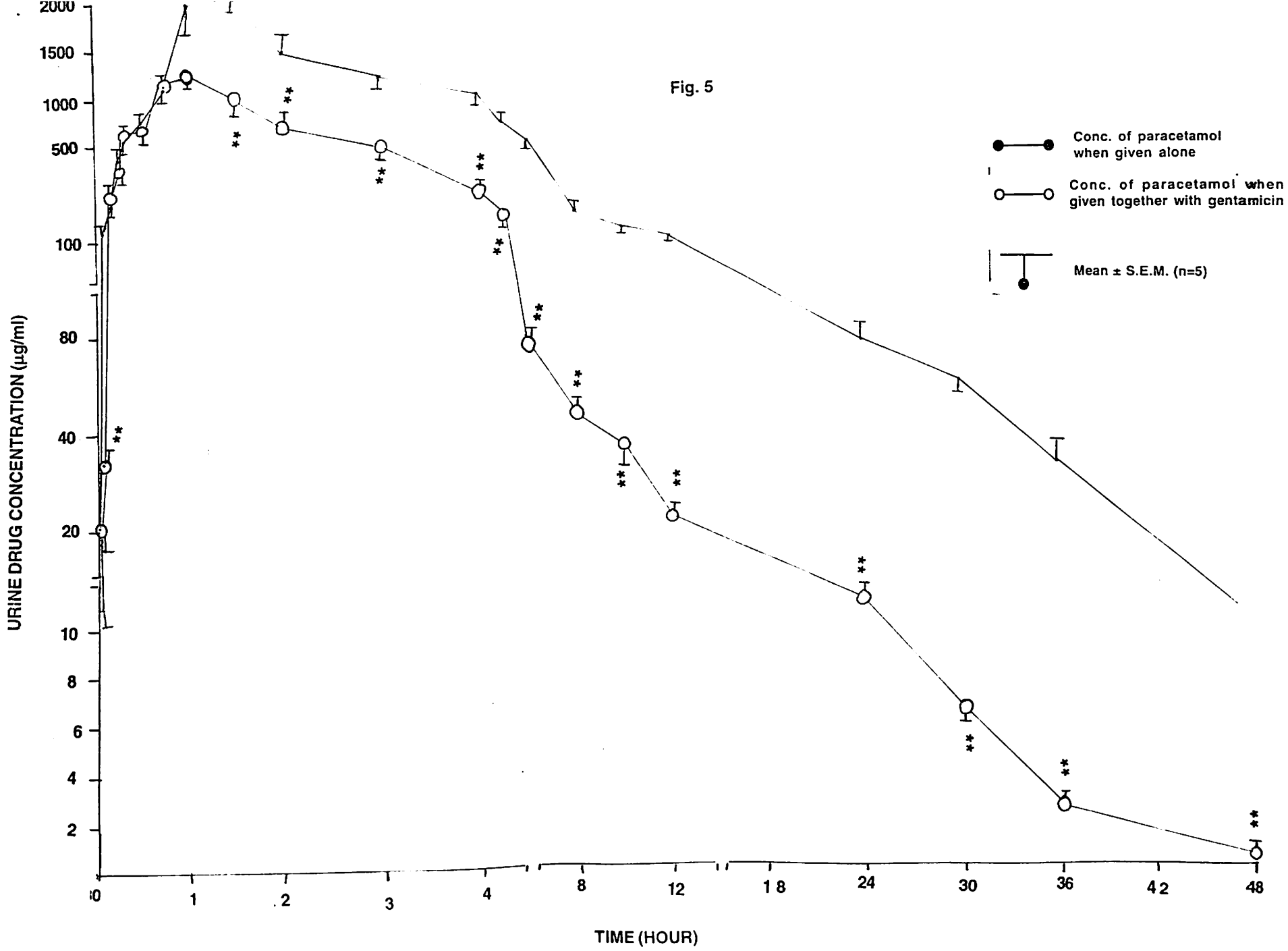
N.D. = Non detectable

TABLE – 6

Urine concentrations (μg/ml) of paracetamol in female healthy buffalo calves following single intravenous dose of 40 mg/kg

Time (h)	ANIMAL NUMBER					Mean ± S.E.M.
	1	2	3	4	5	
0.042	12.28	9.85	15.60	8.12	14.26	12.01±1.38
0.083	189.6	110.4	192.2	105.8	109.6	141.5±20.18
0.167	291.0	220.6	312.4	208.2	244.8	255.4±20.08
0.25	522.4	388.4	570.5	326.6	395.2	440.6±45.47
0.333	575.5	495.2	683.2	455.8	588.4	559.6±39.55
0.50	727.5	688.5	1002	640.2	705.8	752.8±63.94
0.75	1037	912.8	1455	884.8	1286	1115±110.6
1	1249	2255	2650	1226	2456	1967±304.4
1.5	2480	1889	2015	1812	1918	2022±118.8
2	2056	1605	1414	1126	1218	1483±165.2
3	1759	1106	1212	1004	1056	1227±137.3
4	1552	912.4	1012	815.6	902.2	1038±132.0
5	786.0	658.2	810.8	580.4	788.6	724.8±44.99
6	579.8	465.6	605.2	425.2	524.2	520±33.76
8	225.2	168.4	268.4	240.4	170.5	214.6±19.69
10	162.4	124.2	180.2	145.6	110.4	144.6±12.59
12	112.6	93.82	120.5	98.6	94.6	104.0±5.33
24	83.8	68.44	90.8	68.5	70.25	76.36±4.61
30	63.6	50.5	70.4	52.5	56.44	58.69±3.69
36	42.5	32.2	44.8	20.4	24.36	32.85±4.81
48	10.28	9.15	12.26	8.25	14.58	10.90±1.14

Fig. 5


Conc. of paracetamol when given alone
Conc. of paracetamol when given together with gentamicin
Mean ± S.E.M. (n=5)
URINE DRUG CONCENTRATION (µg/ml)
2000
1500
1000
500
100
80
40
20
10
8
6
4
2
0
1
2
3
4
8
12
18
24
30
36
42
48
TIME (HOUR)

3. Kinetic parameters: -

Log plasma drug concentration versus time profile of paracetamol has confirmed a two compartment open model. Hence, kinetic parameters of paracetamol were derived from the formulae of the above noted compartment model and are presented in Table 7.

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^0) were noted to be 9.51 ± 1.94, 13.11 ± 1.08 and 22.62 ± 1.00 μg/ml, respectively. The distribution rate constant (α) ranged from 5.064 to 12.490 h^{-1} with a mean value of 7.148 ± 1.36 h^{-1} while its elimination rate constant (β) ranged from 0.715 to 1.479 h^{-1} with a mean value of 0.965 ± 0.136 h^{-1}. The mean distribution half life ($t_{1/2}$ α) and elimination half life ($t_{1/2}$ β) values of the drug were observed to be 0.11 ± 0.01 and 0.77 ± 0.09 h, respectively. The values of area under curve (AUC) in plasma and area under first moment curve (AUMC) were found to be 15.59 ± 1.07 mg/L.h and 16.09 ± 2.56 mg/L.h^2 with a mean residential time (MRT) of 1.00 ± 0.10 h. The average rate of transfer of drug from central to peripheral (K_{12}), peripherals to central (K_{21}), and elimination from central (Kel) compartment were calculated to be 1.672 ± 0.142, 4.970 ± 1.540 and 1.471 ± 0.089 h^{-1} respectively. The fraction of drug

TABLE – 7

Kinetic parameters of paracetamol in female healthy buffalo calves following single intravenous does of 40 mg/kg

Kinetic parameter (Unit)	ANIMAL NUMBER					Mean ± S.E.M.
	1	2	3	4	5	
A (μg/ml)	2.58	11.34	11.85	13.60	8.17	9.51±1.94
B (μg/ml)	17.26	12.60	12.39	10.93	12.37	13.11±1.08
C_p^0 (μg/ml)	19.84	23.94	24.25	24.53	20.54	22.62±1.00
α (h^{-1})	12.49	5.457	6.559	5.064	6.167	7.148±1.361
$t_{1/2}$ α (h)	0.06	0.13	0.11	0.14	0.11	0.11±0.01
β (h^{-1})	1.479	0.952	0.914	0.715	0.767	0.965±0.136
$t_{1/2}$ β (h)	0.47	0.73	0.76	0.97	0.90	0.77±0.09
AUC (mg./L.h)	11.88	15.31	15.36	17.97	17.45	15.59±1.07
AUMC (mg/L.h^2)	7.91	14.28	15.11	21.91	21.24	16.09±2.56
MRT (h)	0.67	0.93	0.98	1.22	1.22	1.00±0.10
K_{12} (h^{-1})	1.241	1.523	2.097	1.761	1.738	1.672±0.142
K_{21} (h^{-1})	11.06	3.323	3.798	2.653	4.019	4.970±1.540
Kel (h^{-1})	1.671	1.563	1.578	1.365	1.177	1.471±0.089
Fc	0.89	0.61	0.58	0.52	0.65	0.65±0.06
T ≈ P	0.13	0.64	0.73	0.91	0.53	0.59±0.13
Vd_c (L/kg)	2.02	1.67	1.65	1.63	1.95	1.78±0.08
Vd_B (L/kg)	2.32	3.17	3.23	3.66	3.23	3.12±0.22
Vd_{area} (L/kg)	2.28	2.74	2.85	3.11	2.99	2.79±0.14
Vd_{SS} (L/kg)	2.25	2.44	2.56	2.71	2.79	2.55±0.10
Cl_B (ml/kg/min)	56.20	43.47	43.42	37.06	38.22	43.67±3.39

available for elimination from central compartment (Fc) and approximate tissue to plasma concentration ratio (T≈P) were noted to be 0.65 ± 0.06 and 0.59 ± 0.13. The various values of volume of distribution calculated by different methods are shown in Table 8. The mean value of Vd_{area} was calculated to be 2.79 ± 0.14 L/kg. The total body clearance (Cl_B) ranged from 37.06 to 56.20 ml/kg/min with a mean value of 43.67 ± 3.39 ml/kg/min.

II. KINETIC STUDIES OF DRUGS AFTER COMBINED ADMINISTRATION OF GENTAMICIN AND PARACETAMOL

(A) GENTACMICIN

1. Plasma levels: -

Plasma concentrations of gentamicin at different time intervals following combined i.v. administration of gentamicin (5 mg/kg) and paracetamol (40 mg/kg) have been shown in Table 8 and Fig. 1. The drug was present at 0.042 h with a mean of 58.61 ± 4.56 μg/ml and was detectable in plasma samples of all the buffalo calves upto 24 h with a mean value of 0.49 ± 0.04 μg/ml. The mean therapeutic concentration (≥ 2 μg/ml) was maintained around 8 h.

2. Urine levels: -

Urine concentrations of gentamicin at various time intervals following combined i.v. administration of gentamicin (5 mg/kg) and paracetamol (40 mg/kg) have been presented in Table 9 and Fig. 2. The drug appeared in all animals at 0.042 h with the

TABLE – 8

Plasma concentrations (μg/ml) of gentamicin in buffalo calves following combined administration of gentamicin (5mg/kg) and paracetamol (40 mg/kg) after intravenous administration

Time (h)	ANIMAL NUMBER					Mean ± S.E.M.
	1	2	3	4	5	
0.042	75.41	54.26	60.85	50.28	52.25	58.61±4.56
0.083	48.28	40.55	44.28	37.12	38.55	41.76±2.03
0.167	35.86	31.22	34.60	28.15	29.12	31.79±1.50
0.25	30.90	21.25	28.00	19.22	18.76	23.63±2.46
0.333	26.64	17.00	23.10	16.00	15.52	19.65±2.22
0.50	17.05	10.12	15.05	12.95	10.14	13.06±1.36
0.75	12.66	8.82	11.96	10.55	8.54	10.51±0.82
1	10.91	7.14	8.46	8.24	7.20	8.39±0.68
1.5	6.99	6.00	6.50	6.00	6.14	6.33±0.19
2	6.02	4.62	5.25	3.95	4.50	4.87±0.35
3	4.47	4.00	3.60	3.10	4.12	3.86±0.23
4	4.17	3.65	3.22	2.75	3.80	3.52±0.25
5	3.85	2.84	2.46	2.28	3.20	2.93±0.28
6	2.86	2.52	2.30	1.85	2.68	2.44±0.17
8	1.58	2.18	1.68	1.50	2.20	1.83±0.15
10	1.12	1.35	1.35	1.24	1.45	1.30±0.06
12	0.87	0.90	0.80	0.72	1.00	0.86±0.05
24	0.55	0.52	0.42	0.38	0.60	0.49±0.04

TABLE – 9

Urine concentrations (μg/ml) of gentamicin in female buffalo calves following combined administration of gentamicin (5 mg/kg) and paracetamol (40 mg/kg) after intravenous administration

Time (h)	ANIMAL NUMBER					Mean ± S.E.M.
	1	2	3	4	5	
0.042	24.46	12.62	18.56	10.28	11.94	15.57±2.63
0.083	55.42	38.34	42.80	29.40	30.80	39.35±4.71
0.167	157.4	100.4	121.3	99.00	100.1	115.6±11.24
0.25	282.3	198.1	212.5	180.2	185.3	211.7±18.52
0.333	311.5	252.3	295.2	230.5	262.6	270.4±14.64
0.50	350.2	311.6	330.1	305.8	311.4	321.8±8.20
0.75	442.3	394.0	410.0	392.2	460.9	419.9±13.64
1	558.7	496.2	582.2	448.2	590.4	535.1±27.29
1.5	628.0	581.8	498.4	520.6	496.5	545.1±25.85
2	497.0	485.4	410.0	426.5	408.3	445.4±19.04
3	277.1	390.0	281.1	278.4	350.2	315.4±23.23
4	195.1	221.6	195.8	170.2	198.5	196.2±8.15
5	173.6	125.0	108.3	102.8	110.6	124.1±12.92
6	122.3	55.70	76.24	50.92	72.32	75.50±12.64
8	43.52	36.80	39.28	35.00	33.55	37.63±1.76
10	30.64	28.92	29.50	21.56	28.60	27.84±1.61
12	21.60	19.48	20.00	12.75	11.25	17.02±2.09
24	17.08	10.20	11.28	6.28	5.30	10.03±2.09
30	6.76	4.22	5.70	3.65	1.95	4.46±0.83
36	4.24	2.62	3.30	1.28	0.80	2.45±0.63
48	0.50	N.D.	0.46	N.D.	N.D.	0.19±0.12

N.D. = Non detectable

mean of 15.57 ± 2.63 μg/ml. The drug attained its peak concentration of 545.1 ± 25.85 μg/ml at 1.5 h. The drug was detectable in all the animals upto 36 h with a mean value of 2.45 ± 0.63 μg/ml. The concentration of the drug was obtained only in 2 out of 5 animals at 48 h with a mean value of 0.19 ± 0.12 μg/ml. The therapeutic concentration of ≥ 2 μg/ml was maintained upto 36 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 formulae of the above noted compartment model.

Table 10 presents the values of different kinetic parameters of gentamicin after its combined i.v. administration with paracetamol. The mean extrapolated zero time concentration of the drug in plasma during distribution phase (A), elimination phase (B) and the theoretical zero time concentration (C_p^0) were noted to be 34.48 ± 2.35, 4.41 ± 0.21 and 39.03 ± 2.40 μg/ml, respectively. The distribution rate constant (α) ranged from 1.691 to 2.259 h^{-1} with the mean value of 1.935 ± 0.119 h^{-1}, whereas its elimination rate constant ranged from 0.098 to 0.107 h^{-1} with a mean of 0.104 ± 0.01 h^{-1}. The mean distribution half life ($t_{1/2}\,\alpha$) and elimination half life ($t_{1/2}\,\beta$) were observed to be 0.36 ± 0.02 and 6.67 ± 0.11 h. The values of area under curve in plasma (AUC), area under first moment curve

(AUMC) and mean residential time (MRT) were found to be 62.16 ± 2.82 mg/L.h, 433.1 ± 28.63 mg/L.h^2 and 6.97 ± 0.36 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 1.088 ± 0.111 0.323 ± 0.028 and 0.628 ± 0.024 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 0.17 ± 0.01 and 5.04 ± 0.16. 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 0.78 ± 0.03 L/kg. The total body clearance (Cl_B) ranged from 1.18 to 1.57 ml/kg/min with an average of 1.35 ± 0.06 ml/kg/min.

4. Dosage regimen: -

Table 11 presents the calculated dosage regimen of gentamicin following combined administration of this drug with paracetamol in buffalo calves. For maintaining C_p^{∞} min of 1 μg/ml, the loading doses (D*s) were calculated to be 1.79 ± 0.08 and 2.71 ± 0.13 mg/kg, while maintenance doses (D_os) were calculated to be 1.01 ± 0.05 and 1.93 ± 0.10 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 to be 3.58 ± 0.17 and 5.43 ± 0.27 mg/kg, while D_os were noted to be 2.02 ± 0.10 and 3.87 ± 0.21 mg/kg at γ of 8 and 12 h,

TABLE – 10

Kinetic parameters of gentamicin in female buffalo calves following combined administration of gentamicin (5 mg/kg) and paracetamol (40 mg/kg) after intravenous administration.

Kinetic parameter (Unit)	ANIMAL NUMBER					Mean ± S.E.M.
	1	2	3	4	5	
A (μg/ml)	42.15	32.51	37.44	30.95	29.36	34.48±2.35
B (μg/ml)	4.98	4.84	4.16	3.87	4.91	4.41±0.21
C_p^0 (μg/ml)	47.13	37.35	41.60	34.82	34.27	39.03±2.40
α (h^{-1})	1.729	2.259	1.691	1.812	2.185	1.935±0.119
$t_{1/2}$ α (h)	0.40	0.31	0.41	0.38	0.32	0.36±0.02
β (h^{-1})	0.107	0.104	0.104	0.107	0.098	0.104±0.01
$t_{1/2}$ β (h)	6.48	6.66	6.66	6.48	7.07	6.67±0.11
AUC (mg./L.h)	70.92	60.93	62.14	53.25	63.54	62.16±2.82
AUMC (mg/L.h^2)	449.1	453.9	397.7	347.4	517.4	433.1±28.63
MRT (h)	6.33	7.45	6.40	6.52	8.14	6.97±0.36
K_{12} (h^{-1})	0.893	1.367	0.863	0.968	1.347	1.088±0.111
K_{21} (h^{-1})	0.278	0.383	0.263	0.296	0.397	0.323±0.028
Kel (h^{-1})	0.665	0.613	0.669	0.655	0.539	0.628±0.024
Fc	0.16	0.17	0.16	0.16	0.18	0.17±0.01
T ≈ P	5.22	4.90	5.43	5.12	4.51	5.04±0.16
Vd_C (L/kg)	0.11	0.13	0.12	0.14	0.15	0.13±0.01
Vd_B (L/kg)	1.00	1.03	1.20	1.29	1.02	1.11±0.06
Vd_{area} (L/kg)	0.66	0.79	0.77	0.88	0.80	0.78±0.03
Vd_{SS} (L/kg)	0.46	0.59	0.51	0.60	0.66	0.56±0.04
Cl_B (ml/kg/min)	1.18	1.37	1.33	1.57	1.31	1.35±0.06

TABLE – 11

Dosage regimen of gentamicin in female buffalo calves following administration of gentamicin (5 mg/kg) and paracetamol (40 mg/kg) after intravenous 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*	1.55	1.82	1.77	2.07	1.75	1.79±0.08
		D_o	0.88	1.03	1.00	1.19	0.95	1.01±0.05
	12	D*	2.36	2.75	2.68	3.18	2.59	2.71±0.13
		D_o	1.69	1.96	1.91	2.30	1.79	1.93±0.10
2	8	D*	3.10	3.63	3.54	4.14	3.50	3.58±0.17
		D_o	1.76	2.05	2.00	2.38	1.90	2.02±0.10
	12	D*	4.74	5.50	5.36	6.36	5.19	5.43±0.27
		D_o	3.38	3.92	3.84	4.60	3.59	3.87±0.21
4	8	D*	6.21	7.26	7.08	8.29	7.01	7.13±0.33
		D_o	3.53	4.10	4.00	4.77	3.81	4.04±0.21
	12	D*	9.45	11.00	10.73	12.72	10.38	10.80±0.53
		D_o	6.77	7.84	7.68	9.19	7.18	7.69±0.41

D* = Priming or loading dose

D_o = Maintenance dose

γ = dosage interval

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

respectively. For maintaining C_p^{∞} min of 4 μg/ml, the calculated D*s and D_os were noted to be 7.13 ± 0.33 & 10.80 ± 0.53 and 4.04 ± 0.21 & 7.69 ± 0.41 mg/kg, respectively, at γ of 8 and 12 h.

(B) PARACETAMOL

1. Plasma levels: -

Concentrations of paracetamol in plasma after combined i.v. administration of gentamicin (5 mg/kg) and paracetamol (40 mg/kg) are presented in Table 12 and Fig 4. The drug appeared with a mean concentration of 92.51 ± 3.72 μg/ml at 0.042 h. The drug was present in all animals upto 6 h with a mean of 3.51 ± 0.38 μg/ml. The concentration of the drug was obtained in 4 out of 5 animals at 8 h with a mean of 1.88 ± 0.48 μg/ml.

2. Urine levels: -

Table 13 and Fig. 5 depict the urine concentrations of paracetamol in buffalo calves following combined i.v. administration of gentamicin (5 mg/kg) and paracetamol (40 mg/kg). The drug was detectable in all the animals at 0.042 h and it ranged from 9.90 to 28.30 μg/ml with a mean of 20.56 ± 3.23 μg/ml. The drug reached its peak urine concentration of 1227 ± 19.76 μg/ml at 1 h. Thereafter, concentration of the drug declined with time and was present in all animals upto 36 h (2.35 ± 0.56 μg/ml). The drug was present in 3 out of 5 animals at 48 h with a mean of 0.36 ± 0.15 μg/ml.

TABLE – 12

Plasma concentrations (μg/ml) of paracetamol in female buffalo calves following combined administration of gentamicin (5 mg/kg) and paracetamol (40 mg/kg) after intravenous administration

Time (h)	ANIMAL NUMBER					Mean ± S.E.M.
	1	2	3	4	5	
0.042	103.63	98.05	90.12	88.12	82.65	92.51±3.72
0.083	64.88	55.50	61.85	56.54	52.12	58.18±2.29
0.167	44.01	38.12	50.42	48.55	44.06	45.03±2.14
0.25	41.01	29.25	39.16	40.08	36.14	37.13±2.13
0.333	32.08	26.42	32.15	32.96	30.00	30.72±1.18
0.50	29.10	23.18	25.22	28.14	27.85	26.70±1.09
0.75	26.12	20.00	20.22	24.22	24.22	22.96±1.21
1	23.14	17.32	17.55	20.60	22.02	20.13±1.17
1.5	20.16	14.55	14.88	18.24	19.44	17.45±1.16
2	17.18	12.08	12.00	15.55	16.18	14.60±1.08
3	13.20	7.48	8.88	10.00	9.80	9.87±0.94
4	8.23	5.42	6.00	7.68	7.82	7.03±0.55
5	5.25	3.40	4.80	4.82	5.25	4.70±0.34
6	3.17	2.50	3.12	4.14	4.62	3.51±0.38
8	2.27	N.D	2.10	2.52	2.50	1.88±0.48
12	N.D	N.D.	N.D.	N.D.	N.D.	--

N.D. = Non detectable

TABLE – 13

Urine concentrations (μg/ml) of paracetamol in female buffalo calves following combined administration of gentamicin (5 mg/kg) and paracetamol (40mg/kg) after intravenous administration

Time (h)	ANIMAL NUMBER					Mean ± S.E.M.
	1	2	3	4	5	
0.042	9.90	18.40	20.12	26.10	28.30	20.56±3.23
0.083	32.74	35.50	31.90	34.60	35.90	34.13±0.78
0.167	265.3	292.6	298.3	299.7	265.1	284.2±7.85
0.25	390.1	398.8	370.1	350.2	390.3	379.9±8.80
0.333	589.4	590.5	588.6	585.4	582.2	587.2±1.52
0.50	640.4	710.3	690.6	650.3	651.2	668.6±13.52
0.75	1100	1102	1104	1100	1108	1102±1.50
1	1232	1180	1186	1260	1280	1227±19.76
1.5	706.4	1280	1266	810.9	788.0	970.3±124.8
2	575	840.2	848.1	560	570.2	678.7±67.60
3	443.7	562.6	570.3	400	440.4	483.4±34.79
4	282.6	320.6	300.8	295.5	292.8	298.5±6.28
5	180.9	195.9	190.8	191.5	178.2	187.5±3.37
6	72.40	80.20	79.45	70.25	70.10	74.48±2.22
8	49.50	50.52	52.00	45.20	44.60	48.36±1.47
10	40.08	47.82	38.20	30.18	31.90	37.64±3.15
12	19.80	29.45	20.50	20.14	22.25	22.43±1.81
24	10.60	12.40	12.60	11.10	15.10	12.36±0.78
30	6.80	7.32	5.25	5.30	7.30	6.39±0.47
36	3.90	2.45	1.20	1.00	3.22	2.35±0.56
48	0.75	0.50	N.D	N.D	0.55	0.36±0.15

N.D. = Non detectable

3. Kinetic parameters: -

Plasma drug concentration versus time profile had shown biphasic pattern following combined i.v. administration of gentamicin and paracetamol 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^0) were noted to be 24.86 ± 2.41, 22.32 ± 1.44 and 47.18 ± 1.46 μg/ml. The distribution rate constant (α) and elimination rate constant (β) were noted to be 1.350 ± 0.163 and 0.303 ± 0.020 h^{-1}, respectively. Distribution half life ($t_{1/2}$ α) ranged from 0.37 to 0.79 h with a mean of 0.55 ± 0.07 h whereas the elimination half-life ($t_{1/2}$ β) ranged from 1.85 to 2.57 h with an average of 2.33 ± 0.14 h. Area under curve (AUC), area under first moment curve (AUMC) and mean residential time (MRT) were calculated to be 93.57 ± 6.02 mg/L.h, 266.4 ± 25.79 $mg/L.h^2$ and 2.82 ± 0.13 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 0.345 ± 0.063, 0.797 ± 0.103 and 0.511 ± 0.028 h^{-1}, respectively. The mean value for fraction of drug available for elimination from

TABLE – 14

Kinetic parameters of paracetamol in female buffalo calves following combined administration of gentamicin (5 mg/kg) and paracetamol (40 mg/kg) after intravenous administration

Kinetic parameter (Unit)	ANIMAL NUMBER					Mean ± S.E.M.
	1	2	3	4	5	
A (μg/ml)	19.80	19.41	31.02	29.65	24.41	24.86±2.41
B (μg/ml)	26.43	23.34	17.46	22.04	22.31	22.32±1.44
C_p^0 (μg/ml)	46.23	42.75	48.49	51.69	46.72	47.18±1.46
α (h^{-1})	0.872	1.879	1.337	1.441	1.219	1.350±0.163
$t_{1/2}$ α (h)	0.79	0.37	0.52	0.48	0.57	0.55±0.07
β (h^{-1})	0.320	0.375	0.270	0.277	0.272	0.303±0.020
$t_{1/2}$ β (h)	2.17	1.85	2.57	2.50	2.55	2.33±0.14
AUC (mg./L.h)	105.3	72.57	87.87	100.1	102.0	93.57±6.02
AUMC (mg/L.h^2)	284.1	171.5	256.9	301.5	318.0	266.4± 25.79
MRT (h)	2.70	2.36	2.92	3.01	3.12	2.82±0.13
K_{12} (h^{-1})	0.117	0.469	0.402	0.429	0.309	0.345±0.063
K_{21} (h^{-1})	0.636	1.196	0.654	0.773	0.724	0.797±0.103
Kel (h^{-1})	0.439	0.589	0.552	0.516	0.458	0.511±0.028
Fc	0.73	0.64	0.49	0.54	0.59	0.60±0.04
T ≈ P	0.37	0.57	1.05	0.86	0.68	0.71±0.12
Vd_C (L/kg)	0.87	0.94	0.82	0.77	0.86	0.85±0.03
Vd_B (L/kg)	1.51	1.71	2.29	1.81	1.79	1.82±0.13
Vd_{area} (L/kg)	1.19	1.47	1.69	1.44	1.44	1.45±0.08
Vd_{SS} (L/kg)	1.03	1.31	1.32	1.20	1.23	1.22±0.05
Cl_B (ml/kg/min)	6.35	9.19	7.61	6.65	6.53	7.26±0.53

central compartment (Fc) and approximate tissue to plasma concentration ratio (T≈P) were observed to be 0.60 ± 0.04 and 0.71 ± 0.12, respectively. The different values of volume distribution calculated by different methods are shown in Table 14. The mean Vd_{area} of 1.45 ± 0.08 L/kg was observed. The total body clearance (Cl_B) ranged from 6.35 to 9.19 ml/kg/min with the mean value of 7.26 ± 0.53 ml/kg/min.

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

1. Plasma levels: -.

Comparison of plasma concentrations of gentamicin (5 mg/kg) when given alone and when given together with paracetamol (40 mg/kg) after i.v administration are presented in Table 15 and Fig. 1. Concentrations of gentamicin were found to be significantly higher when it was given along with paracetamol as compared to its single administration from 0.042 to 0.333 h and at 24 h. No significant difference was observed from 0.50 to 12 h. The therapeutic concentration (≥ 2 μg/ml) was maintained upto 6 h in both the groups. Concentrations of gentamicin were detected up to 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 paracetamol. Concentrations of the drug in urine were significantly higher from 0.083 to 24 h when gentamicin was given along with paracetamol. The drug was detected up to 48 h in both the groups. The drug attained its peak level at the same time interval of 1.5 h in both the groups with a concentration of 83.42 ± 3.17 μg/ml when given alone as compared to the concentration of 545.1 ± 25.85 μg/ml when given in combination with paracetamol. The mean therapeutic concentration in urine (≥ 2 μg/ml) was maintained upto 24 h in case of single administration and 36 h in case of combined administration.

3. Kinetic parameters: -

Table 16 presents the kinetic parameters of gentamicin when it was given alone (5 mg/kg) and when given together with paracetamol (40 mg/kg) following i.v. administration. The values of extrapolated zero time concentration during distribution phase (A), and theoretical zero time concentration (C_p^0) were noted to be significantly higher ($p < 0.01$) in case of combined administration as compared to single administration of gentamicin. Area under curve (AUC), area under first moment curve (AUMC) and micro rate constant of drug transfer from central to peripheral compartment (K_{12}) were significantly higher ($p < 0.01$) in case of combined

TABLE – 15

Comparison of plasma and urine concentrations (μg/ml) of gentamicin (5 mg/kg) when given alone and when given together with paracetamol (40 mg/kg) in buffalo calves following intravenous administration.

Time (h)	Gentamicin given alone		Gentamicin + paracetamol given together	
	Plasma	Urine	Plasma	Urine
0.042	19.80±1.36	7.66±0.82	58.61±4.56**	15.57±2.63$^{N.S}$
0.083	16.64±1.21	12.57±0.32	41.76±2.03**	39.35±4.71**
0.167	14.69±1.26	16.09±1.16	31.79±1.50**	115.6±11.24**
0.25	12.63±0.90	45.49±2.68	23.63±2.46*	211.7±18.52**
0.333	10.88±0.67	59.21±2.66	19.65±2.22*	270.4±14.64**
0.50	9.62±0.37	66.41±4.22	13.06±1.36$^{N.S}$	321.8±8.20**
0.75	8.80±0.43	71.93±4.57	10.51±0.82$^{N.S}$	419.9±13.64**
1	7.72±0.40	80.34±6.45	8.39±0.68$^{N.S}$	535.1±27.29**
1.5	6.47±0.55	83.42±3.17	6.33±0.19$^{N.S}$	545.1±25.85**
2	5.28±0.50	68.10±2.65	4.87±0.35$^{N.S}$	445.4±19.04**
3	3.74±0.12	45.83±3.54	3.86±0.23$^{N.S}$	315.4±23.23**
4	3.13±0.17	34.55±2.37	3.52±0.25$^{N.S}$	196.2±8.15**
5	2.50±0.09	21.62±2.70	2.93±0.28$^{N.S}$	124.1±12.92**
6	2.15±0.12	16.17±1.44	2.44±0.17$^{N.S}$	75.50±12.64*
8	1.39±0.15	12.04±1.68	1.83±0.15$^{N.S}$	37.63±1.76**
10	1.00±0.14	7.55±0.95	1.30±0.06$^{N.S}$	27.84±1.61**
12	0.65±0.10	4.73±0.43	0.86±0.05$^{N.S}$	17.02±2.09**
24	0.23±0.03	2.81±0.08	0.49±.0.04*	10.03±2.09*
30	N.D	1.22±0.15	N.D	4.46±0.83$^{N.S}$
36	N.D	0.75±0.07	N.D	2.45±0.63$^{N.S}$
48	N.D	0.10±0.04	N.D	0.19±0.12$^{N.S}$

N.D = Non detectable

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

TABLE – 16

Comparison of kinetic parameters of Gentamicin when it was given alone (5 mg/kg) and when given together with paracetamol (40 mg/kg) in buffalo calves following i.v. administration

Kinetic Parameter (unit)	Gentamicin given alone	Gentamicin + paracetamol given together
A (μg/ml)	11.08±0.86	34.48 ± 2.35**
B (μg/ml)	4.81±0.31	4.41 ± 0.21$^{N.S}$
C_p^0 (μg/ml)	15.89±0.85	39.03 ± 2.40**
α (h^{-1})	1.058±0.10	1.935 ±0.119*
$t_{1/2}$ α (h)	0.69±0.08	0.36 ± 0.02*
β (h^{-1})	0.139±0.008	0.104±0.01$^{N.S}$
$t_{1/2}$ β (h)	5.05±0.30	6.67±0.11$^{N.S}$
AUC (mg./L.h)	45.90±3.10	62.16 ±2.82*
AUMC (mg/L.h^2)	268.2±27.86	433.1±28.63*
MRT (h)	5.79±0.34	6.97±0.36$^{N.S}$
K_{12} (h^{-1})	0.425±0.054	1.088 ±0.111*
K_{21} (h^{-1})	0.421±0.042	0.323 ±0.028$^{N.S}$
Kel (h^{-1})	0.351±0.023	0.628 ±0.024**
Fc	0.40±0.02	0.17 ±0.01**
T ≈ P	1.54±0.16	5.04±0.16**
Vd_C (L/kg)	0.32±0.02	0.13±0.01**
Vd_B (L/kg)	1.05±0.06	1.11±0.06*
Vd_{area} (L/kg)	0.80±0.04	0.78±0.03$^{N.S}$
Vd_{SS} (L/kg)	0.64±.0.4	0.56±0.04^{NS}
Cl_B (ml/kg/min)	1.86±0.16	1.35±0.06^{NS}

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

administration as compared to single administration of gentamicin. The value of rate constant of drug elimination from central compartment (Kel), and approximate tissue to plasma concentration ratio (T≈P) were noted to be 0.628 ± 0.024 and 5.04 ± 0.16 for gentamicin when it was given along with paracetamol. These values were significantly higher ($p<0.01$) as compared to alone administration of gentamicin. The value of distribution half life is significantly lower in case of combined administration of gentamicin as compared to its single administration. The other kinetic parameters like extrapolated zero time concentration during elimination phase (B), elimination rate constant (β), elimination half life ($t_{1/2}\,\beta$), mean residential time (MRT), rate of transfer of drug from peripheral to central compartment (K_{21}) and Vd_{area} etc. did not differ significantly between both the groups (Table 16).

4. Dosage regimen: -

The comparison of calculated dosage regimen of gentamicin when given alone and when given together with paracetamol in buffalo calves following i.v. administration are shown in Table 17. No significant difference was observed for loading doses (D*s) for maintaining (C_p^{∞} min) of 1, 2 and 4 μg/ml at the selected dosage intervals (γ) 8 and 12 h, but significantly lower maintenance doses (D_os) were required in combined administration of gentamicin as compared to its single administration.

TABLE – 17

Comparison of calculated dosage regimen of gentamicin when given alone and when given together with paracetamol in buffalo calves following i.v. administration

C_p^{∞} min (μg/ml)	γ (h)	Dose (mg/kg)	Gentamicin given alone	Gentamicin + Paracetamol
1	8	D*	2.47 ± 0.23	1.79±0.08$^{N.S}$
		D_o	1.67 ± 0.20	1.01±0.05*
	12	D*	4.37 ± 0.54	2.71±0.13$^{N.S}$
		D_o	3.57 ± 0.53	1.93±0.10*
2	8	D*	4.94±0.45	3.58±0.17$^{N.S}$
		D_o	3.34±0.41	2.02±0.10*
	12	D*	8.74±1.09	5.43±0.27$^{N.S}$
		D_o	7.14±1.05	3.87±0.21*
4	8	D*	9.88±0.90	7.13±0.33$^{N.S}$
		D_o	6.68±0.81	4.04±0.21*
	12	D*	17.48±2.17	10.80±0.53$^{N.S}$
		D_o	14.28±2.10	7.69±0.41*

D* = Priming or loading dose

D_o = Maintenance dose

γ = dosage interval

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

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

IV. COMPARISION OF KINETICS OF PARACETAMOL WHEN GIVEN ALONE AND WHEN GIVEN TOGETHER WITH GENTAMICIN

1. Plasma levels: -

Plasma concentrations of paracetamol when given alone (40 mg/kg) and when given together with gentamicin (5 mg/kg) after i.v. administration in buffalo calves are presented in Table 18 and Fig. 5. The drug concentrations were noted to be significantly higher ($p < 0.01$) from 0.042 to 8 h when paracetamol was given in combination with gentamicin.

2. Urine levels: -

Table 18 and Fig. 6 depict the comparison of urine concentrations of paracetamol when given alone and when together with gentamicin. The drug concentration was observed to be significantly ($p < 0.01$) higher at 0.083 h while significantly ($p < 0.01$) lower concentrations were noted from 1.5 to 48 h in case of combined administration as compared to single i.v. administration of paracetamol. No significant difference was observed at 0.042 and from 0.167 to 1 h between both the groups.

3. Kinetic parameters: -

Kinetic parameters of paracetamol when given alone and when given together with gentamicin after single i.v. administration are presented in Table 19. The values of extrapolated zero time

TABLE – 18

Comparison of plasma and urine concentrations of paracetamol when given alone (40 mg/kg) and when given together with gentamicin (5 mg/kg) in buffalo calves following intravenous administration.

Time (h)	Paracetamol alone		Paracetamol+Gentamicin	
	Plasma	Urine	Plasma	Urine
0.042	20.23±0.84	12.01±1.38	92.51±3.72**	20.56±3.23$^{N.S}$
0.083	17.53±0.68	141.5±20.18	58.18±2.29**	34.13±0.78**
0.167	14.79±0.35	255.4±20.08	45.03±2.14**	284.2±7.85$^{N.S}$
0.25	12.28±0.20	440.6±45.47	37.13±2.13**	379.9±8.80$^{N.S}$
0.333	9.93±0.45	559.6±39.55	30.72±1.18**	587.2±1.52$^{N.S}$
0.50	8.07±0.26	752.8±63.94	26.70±1.09**	668.6±13.52$^{N.S}$
0.75	6.55±0.12.	1115±110.6	22.96±1.21**	1102±1.50$^{N.S}$
1	4.80±0.30	1967±304.4	20.13±1.17**	1227±19.76$^{N.S}$
1.5	3.10±0.43	2022±118.8	17.45±1.16**	970.3±124.8**
2	1.87±0.48	1483±165.2	14.60±0.08**	678.7±67.6**
3	N.D	1227±137.3	9.87±0.94**	483.4±34.79**
4	N.D	1038±132.0	7.03±0.55**	298.5±6.28**
5	N.D	724.8±44.99	4.70±0.34**	187.5±3.37**
6	N.D	520±33.76	3.51±0.38**	74.48±2.22**
8	N.D	214.6±19.69	1.88±0.48*	48.36±1.47**
10	-	144.6±12.59	-	37.64±3.15**
12	-	104.0±5.33	-	22.43±1.81**
24	-	76.36±4.61	-	12.36±0.78**
30	-	58.69±3.69	-	6.39±0.47**
36	-	32.85±4.81	-	2.35±0.56**
48	-	10.90±1.14	-	0.36±0.15**

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

TABLE – 19

Comparison of kinetic parameters of paracetamol (40 mg/kg) when it was given alone and when given together with gentamicin (5 mg/kg) in buffalo calves following i.v. administration.

Kinetic Parameter (unit)	Gentamicin given alone	Gentamicin + paracetamol given together
A (μg/ml)	9.51±1.94	24.86±2.41 $^{N.S}$
B(μg/ml)	13.11±1.08	22.32±1.44**
C_p^0 (μg/ml)	22.62±1.00	47.18±1.46**
α (h^{-1})	7.148±1.361	1.350±0.163*
$t_{1/2}$ α (h)	0.11±0.01	0.55±0.07**
β (h^{-1})	0.965±0.136	0.303±0.020**
$t_{1/2}$ β (h)	0.77±0.09	2.33±0.14**
AUC (mg./L.h)	15.59±1.07	93.57±6.02**
AUMC (mg/L.h^2)	16.09±2.56	266.4±25.79**
MRT (h^{-1})	1.00±0.10	2.82±0.13**
K_{12} (h^{-1})	1.672±0.142	0.345±0.063**
K_{21} (h^{-1})	4.970±1.540	0.797±0.103$^{N.S}$
Kel (h^{-1})	1.471±0.089	0.511±0.028**
Fc	0.65±0.06	0.60±0.04*
T ≈P	0.59±0.13	0.71±0.12$^{N.S}$
Vd_C (L/kg)	1.78±0.08	0.85±0.03**
Vd_B (L/kg)	3.12±0.22	1.82±0.13**
Vd_{area} (L/kg)	2.79±0.14	1.45±0.08**
Vd_{SS} (L/kg)	2.55±0.10	1.22±0.05**
Cl_B (ml/kg/min)	43.67±3.39	7.26±0.53**

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

concentration during elimination phase (B), theoretical zero time concentration (C_p^0), distribution half life ($t_{1/2}$ α), elimination half life ($t_{1/2}$ β) were found to be significantly higher in case of combined administration of the paracetamol as compared to its alone administration. Similarly, area under curve (AUC), area under first moment curve (AUMC), and mean residential time (MRT) were noted to be significantly higher when given in combined administration as compared to single administration of paracetamol. Distribution rate constant (α), elimination rate constant (β), micro rate constant of drug transfer from central to peripheral compartment (K_{12}), elimination from central compartment (Kel), fraction of drug available for elimination from central compartment (Fc), different values of volume distribution and total body clearances (Cl_B) were observed to be significantly lower in case of combined administration as compared to single i.v. administration of paracetamol. Rest of the kinetic parameters did not differ significantly between both groups.

✪✪✪✪✪

Chapter - 5

Discussion

DISCUSSION

Gentamicin, a valuable member of aminoglycosides, is clinically used on account of its many advantages such as bactericidal effects on aerobic gram negative bacillary organisms, broader spectrum of activity and available in all forms *viz.*, parenteral, oral and local application. It is widely used in veterinary and human medicine to treat various infections. Its best actions are seen in on urinary tract infections, mammary gland infections and eye infections. Pharmacokinetic studies of gentamicin have been conducted in many species of animals including buffalo calf but so far, it seems little work has been done on kinetic interaction of gentamicin with NSAIDs, particularly with paracetamol in buffalo calf.

Paracetamol, a potent antipyretic agent, having analgesic and anti-inflammatory properties is frequently employed in treating febrile conditions of animals. Antimicrobial agents are concurrently used along with paracetamol for treating microbial infections as well as to treat pyrexia. Though pharmacokinetic interactions between antimicrobials and NSAIDs were studied in different species of animals but interaction between gentamicin and paracetamol was so far not carried out, particularly in buffalo calf. Therefore, the present study was undertaken to know the kinetic interactions of gentamicin with paracetamol in buffalo calves.

I. KINETIC STUDY OF GENTAMICIN

(a) Distribution in Plasma: -

Concentrations of gentamicin at different time intervals post i.v. injection of gentamicin (5 mg/kg) when given along with paracetamol (40 mg/kg) was significantly higher as compared to its single administration from 0.042 to 0.333 h and at 24 h; rest of the time (0.50 to 12 h) the concentrations of the drug did not differ significantly (Table 15 and Fig. 1). The therapeutic concentration (≥ 2 μg/ml) was maintained upto 6 h in both the groups. Serum concentrations of gentamicin were detected upto 24 h in both the groups of buffalo calves, whereas it was detectable upto 6h in febrile and afebrile conditions, when gentamicin (5 mg/kg) was given in goats (Ahmad *et al.*, 1994).

(b) Urinary excretion: -

Concentrations of gentamicin in urine were significantly higher from 0.083 to 24 h when it was given in combination with paracetamol as compared to its alone administration. The drug was detected upto 48 h in both the groups. The drug attained its peak level at the same time interval (1.5 h) in both the groups with a concentration of 83.42 ± 3.14 μg/ml when gentamicin was given alone as compared to the concentration of 545.1 ± 25.85 μg/ml, when given in combination with paracetamol. The mean therapeutic

concentration in urine (≥ 2 μg/ml) was maintained upto 24 h in alone administration as compared to a longer period of around 36 h when it was given in combination with paracetamol.

(c) Kinetic parameters: -

The distribution half-life ($t_{1/2}$ α) was significantly lower (0.36 ± 0.02 h) in case of combined administration of gentamicin as compared to its single administration (0.69 ± 0.08 h). This clearly indicates that paracetamol influences the rate of distribution of gentamicin that is gentamicin is distributed quicker in tissues and body fluids when given along with paracetamol. On the other hand, Sudha Kumari (1998) noted no influence of paracetamol on the rate of distribution of enrofloxacin. Similarly, Nitesh Kumar (2003) noted no influence of diclofenac in the rate of distribution of enrofloxacin. Distribution half life ($t_{1/2}$ α) of 0.05 ± 0.01 in cow (Satish *et al.*, 1989), 0.1 ± 0.1 h in horse (Swan *et al.*, 1995), 0.38 ± 0.07 h in rabbit (Uppal *et al.*, 1992) and 10.25 ± 1.42 min in chicken (Garg *et al.*, 1989) were found to be lower as compared to the $t_{1/2}$ α value noted in the present study (0.69 ± 0.08 h) in buffalo calves. This shows that gentamicin may be distributed comparatively slowly in buffalo calves as compared to other species noted above.

The elimination rate constant of (β) was noted to be 0.139 ± 0.008 h^{-1}, while the elimination half-life ($t_{1/2}$ β) was noted to be 5.05

± 0.30 h following single i.v. administration of gentamicin (5 mg/kg). These values did not differ significantly in buffalo calves when gentamicin was given along with paracetamol. This denotes that similar rate of elimination occurred in both the groups and paracetamol has no influence in the elimination of gentamicin. Due to this, the values of mean residential time (MRT) between gentamicin when given alone and when given together did not differ significantly (Table 16). 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 whereas Nitesh Kumar (2003) noted non significant influence of diclofenac in $t_{1/2}$ β of enrofloxacin when given together with diclofenac as compared to alone administration of enrofloxacin. Lower $t_{1/2}$ β of 1.12 ± 0.25 h in cow (Satish *et al.*, 1989), 1.2 ± 0.2 h in horse (Swan *et al.*, 1995), 2.88 ± 0.33 h in rabbit (Uppal *et al.*, 1992), 131.60 ± 15.14 min in White Leghorn chicken (Garg *et al.*, 1989) were noted. The above data showed that gentamicin is comparatively removed slower in buffalo calves as compared to other species.

In the present investigation, AUC and AUMC values of 62.16 ± 2.82 mg/L.h and 433.1 ± 28.63 mg/L. h^2 obtained for gentamicin when given along with paracetamol were significantly higher as compared to the values of 45.90 ± 3.10 mg/L.h and 268.2 ± 27.86 mg/L.h^2 when gentamicin was given alone. The significantly

higher values may be due to higher plasma drug concentrations of gentamicin obtained from 0.042 to 0.333 h and at 24 h, when gentamicin was given along with paracetamol as compared to its alone administration.

The value of rate constant of drug transfer from central to peripheral (K_{12}) compartment was noted to be significantly higher when gentamicin was given along with paracetamol (1.088 ± 0.11h) as compared to its alone administration (0.425 ± 0.054 h). This indicates faster movement of drug from central (plasma) to peripheral (tissue) compartment. The values of rate constant of drug transfer from peripheral to central compartment (K_{21}) did not differ significantly in buffalo calves when gentamicin was given alone and when given together with paracetamol after i.v. administration. Signficantly lower fraction of drug available for elimination from central compartment (Fc) was obtained for gentamicin when it was given with paracetamol (0.17 ± 0.01) as compared to its alone administration (0.40 ± 0.02). This value along with significantly higher (K_{12}) value obtained when gentamicin was given along with paracetamol led to significantly higher approximately tissue to plasma concentration (T≈ P) value of 5.04 ± 0.16 when gentamicin was given along with paracetamol as compared to the value of 1.54 ± 0.16 when gentamicin was given alone. This shows that gentamicin may be distributed to a greater amount in peripheral tissues and fluids when

given in combination with paracetamol as compared to its alone administration.

Notari (1980) stated that for a two-compartment open model, the value of Vd_B > Vd_{area} > Vd_{SS} and Vdc. 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 overestimates and Vd_{SS} and Vd_C underestimate the amount of drug in the body. Vd_{area} of 0.80 ± 0.04 and 0.78 ± 0.03 L/Kg were obtained when gentamicin was given alone and when given together with paracetamol. These values did not differ significantly. Vd_{area} of 0.37 ± 0.13 L/kg in cow (Satish *et al.*, 1989), 0.26 ± 0.04 L/kg in goats (Garg *et al.*, 1995), 0.45 ± 0.11 L/kg in rabbit (Uppal *et al.*, 1992) and 0.32 ± 0.05 L/Kg in White Leghorn chicken (Garg *et al.*, 1989) were noted to the lower as compared to be present value obtained in buffalo calves. This may indicate that gentamicin may be distributed to a greater amount in body of buffalo calves as compared to the above noted species.

(d) Dosage regimen: -

In the present study, calculations of dosage regimen of gentamicin when given alone and when given together with paracetamol were carried out at (3) three different therapeutic level (C_p^{∞} min = 1, 2 and 4 μg/ml) in order to combat mild, moderate and

severe infections, respectively, at convenient dosage intervals (γ) of 8 and 12 h. Though gentamicin required non-significantly lower loading doses (D*s), it required significantly lower maintenance doses (D_os), at all dosage intervals when given with paracetamol as compared to its alone administration. Gentamicin can be administered either alone or along with paracetamol for treating mild or moderate infections at the calculated loading and maintenance doses at γ of 12 h. In case of severe infections (C_p^{∞} min = 4 μg/ml), the calculated D* and D_0 of 10.80 ± 0.53 and 7.69 ± 0.41 can be used when given with paracetamol, while the calculated D* (17.48 ± 2.17 mg/kg) and D_0 (14.28 ± 2.10 mg/kg) when given alone may be very high, which may cause higher plasma levels of gentamicin leading to toxicity. Plasma levels of gentamicin in the range of 7 to 10 μg/ml were non-toxic (Gyselynek et al., 1971). Hence shorter γ of 8 h can be recommended when given alone where D* and D_0 of 9.88 ± 0.90 and 6.68 ± 0.81 mg/kg were calculated. From the study, it seems that gentamicin may effectively be combined with paracetamol for treating susceptible bacterial infections accompanied by pyrexia.

II. KINETIC STUDY OF PARACETAMOL

Kinetic studies of paracetamol were conducted in cross-bred calf (Sharma *et al.*, 1995) goat (Manna *et al.*, 1994l Sudha Kumari, 1998) and buffalo calf (Sidhu *et al.*, 1993; Choudhary *et al.*, 2002).

(a) Distribution in Plasma: -

Concentrations of paracetamol in plasma were found to be significantly higher at all time intervals (0.042 to 8 h) in buffalo calves when gentamicin was administered in combination with paracetamol. In contrast, Sudha Kumari (1998) studied concentrations of paracetamol in plasma were significantly higher initially only from 5 to 30 min in goats when paracetamol was administered in combination with enrofloxacin as compared to its single administration by i.v. route.

(b) Urinary excretion: -

Significantly lower urine concentrations of paracetamol were noted at most of the time intervals when it was given in combination with gentamicin as compared to its alone administration. Due to lower excretion of paracetamol in urine in combined administration, the drug was present for a longer period in plasma. On the other hand, Sudha Kumari (1998) established that concentrations of paracetamol in urine were significantly higher form 5 to 45 min and at 30 h only when administered with enrofloxacin as compared to its single administration of paracetamol by i.v. route. Peak concentration in urine was noted earlier at 45 min in case of combined administration of paracetamol and enrofloxacin as compared to 1 h noted in case of single adminstration of paracetamol by i.v. route.

(c) Kinetic parameters: -

Significantly higher value for extrapolated zero time concentration during elimination phase (B) and theoretical zero time concentration (C_p^0) for paracetamol were obtained when administered in combination with gentamicin as compared to single administration of paracetamol by i.v. route in buffalo calves (Table 19).

Distribution rate constant (α) of 1.350 ± 0.163 h^{-1} was significantly lower ($p < 0.05$) while distribution half-life ($t_{1/2}\ \alpha$) of 0.55 ± 0.07 h was significantly ($p < 0.01$) higher in case of combined administration of paracetamol as compared to its single administration (7.148 ± 1.361 h^{-1} and 0.11 ± 0.01 h, respectively). This indicates that the drug may be distributed faster when paracetamol given alone as compared to its combined administration with gentamicin. In contrast, Sudha Kumari (1998) noted the values of distribution rate constant (α) of 3.293 ± 0.492 h^{-1} and distribution half life ($t_{1/2}\ \alpha$) of 0.24 ± 0.04 h, which did not differ significantly in goats when paracetamol was co-administered with enrofloxacin denoting similar rate of distribution of drug occurred in goats of both the groups, while lower mean $t_{1/2}\ \alpha$ of 0.10 h was noted in black bengal goats (Manna *et al.*, 1994).

The elimination rate constant (β) of 0.965 ± 0.136 h^{-1} and elimination half life ($t_{1/2}$ β) of 0.77 ± 0.09 h were obtained after single i.v. administration of paracetamol. Significantly decreased β (0.303 ± 0.020 h^{-1}) and highly increased $t_{1/2}$ β (2.33 ± 0.14 h) were noted for paracetamol when it was given in combination with gentamicin. This indicates that the drug may be slowly eliminated and remained for a longer period in the body of buffalo calf. This is further supported by significantly lower Kel (0.511 ± 0.028 h^{-1}) and Cl_B (7.26 ± 0.53 ml/kg/min) and higher MRT value (2.82 ± 0.13 h) in combined administration of paracetamol as compared to the values of 1.471 ± 0.089, 43.67 ± 3.39 and 1.00 ± 0.10 for Kel, Cl_B and MRT, respectively, when given alone. The $t_{1/2}$ β value of 3.56 ± 0.13 h after i.v. administration in goats (Sudha Kumari, 1998), 8.69 ± 0.83 h after i.m. route of paracetamol in buffalo calves (Sidhu *et al.*, 1993), 4.84 ± 1.26 h after i.m. administration of paracetamol in cross bred calves (Sharma *et al,*. 1995) were noted to be longer than the value obtained in buffalo calf in the present investigation, while a very low mean $t_{1/2}$ β of 0.53 h in black bengal goats (Manna *et al.*, 1994) was noted after 50 mg/kg i.v. administration of paracetamol. Choudhary *et al.* (2002) described $t_{1/2}$ β of 1.91 ± 0.07 h in buffalo calves after post i.v. dose of 10 mg/kg of paracetamol.

The value of rate of transfer of drug from central to peripheral (K_{12}) compartment was significantly lower in combined

administration of paracetamol as compared to single administration, but peripheral to central compartment (K_{21}) did not differ significantly. Fraction of drug available for elimination from central compartment (Fc) was significantly lower in case of combined administration while approximate tissue to plasma concentration (T≈P) did not differ significantly when paracetamol was given alone and when given together with gentamicin in the present study.

The values of area under plasma concentration time curve (AUC) and total area under first moment of plasma drug concentration time curve (AUMC) were noted to be 93.57 ± 6.02 ml/L.h. and 266.4 ± 25.79 ml/L.h^2, when paracetamol was given in combination with gentamicin. These values were significantly higher ($p < 0.01$) as compared to alone administration of paracetamol (Table 19). Significantly higher AUMC and MRT in buffalo calves reflect that the drug may remain for a longer duration in the body of buffalo calves when paracetamol was concurrently administered with gentamicin.

The various values of volume of distribution were significantly lower in buffalo calves when paracetamol was given together with gentamicin as compared to its single administration (Table 19). The value of Vd_{area} was 2.79 ± 0.14 L/kg in combined administration as compared to alone administration (1.45 ±

0.08 L/kg). Sidhu *et al.*(1993) noted more or less similar Vd_{area} of 1.22 ± 0.23 L/kg in buffalo calf while lower Vd_{area} of 0.48 ± 0.11 L/kg was obtained in cross-bred calf (Sharma *et al.*, 1995). High Vd_{area} of 5.48 ± 1.40 L/Kg in goats (Sudha Kumari, 1998) and low value of 0.55 ± 0.12 L/kg in buffalo calves (Choudhary *et al.*, 2002) were noted.

The total body clearance (Cl_B) value of 7.26 ± 0.53 and 43.67 ± 3.39 ml/kg/min in buffalo calves when paracetamol was given together with gentamicin and when given alone, respectively were noted in the present study. Cl_B value of 79.6 ± 22.7 ml/kg/h in cross-bred calf (Sharma *et al.*, 1995), 17.37 ± 4.24 ml/kg/min in goat (Sudha Kumari, 1998) and 113.1 ± 39.8 ml/kg/h in buffalo calf (Sidhu *et al.*, 1993).

III. KINETIC INTERACTIONS BETWEEN GENTAMICIN AND PARACETAMOL

Distribution in plasma and urinary excretion as well as different kinetic parameters of gentamicin and paracetamol when given alone or in combination, following i.v. administration have been described in detail under results. Definite kinetic interactions between the drugs occurred in buffalo calves and the salient features are discussed below:

The results of the present study clearly established that paracetamol influences the kinetics of gentamicin. The plasma

concentrations of gentamicin were found to be significantly higher initially (0.042 to 0.333 h) when it was given in combination with paracetamol as compared to its alone administration. This has resulted significantly higher values of extrapolated zero time concentration during distribution (A) and theoretical zero time concentration (C_p^o) but not during elimination phase (B), when gentamicin was given in combination with paracetamol. This is further resulted in significantly higher values of AUC and AUMC, when gentamicin was given together with paracetamol as compared to its alone administration. Similarly, Sudha Kumari (1998) showed significantly higher values of A and C_p^o for enrofloxacin, when it was given in combination with paracetamol as compared to its alone administration.

Gentamicin is rapidly distributed when it was given along with paracetamol as shown by significantly higher α (1.935 ± 0.119 h^{-1}) and lower $t_{1/2}\,\alpha$ (0.36 ± 0.02 h) as compared to its alone administration of (α = 1.058 ± 0.10 h^{-1} & $t_{1/2}\,\alpha$ = 0.69 ± 0.08 h). In contrast, Sudha Kumari (1998) showed non-influence of paracetamol in the rate of distribution of enrofloxacin as shown by non significant difference in the values of α and $t_{1/2}\,\alpha$ between when enrofloxacin was given alone or when given with paracetamol.

Gentamicin is eliminated from the body of buffalo calf at the similar rate when it was given alone or it was given along with paracetamol as shown by non significant difference in the values of β and $t_{1/2}$ β in both the cases (Table 16). In contrast, paracetamol caused increase in the rate of elimination of enrofloxacin as shown by significantly higher β and lower $t_{1/2}$ β, when it was given in combination with paracetamol as compared to its single administration in goats (Sudha Kumari, 1998).

Gentamicin is distributed to a greater extent in the peripheral tissues when given in combination with paracetamol as shown by significantly higher ($p < 0.01$) $T \approx P$ value of 5.04 ± 0.16 as compared to $T \approx P$ value of 1.54 ± 0.16, when it was given alone. The higher value of $T \approx P$ obtained for gentamicin when it was given with paracetamol may be due to significantly higher value of K_{12} obtained when both the drugs are given together as compared to single administration.

The calculated loading doses (D*s) for treating mild, moderate and severe systemic infections (C_p^{∞} min =1, 2 and 4 μg/ml, respectively) when gentamicin was given alone or when given together, did not differ significantly, while maintenance doses (D_os) were noted to be significantly lower for the dosage interval (γ) of both 8 and 12 h when gentamicin was given along with paracetamol as compared to its alone administration (Table 17). Thus, the study

clearly shows that gentamicin can be effectively combined with paracetamol for treating infections accompanied with pyrexia and other inflammatory conditions. In contrast, Sudha Kumari (1998) recommended enrofloxacin at the dose rate of 5 mg/kg i.v. every 12 hourly given for treating septicemia and other systemic infections when it was given alone, but at the reduced dosage intervals of 8 h at the same dose rate by i.v. route when enrofloxacin was combined with paracetamol.

In the present study, the mean therapeutic concentration ($\geq$ 2 μg/ml) in urine was maintained upto 24 h and 36 h when gentamicin was given alone (5 mg/kg i.v.) and when given together with paracetamol (40 mg/kg i.v.), respectively. Hence, the study recommends that gentamicin can be given by systemic route at @ 5 mg/kg i.v. daily while every 36 hourly when given along with paracetamol for treating urinary tract infections.

Paracetamol produced beneficial interaction (lower $t_{1/2}\ \alpha$ and higher $T \approx P$) and also lower calculated doses for treating systemic infections as well as longer dosage intervals for treating urinary tract infections. Hence, the combination may be recommended in clinical cases of infections where pyrexia and other inflammatory conditions occur.

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Chapter - 6

Summary

SUMMARY

A detailed pharmacokinetic study of gentamicin and paracetamol when given alone and their interactions when given concurrently was carried out in buffalo calves weighing between 102-180 kg following intravenous administration. 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 findings were obtained: -

1. The results of the present investigation clearly established that after combined i.v. administration of gentamicin (5 mg/kg) with paracetamol (40 mg/kg), the plasma concentrations of gentamicin were significantly higher when it was given along with paracetamol as compared to its single administration from 0.042 to 0.333 h and at 24 h. No significant difference was observed from 0.50 to 12 h. The therapeutic concentration (≥ 2 μg/ml) was maintained upto 6 h in both the groups. In case of urine, significantly higher concentrations of gentamicin were observed from 0.083 to 24 h following combined administration of gentamicin as compared to its alone administration. The

drug attained its peak level in urine at the same time interval of 1.5 h in both the groups with a concentration of 83.42 ± 3.17 μg/ml when given alone as compared to the concentration of 545.1 ± 25.85 μg/ml in combination with paracetamol. The mean therapeutic concentration in urine (≥ 2 μg/ml) was maintained upto 24 h in case of single administration and 36 h in case of combined administration.

2. Variations occurred in different kinetic parameters of gentamicin, when it was given in combination with gentamicin. The extrapolated zero time concentration during distribution phase (A) and theoretical zero time concentration (C^o_p) were significantly higher in case of combined administration whereas the extrapolated zero time concentration during elimination phase (B) did not differ significantly.

3. Significantly higher distribution rate constant (α) and significantly lower distribution half-life ($t_{1/2}$ α) values were observed when gentamicin was given in combination with paracetamol as compared to its single administration. This indicates that gentamicin is rapidly distributed in the body of buffalo calves when it was given along with paracetamol. This is further supported by significantly higher value of rate constant of drug transfer from central to peripheral compartment (K_{12}) obtained for gentamicin, when it was given in combination with paracetamol.

4. No significant difference was observed for both elimination rate constant (β) and elimination half-life ($t_{1/2}$ β), when gentamicin was given alone or when it was given along with paracetamol, which denotes that gentamicin is eliminated from the body of buffalo calves at similar rate in both the groups. This led to non significant difference in mean residential time (MRT) in both the groups.

5. Significantly higher values of AUC and AUMC were 62.16 ± 2.82 mg/L.h and 433.1 ± 28.63 mg/L.h^2 when gentamicin was given along with paracetamol as compared to the values of 45.90 ± 3.10 mg/L.h and 268.2 ±27.86 mg/L.h^2 respectively, after alone administration of gentamicin.

6. Approximate tissue to plasma concentration ratio (T≈P) was noted to be significantly ($p < 0.01$) higher for gentamicin (5.04 ± 0.16) in case of combined administration as compared to its alone administration (1.54 ± 0.17). Due to this, the drug is expected to be distributed more in peripheral tissues and body fluids when it was given along with paracetamol.

7. No significant difference was observed for various values of volume distribution (Vd_B, Vd_{area} & Vd_{SS}) and total body clearance (Cl_B) for gentamicin when it was given alone or in combined administration with paracetamol, but Vd_c was significantly lower when gentamicin was given together with paracetamol as compared to its single administration.

8. Concentrations of paracetamol in plasma were found to be significantly higher at all time intervals (0.042 to 8 h) when paracetamol was given in combination with gentamicin as compared to its alone administration. In case of urine, concentrations of paracetamol was found to be significantly higher at 0.083 h while significantly lower concentrations were observed from 1.5 to 48 h when paracetamol was administered along with gentamicin as compared to alone administration of paracetamol.

9. The extrapolated zero time concentration during elimination (B) phase and theoretical zero time concentration (C_p^0) were found to be significantly ($p < 0.01$) higher for paracetamol when it was given together with gentamicin as compared to its alone administration, but extrapolated zero time concentration during distribution phase (A) was noted to be non significant. The values of distribution half life ($t_{1/2}\ \alpha$) and elimination half life ($t_{1/2}\ \beta$) were significantly higher for paracetamol when it was given in combination with gentamicin as compared to its alone administration, which indicate that the drug is expected to be distributed as well as eliminated slowly when given along with gentamicin.

10. Rate constant of drug transfer from central to peripheral compartment (K_{12}), rate constant of drug elimination from central compartment (Kel) and fraction of drug available for

elimination from central compartment (Fc) were significantly lower. On the other hand, the values of area under curve (AUC), area under first moment curve (AUMC) and mean residential time (MRT) were noted to be significantly higher for paracetamol when it was given together with gentamicin as compared to its single administration. There was no significant changes in rate constant of drug transfer from peripheral to central compartment (K_{21}) as well as approximate tissue to plasma concentration ratio (T≈P) when paracetamol was given alone or in combination with gentamicin.

1. Various volume of distribution (Vd_c, Vd_B, Vd_{area} and Vd_{ss}) and total body clearance were significantly lower for paracetamol when it was given together with gentamicin as compared to its alone administration.

2. No significant difference was observed for loading doses (D*) (C_p^{∞} min = 1, 2 and 4 μg/ml) at both dosage intervals (γ) of 8 and 12 h, when gentamicin was given alone or in combination with paracetamol whereas significantly ($p < 0.05$) lower maintenance (D_o) doses were required for C_p^{∞} min of 1, 2 & 4 μg/ml at both dosage intervals (γ) of 8 and 12 h when gentamicin was given in combination with paracetamol. For treating mild systemic infections (C_p^{∞} min = 1 μg/ml) loading doses (D*s) and maintenance doses (D_os) of 4.4 and 3.6 mg/kg at

dosage intervals (γ) of 12 h may be used when gentamicin was given alone while Ds* and D_o of 2.7 and 1.9 mg/kg can be used when gentamicin was given together with paracetamol. For treating moderate (C_p^∞ min = 2 μg/ml) and severe (C_p^∞ min = 4 μg/ml) infections at γ of 8 h D*s of 4.9 & 9.9 and D_os of 3.3 & 6.7 mg/kg were needed when gentamicin was given alone while lower D*s of 3.6 & 7.1 and D_os of 2.0 and 4.0 mg/kg, were required when gentamicin was given in combination with paracetamol. Similar is the trend at γ of 12 h for calculated D* and D_o for maintaining C_p^∞ min = 2 μg/ml and C_p^∞ min = 4 μg/ml. For treatment of moderate infections (C_p^∞ min = 2 μg/ml), the required D* and D_o at γ of 12 h are 8.7 and 7.1 mg/kg when gentamicin given alone while lower D* and D_o 5.4 and 3.9 mg/kg are calculated when gentamicin given with paracetamol In case of severe infections (C_p^∞ min = 4 μg/ml), the calculated D* and D_o of 10.8 and 7.7 mg/kg can be used when given with paracetamol, while the calculated D* and D_o are 17.5 and 14.3 mg/kg at γ of 12 h when given alone which, may be very high that may cause higher plasma levels of gentamicin leading to toxicity. Plasma levels of gentamicin in the range of 7 to 10 μg/ml were non toxic (Gyselynek *et al.*, 1971) and above this level may cause toxicity. Hence, shorter γ of 8 h can be

recommended as noted above. Since paracetamol reduces the doses of gentamicin and thus, expected to prevent severe toxicity in buffalo calves. Hence, gentamicin can be successfully used along with paracetamol simultaneously for treating various systemic infections associated with pyrexia.

The present investigation established that both gentamicn and paracetamol interact with one another and cause many changes in their kinetic behaviour. The study further points out that the combination with paracetamol may be beneficial because paracetamol reduces the doses of gentamicin, which may be much advantageous in the field of veterinary practice. Apart from kinetic interaction study carried out in the present work, further studies should be carried out regarding pharmacodynamic interactions such as changes in antimicrobial efficacy, immunological changes etc. when these drugs are given simultaneously.

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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 buffalo calf no. 4 obtained after a single i.v. injection of gentamicin (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 2 h.

Sl. No.	Time (h) X	X^2	Plasma drug concentration (Y) (μg/ml)	Log Y	XY
1	2	4	5.20	0.7160	1.432
2	3	9	3.95	0.5966	1.790
3	4	16	3.70	0.5682	2.273
4	5	25	2.62	0.4183	2.092
5	6	36	2.50	0.3979	2.387
6	8	64	1.60	0.2041	1.633
7	10	100	1.15	0.0607	0.603
8	12	144	0.68	-0.1675	-2.01
9	24	576	0.20	-0.6990	-16.78
$\Sigma n = 9$	$\Sigma x = 74$ $\overline{X} = 8.222$	$\Sigma x^2 = 974$		$\Sigma \log Y = 2.0953$ $\overline{Y} = 0.2328$	$\Sigma XY = - 6.576$

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

$$= \frac{9 \times (-)6.576 - 74. \times 2.095}{9 \times 974 - (74)^2}$$

$$= \frac{-59.184 - 155.03}{8766 - 5476}$$

$$= \frac{214.214}{3290} = 0.065$$

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

$$= 0.1499 \text{ or } 0.150 \text{ h}^{-1}$$

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,

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

$$= 0.2328 - (0.065) \times 8.222$$

$$= 0.2328 + 0.5344$$

Log 0.7681

Zero time concentration (B) = antilog of 0.7681

$$= 5.86\ \mu g/ml$$

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 11.90 μg/ml and α is 1.108 h^{-1}.

The theoretical plasma concentration at zero-time

C_p^o = A + B = 11.90 + 5.86 = 17.76 μg/ml.

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

$$t_{1/2}\alpha = \frac{0.693}{\alpha} = \frac{0.693}{1.108} = 0.63h$$

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

$$t_{1/2}\beta = \frac{0.693}{\beta} = \frac{0.693}{0.150} = 4.62h$$

Area under curve, AUC

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

$$= \frac{11.90}{1.108} + \frac{5.86}{0.150}$$

$$= 10.7401 + 39.067$$

$$= 49.81 \text{ mg/ L.h}$$

rea under first moment curve. Plasma drug concentration time ırve, AUMC

$$\text{AUMC} = \frac{A}{\alpha^2} + \frac{B}{\beta^2} = \frac{11.90}{1.108} + \frac{5.86}{0.150}$$

$$= \frac{11.90}{1.2277} + \frac{5.86}{0.0225}$$

$$= 9.6929 + 260.44$$

$$= 270.14 \text{ mg/L.h}^2$$

Iean residential time, MRT

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

$$= \frac{270.14}{49.81} = 5.42\text{h}$$

ate constant of drug transfer from peripheral to central ompartment, K_{21}

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

$$= \frac{11.90 \times 0.150 + 5.86 \times 1.108}{17.76}$$

$$= \frac{1.785 + 6.493}{17.76} = \frac{8.2779}{17.76}$$

$$= 0.466 \text{ h}^{-1}$$

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

$$Kel = \frac{\alpha \times \beta}{K_{21}} = \frac{1.108 \times 0.150}{0.466}$$

$$= 0.357\ h^{-1}$$

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

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

$$= 1.108 + 0.150 - 0.466 - 0.357$$

$$= 1.258 - 0.823$$

$$= 0.435\ h^{-1}$$

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

$$Fc = \frac{\beta}{Kel} = \frac{0.150}{0.357} = 0.42$$

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

$$T \approx P = \frac{K_{12}}{K_{21} - \beta} = \frac{0.435}{0.466 - 0.150}$$

$$= \frac{0.435}{0.316} = 1.38$$

Volume of distribution based on both distribution and elimination, Vd_C

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

$$= \frac{5}{17.76} = 0.28 L/Kg$$

Volume of distribution based on elimination, Vd_B

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

$$= \frac{5}{5.86} = 0.85\ L/Kg$$

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

$$Vd_{area} = \frac{D}{AUC.\beta} = \frac{5}{49.81 \times 0.150} = \frac{5}{7.4715} = 0.67 L/Kg$$

Volume of distribution at steady state, Vd_{SS}

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

$$= \frac{0.435 + 0.466}{0.466} \times 0.28 = 0.54 L/Kg$$

Total body clearance, Cl_B

$$Cl_B = Vd_{area} \times 0.150$$

$$= 0.67 \times 0.150$$

$$= 1.675\ L/kg/h$$

$$= 1.68\ ml/Kg/min$$

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. 4 obtained after a single i.v. injection of gentamicin in healthy buffalo calf 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 at the onset to reach the target concentration rapidly. The maintenance (D_o) dose is the dose given at particular dosage interval (γ) 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. 4 after i.v. administration of the drug. and D_0 were calculated as shown below: -

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

$$= 1 \times 0.67\ e^{0.150 \times 8}$$

$$= 0.67 \times 3.32$$

$$= 2.22 \text{ mg/kg}$$

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

$$= 1 \times 0.67\ (e^{0.150 \times 8} - 1)$$

$$= 0.67 \times 2.32$$

$$= 1.55 \text{ mg/kg.}$$

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