PHARMACOKINETICS AND TOXICITY OF MELOXICAM IN GOATS

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

PUSA (SAMASTIPUR) BIHAR

(FACULTY OF POST-GRADUATE STUDIES)

In the partial fulfilment of the requirement

FOR THE DEGREE OF

Master of Veterinary Science

VETERINARY PHARMACOLOGY AND TOXICOLOGY

By

Sanjeev Kumar

Reg. No. - M/V. Phar./36/2002-2003

DEPARTMENT OF VETERINARY PHARMACOLOGY AND TOXICOLOGY

BIHAR VETERINARY COLLEGE

P A T N A - 800 014

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Dedicated to

my benevolent
and
adorable
PARENTS

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and
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PARENTS

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

Dr. S. D. Singh
M.V.Sc., Ph.D.
Associate Professor & Head
Deptt. of Pharmacology & Toxicology
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Bihar Veterinary College, Patna-800014

CERTIFICATE - I

This is to certify that the thesis entitled "**PHARMACOKINETICS AND TOXICITY OF MELOXICAM IN GOATS**" submitted in partial fulfillment of the requirement for the degree of "**Master of Veterinary Science (Veterinary Pharmacology & Toxicology)**" of the faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar, is the record of bonafide research carried out by **DR. SANJEEV KUMAR**, under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

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Major Advisor

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(Chairman / Head of the Department)

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

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CERTIFICATE - III

This is to certify that the thesis entitled "**PHARMACOKINETICS AND TOXICITY OF MELOXICAM IN GOATS**" submitted by **DR. SANJEEV KUMAR**, 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 ...17-08-2004.....

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Sanjeev Kumar

(Sanjeev Kumar)

Date : 08/05/04

Place : Patna

Chapter - 1

Introduction

INTRODUCTION

Meloxicam is a novel non steroidal anti-inflammatory drug (NSAID) of oxicam group. It acts by blocking the synthesis of prostaglandins preferably by inhibiting COX-2 and thereby exerting its potent anti-inflammatory, anti-exudative, analgesic and antipyretic actions. Meloxicam is used in the treatment of both osteoarthritis and rheumatoid arthritis (Engelhardt *et al.*, 1995) apart from other inflammatory conditions.

Inflammation of different tissues is the commonest single problem faced by clinicians. Inflammation in fact is a normal physiological response against causative agents and the clinical signs produced in inflammatory disorders are due to the presence of mediators of inflammation, particularly prostaglandins. Although the inflammation may be caused by infectious and non-infectious agents, but irrespective of the causative factors, the response to inflammation is more or less similar and mediated through a number of biochemical cascades including prostaglandins as a major mediator.

Administration of anti-inflammatory agents to alleviate signs of inflammation is a standard therapeutic approach. A number of anti-inflammatory drugs are known to exert the desired action by inhibiting the synthesis of prostaglandins (Brander *et al.*, 1991).

Recent studies have shown that two distinct enzyme classes are involved in prostaglandins synthesis (Vane and Botting, 1996), which are cyclooxygenase-1 (COX-1) and the cyclooxygenase-2 (COX-2). COX-1 is responsible for normal physiological turnover of prostaglandins whereas COX-2 is produced in response to inflammation. The older group of NSAIDs mainly acts on COX-1, due to which normal physiology of body is disturbed. Thus, these drugs have narrow safety of margin and have more adverse effects on gastrointestinal and renal system with antithrombogenic activity (Vane and Botting, 1996). It is therefore desirable to use such drugs that have lesser toxicity with increased margin of safety such as COX-2 selective inhibitors.

In veterinary practice, a number of anti-inflammatory drugs such as phenylbutazone and diclofenace sodium are frequently and routinely being used for symptomatic relief. But, these drugs are not COX-2 specific and hence their use is not without side effects, particularly gastric irritation.

Meloxicam, a new NSAID, is a COX-2 specific (Barner, 1996) and has a high intrinsic activity combined with a low ulcerogenic potential along with high therapeutic index (Engelhardt *et al.,* 1996 b). The therapeutic index of meloxicam is higher than that of other NSAIDs including piroxicam, diclofenac and indomethacin (Engelhardt *et al.*, 1996 b). Although pharmacokinetic and toxicity

studies of meloxicam have been carried out in different species of animals, but very little information is available in goats.

During recent time, much attention is being paid in goat rearing in tropical countries as an easy alternative for uplifting the economic conditions of poor farmers. Because of its versatile traits like greatest adaptability, maintenance on low grade ration, least rearing expenses and early economic returns, goat rearing is encouraged to a greater extent in under developed and developing countries like India. The optimal economic gain by goat rearing is only possible when effective health coverage is provided to this species.

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

1. Estimation of concentrations of meloxicam at different time intervals in body fluids following its i.v. administration in goats.
2. Determination of different kinetic parameters such as distribution half life, elimination half life, volume of distribution etc.
3. Toxicity study of meloxicam by estimating different haematological and biochemical parameters after repeated i.m. injection of meloxicam.

Chapter - 2

Review of Literature

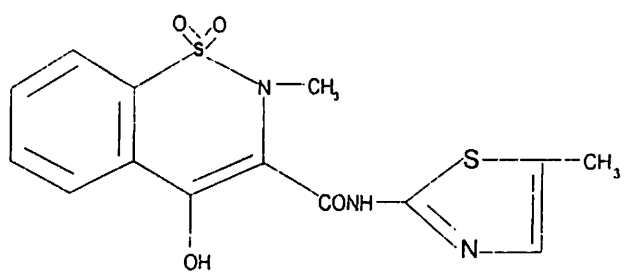
REVIEW OF LITERATURE

Meloxicam is a novel non-steroidal anti-inflammatory drug (NSAID), which is widely used in many inflammatory conditions in man and animals, particularly in rheumatic conditions in human beings. It is a drug of acid enolcarboxamide.

CHEMISTRY

Chemically, meloxicam is a 4-hydroxy –2- methyl –N-(5-methyl-2-thiazolyl) – 2H-1,2 benzothiazine – 3- carboxamide –1, 1-dioxide.

Fig. Showing chemical structure of meloxicam



Molecular Formula = $C_{14}H_{13}O_4N_3S_2$

Molecular weight = 351.35.

MECHANISM OF ACTION

Cyclooxygenase (COX) is the pivotal enzyme in prostaglandins biosynthesis. It exists in two isoforms viz., constitutive

COX-1 (responsible for physiological functions) and inducible COX –2 (involved in inflammations). Inhibition of COX explains both the therapeutic effects (Inhibition of COX-2) and side effects (inhibition of COX-1) of NSAIDs. The activity of a number of NSAIDs has been investigated in several trial systems, showing that most of those marketed have higher activities against COX-1 or are equipotent against both isoforms. Adverse event data of marketed NSAIDs show a relationship between a poor safety profile and more potent inhibition of COX-1 relative to COX-2. There are several new non-steroidal COX-2 inhibitors under development. The most clinically advanced one is meloxicam, which consistently demonstrates higher activity against COX-2 than COX-1 in several test systems (Vane and Botting, 1996).

In vitro, meloxicam is found to be three times more effective in inhibiting the inducible COX-2 in cultured guinea pig peritoneal macrophages as compared to constitutive COX-1 of these cells (Engelhardt *et al.,* 1995).

METABOLISM

Despite the structural relationship to other NSAIDs (Woolf and Radulovie, 1989; Olkkala *et al.*, 1994), the introduction of

methyl group in the thiazolyl moiety of the oxicam has facilitated the formation of metabolities (Schmid *et al.*, 1995 b) that undergo fast elimination, leading to a shorter $t_{1/2}$ in comparison with piroxicam and tenoxicam.

Because of the enolic hydroxyl group confers acid properties on meloxicam, the absorption may vary along the gastrointestinal tract because of the changing p^H of the environment (Busch *et al.*, 1998).

Meloxicam is well absorbed in all species and is cleared exclusively metabolically, because only low levels of parent compound were detected in bile, urine and feces. Therefore, bio-transformation governs the elimination of parent compound in all species. The main metabolites of meloxicam in human, rats, mice and mini-pigs were a 5-hydroxy methyl derivative and 5-carboxy derivative. Other metabolities have also been detected in rats and human (Schmid *et al.*, 1995 a,b), the major metabolite is (5-methyl-1-2-thiazolyl) aminooxoacetic, which is typical for oxicams (Woolf and Radulovic, 1989).

Studies Conducted in rats and mini-pigs revealed the highest tissue concentrations of meloxicam to be in the liver, kidneys and lungs with low levels in the CNS. Meloxicam and its metabolites show no affinity for pigmented tissues (Busch *et al.*, 1998).

A. KINETIC STUDIES

General Pharmacokinetics

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

The compartment model is a hypothetical structure and is used to characterise with reproducibility of behaviour and fate of a drug in a biological system, when administered by certain route in a particular dosage form. In pharmacokinetic studies, compartment is an entity, which has definite volume and in that concentration a drug exists at any time.

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

One compartment open model

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

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

where ,

Cp = Concentration of drug in plasma

B = Extrapolated zero time intercept of mono exponential curve.

β = Overall elimination rate constant.

t = Time elapsed after drug administration

e = Base of natural logarithm.

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

Two compartment open model

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

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

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

where,

C_p = Plasma concentration of the drug

A = Zero time intercept of distribution phase

B = Zero time intercept of elimination phase

α = Distribution rate constant

β = Elimination rate constant

t = Time elapsed after drug administration

e = Base of natural logarithm

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

Three or multiple compartment open model

The disposition kinetics of some drugs may also follow three or multi compartment. A semilogarithmic plot of plasma drug concentration against time shows triphasic or multiphasic curve. The initial steep decline in plasma drug concentration against time is due to distribution of drug from blood to highly perfused tissue known as peripheral – I. After that, gradual decline is due to distribution of a drug from central to less perfused tissue known as peripheral - II. The drug concentration in plasma following single i.v. administration is expressed by following triexponential mathematical formula as a function of time.

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

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

Clinical importance of pharmacokinetic study

Clinically, the pharmacokinetic studies consist of: -

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

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

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

Some important pharmacokinetic parameters

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

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

3. Elimination rate constant (β)

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

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

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

b. The total body clearance (Cl_B)

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

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

4. Elimination or biological half life ($t_{1/2}$ β)

Gibaldi and Weintraub (1971) defined that the elimination half life is the time required to reduce the drug concentration in plasma or serum to its half during the elimination phase of the drug concentration time profile. This clearly shows that doubling the dose does not double the duration of action of drug but increases it by one half life. It is inversely proportional to the overall elimination rate constant. The elimination half life follows the first

order process and hence is not dependent on the dose of the drug as well of route of administration. It is used to calculate the duration of drug action in the body and designing the rational dosage regimen.

5. Volume of distribution

The apparent volume of distribution is a hypothetical volume of body fluid that would be required to dissolve the total amount of the drug to attain the same concentration as that found in the blood. Riegelman *et al.* (1968) stated that the calculated value of volume of distribution is not dependent upon the method used for its calculation, if the drug distributes truly according to one compartment. The apparent volume of distribution indicates the amount of distribution of a drug without providing any clue whether the dug 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 is due to high protein binding or low lipid solubility of a drug and indicates that the drug is restricted to certain fluid compartment, like plasma water, extracellular fluids *etc.* This is due to high protein binding or low solubility of drug.

6. Total body clearance (Cl_B)

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

7. Bioavailability

The extent of absorption (F) is generally known as bioavailability. Bioavailability of a drug indicates the rate of drug absorption as well as the amount of absorption of a drug in pharmacologically active from. It is a measure of the fraction of administered dose of a drug that reaches the systemic circulation in the unchanged from. It is calculated experimentally by the ratio of the area under plasma concentration time curve after extravascular and intravenous administration (Baggot, 1977; Sams, 1978).

8. Protein binding

Some drugs have tendency to get bound with plasma protein mainly with albumin. Binding of a drug with plasma protein

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

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

Kinetic studies of meloxicam in animals

Kinetic studies on meloxicam were conducted in different species. They are noted as follows

(i) ***Horse*** :- The plasma concentration time curve was defined by a three compartment open model in one pony and by a two compartment model in five ponies. Mean value for the five ponies were $t_{1/2}$ α 0.40 h; $t_{1/2}$ β 2.70 h; Vd_{area} 0.158 L/kg; Cl_B 11.87 ml/kg/h. Exudate concentrations of meloxicam were initially similar to and eventually greater than concentrations in plasma and this may explain the more prolonged inhibition of eicosanoid synthesis in exudate than in serum (Lees *et al.*, 1991).

(ii) ***Dog*** :- After a review of literature on the pharmacokinetics and pharmacodynamics of NSAIDs, plasma concentrations of meloxicam were determined in 6 dogs after a s.c. injection (0.2 mg/kg) followed by oral treatment (0.1 mg/kg) for 13 days. Result confirmed the recommended dosage regimen of the initial dose and the subsequent maintenance dose, which resulted in a highly favourable curve of concentration with a rapidly attained steady state after 2 days, without accumulation (Nautrup *et al.*, 1999).

Meloxicam @ 0.2 mg/kg was chosen for administration by oral, i.v and s.c. route to dog (Busch *et al.*,1998). The s.c. route of administration was chosen because this was the parenteral route of dosing intended for the therapeutic treatment of osteoarthritis in dogs. Values for $t_{1/2}$, $AUC_{0-\infty}$, AUC to the final measurable sample, CL/F and Vc/F were comparable for all routes of administration. The absorption kinetic of meloxicam i.e., C_{max} and t_{max} showed marked defferences between the oral and s.c. routes. Values for t_{max} were higher after oral dosing, as compared with s.c. dosing (7.5 vs 2.5 h), whereas C_{max} was higher after s.c. dosing (0.734 Vs. 0.464 mg/L), Because of this difference in absorption, the $MRT_{[oral]}$ was also some what higher after oral dosing.

Despite a relatively wide range of $t_{1/2}$ values, the mean value of 24 hr was within the range seen for male and female rats. However, in contrast to the results obtained with rats, female dogs showed a trend toward lower AUC and $t_{1/2}$ values.

A compartmental analysis using the TOPFIT program (Heizel *et al.*, 1993) was performed to predict the time curve of plasma concentration after repeated daily oral dosing. Simulation plasma-time profiles revealed an accumulation factor of (2.1-2.3) and thus steady-state conditions would be reached after three to five doses, using a dosing interval of 24 hr because C_{max} is achieved earlier after s.c. dosing. This route has advantages over the oral route for administration of the first dose in dogs.

Complete absorption was demonstrated for both the oral and s.c. routes, with absolute bioavailability values of 106% and 112%, respectively. Therefore, the two routes should be associated with similar therapeutics efficacies.

Plasma protein binding data *in-vitro* showed that 97% of meloxicam was bound to plasma protein. The level of unbound, pharmacodynamically active meloxicam is 10 times higher than that determined in rat, after a dose of 0.2 mg /kg in each species (Busch *et al.*, 1998).

(iii) ***Mini-pig*** : Busch *et al.* (1998) conducted pharmacokinetic study of meloxicam in mini pig after its oral administration. They obtained pharmacokinetic parameters through non-compartmental analysis from plasma concentration versus time data. The plasma t_{max} after oral administration was 3h; the long terminal elimination $t_{1/2}$ for total radioactivity of about 145 h was the result of the presence of metabolites in plasma. By measuring only the parent compound in plasma, a terminal $t_{1/2}$ of about 8 h was estimated, which is the most relevant $t_{1/2}$ value (Busch *et al.*, 1998).

(iv) ***Baboons*** :- After oral administration of meloxicam to baboons, mean peak plasma concentrations of radioactivity were attained after approximately 6 h, and the majority of the radioactivity was eliminated with in 24 h. By 72 h after dosing, elimination was virtually complete. The elimination $t_{1/2}$ for total radioactivity was 6 h. As seen in other animal species, the majority of the radioactivity was found in plasma rather than the erythrocytes. (Busch *et al.*, 1998)

(v) ***Mice*** :- Busch *et al.* (1998) carried out pharmacokinetic study of meloxicam in mice after a single oral administration of the drug @ 10mg/kg. They obtained t_{max} values of 0.7 and 0.6 h in male

and female mice, respectively. After a single i.v. dose of 10mg/kg meloxicam, an initial concentration of 36.6 mg-eq/litre was attained after 5 min and plasma concentrations were similar to those seen after oral dosing within 1 h. Thereafter, the i.v. and oral concentration time profiles were comparable, both showing bi-phasic elimination between 1 and 8 h after dosing. The bio-availability (Calculated as AUC_{oral}/ $AUC_{i.v.}$) was 94%.

(vi) ***Rat*** :- Velpandian *et al.* (2000) conducted pharmacokinetic study of meloxicam after its single i.v. administration of 0.2 mg/kg in four male wistar rats weighing 200 gm. They noted $t_{1/2}\,\alpha$ of 0.058 h; $t_{1/2}\,\beta$ of 6.10 h; MRT of 8.28 h and Vdss of 1.43 L/kg.

(vii) ***Bird*** :- Information on the pharmacokinetics and pharmacodynamics of inflammatory drugs in birds is scarce. Choice of drug and dosage is usually empirical, since studies of anti-inflammatory drugs are lacking. In this study, three common veterinary non-steroidal anti-inflammatory drugs, (NSAIDs) were administered intravenously to five different bird species. Sodium salicylate, flunixin and meloxicam were selected as anti-inflammatory drugs. These NSAIDs were administered intravenously to chickens (*Gallus gallus*)

ostriches (*Struthio camelus*), ducks (*Anas platyrhychos*), Turkeys (*Meleagris gallopava*) and pigeons (*Columba livia*). Plasma concentrations of these drugs were determined by validated high performance liquid chromatography (HPLC) methods and pharmacokinetic parameters were calculated. Most bird species exhibited rapid elimination of these drugs. Ostriches had the fastest elimination rate for all the NSAIDs, but there were some interesting species differences. Chicken had a half-life that was approximately 10 fold as long as the other bird species for flunixin. The half-life of chickens and pigeons was three fold as long as the other bird species for meloxicam, and, for salicylic acid, the half life in pigeons was at least three-five fold longer than in the other bird species (Baert and Backer, 2003).

B. TOXICITY STUDIES

Only a few report on toxicological studies of meloxicam have been reported so far.

(i) Human :- Meloxicam induced liver toxicity in female patient with rheumatoid arthritis who develop acute cytolytic hepatitis. Meloxicam has recently been introduced in Belgium as the first NSAID with selective action on the inducible form of cyclooxygenase –2. The acute

cytolytic hepatitis occurred rapidly after meloxicam administration and was associated with the development of antinuclear antibodies suggesting hypersensitivity mechanism. The first case of meloxicam related liver toxicity demonstrated the potential of this drug to induce hepatic damage (Stacrkel et al., 1999).

(ii) Buffalo calves :- Meloxicam has not been reported to induce any change in Hb% and differential leucocytic count in buffalo calves.

The effect of drug on some of the blood biochemical parameter viz. total protein and albumin were reported. In comparison to 0-hour value, significantly higher plasma total protein values were found at 48, 120 and 144 hrs while for plasma albumin significantly higher values were found at 48, 96, 144 hours of the drug administration (Magarwaidya *et al.*, 2002)

(iii) Dogs :- Cardini *et al.* (1997) administered an oral meloxicam suspension (Metacam) daily to three year - old dogs for 91 days at 0.2 mg/kg body weight. The results of blood and urinary examinations, carried out every 10 days, differed little from those in 3 controls. Endoscopy at the end of the trial showed some gastric erythema and prominent antral folds in the treated dogs, but there were no other signs of toxicity.

(v) ***Rat*** :- Sub-acute toxicity of repeated (14 day) intravenous injections of meloxicam in male and female sprague – Dawley rats was assessed. Twenty four male and female sprague – Dawley rats were divided into four groups of six animals each and were administered meloxicam intravenously (0.25, 2.5 and 7.5 mg/kg body weight) daily for 14 days control group received vehicle. Sub-acute toxicity study on meloxicam showed that there was no significant changes in haematological and biochemical parameters in rats, even at high dose level (7.5mg / kg body weight). No mortality was observed and histological examination confirmed these results. Meloxicam injection did not show any toxicity in rats upto 7.5 mg/kg body weight as an i.v. injection for 14 days in sprague – Dawley male and female rats (Cherian *et al.*, 2001).

❑❑❑❑❑

Chapter - 3

Materials and Methods

MATERIALS AND METHODS

In the present study, five clinically healthy female goats of non-descript breed between 12 to 18 months of age and 20-22 kg body weight were used. The goats were maintained on dry fodder, cattle feed, and greens. Water was given *ad lib.*

EXPERIMENTAL DESIGN

Meloxicam was administered separately in each of five healthy goats by i.v. route for pharmacokinetics study. An interval of 15 days was allowed to elapse before administration of next dose of the drug for toxicity study. After conducting the kinetic study, the drug was administered by i.m. route to investigate the toxicity of drug.

DRUG USED

Meloxicam was used in the present experiment Melonex(R) - an injectable commercial preparation containing meloxicam in concentration of 5mg/ml marketed by Intas Pharmaceuticals Ltd., Ahmedabad, India was used.

COLLECTION OF BIOLOGICAL FLUIDS AND THEIR TIMINGS

(i) Pharmacokinetic study : -

Samples of plasma and urine were collected after i.v. administration of melxociam at the dose rate of 0.5 mg/kg body

weight in each healthy goat. The sample of plasma and urine were collected at 0.042, 0.083, 0.167, 0.25, 0.333, 0.50, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 h but samples of urine were collected further upto 48 h (at 30, 36 and 48 h)

(ii) Toxicity study : -

Blood samples only were collected after i.m. administration of drug in each of five healthy goat for seven consequent days at the dose rate of 1 mg/kg body weight. The blood samples were collected on 0, 2, 4 and 8 days.

(A) BLOOD

Before collection of blood, the sites around the jugular vein on either side of the neck of animals were aseptically prepared. Blood samples were collected in centrifuge tubes containing appropriate amount of sodium oxalate by vene puncture with disposable 18G needles at various above noted time intervals after drug administration. The blood samples were centrifuged at 3000 rpm for 15 min for separation of plasma. For the preparation of standards, normal plasma prior to drug administration was also collected. The standards of meloxicam in plasma and test samples were then kept in a refrigerator until assay was carried out.

For toxicity study, blood was collected in clean test tubes for serum separation. Approximate amount of suitable anti-coagulant

was added to each vial for different haematological and biochemical tests. Blood and serum samples were then kept in a refrigerator until various tests were carried out.

For total leukocyte count and differential leucocyte count, blood was taken from ear vein.

[B] URINE

Urine samples were collected for analysis by introducing a sterile Foley's balloon catheter (No-12) lubricated with glycerine through urethra into the urinary bladder of the experimental goats with the aid of a flexible metal probe. The balloon of the catheter was inflated by injecting 20 ml of sterile water through a syringe to keep the catheter in position. The opening of the catheter was locked 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 then kept in a refrigerator and were analyzed in successive days.

ADMINISTRATION OF DRUGS

(i) Kinetic study :-

Meloxicam (Melonex$^{(R)}$) injection containing 5 mg of meloxicam per ml was injected at the dose rate of 0.5 mg/kg body weight by i.v. route in each goat.

(ii) Toxicity study :

Meloxicam injection containing 5mg of meloxicam per ml was injected at the dose rate of 1mg/kg body weight once daily by i.m. route in each goat for 7 consecutive days.

I. PHARMACOKINETIC STUDIES

Estimation of meloxicam by using high performance liquid chromatography (HPLC) :

Concentrations of meloxicam in plasma and urine were estimated by reverse phase partition chromatography by using HPLC as per the method described by Velpandian *et al.* (2000) with slight modification. The details of the procedure are as follows.

Apparatus :

The HPLC equipment (Waters$^{(R)}$ – supplied by Waters (India) Pvt., Ltd.) used comprised of a HPLC pump (Model 515), a dual wave length absorbance detector (Model 7486), a rheodyne manual injector (Model 746) with a 20 µl loop size and a data module (integrator). Chromatographic seperations were performed using C18 column (3.9 × 300 mm size).

Chromatographic conditions

For HPLC analysis of meloxicam in biological samples, the flow rate was 0.80 ml/min, the effluent was maintained at 364 nm, loop size was 20 µl, injection volume was 100 µl, chart speed was 0.25

mm/min and the detector sensitivity was monitored at 2.000 A.U.F.S. (Area Under Full Scale).

Reagents :

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

Mobile phase :-

The mobile phase comparised of methanol, acetonitrile and an aqueous solution of diammonium hydrogen orthophosphate (50 mM) in the ratio of 4 : 1 : 5.

Preparation of standard of Meloxicam in biological samples :-

Melonex, an injectable commercial preparation containing meloxicam in concentration of 5 mg/ml was used in the present study. Meloxicam was diluted in triple distilled water to have different strengths *viz.*, 10, 5, 2 and 1 μg/ml.

From each standard solution 0.1 ml was added to a centrifuge tube containing 0.9 ml of plasma or urine prior to drug administration. This yielded meloxicam of 1, 0.5, 0.2 and 0.1 μg/ml. Blank plasma/blank urine containing no drug was also prepared. These standards were used simultaneously with test samples for determination of the drug concentrations in test samples.

Analytical Method

1. In a clean and dry centrifuge tube, 1 ml of plasma sample was taken and 2 ml of trichloroacetic acid (TCA) and 2 ml of chloroform ($CHCl_3$) was added for precipitation of plasma protein.

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

3. The lower phase was transferred to a clean tube and evaporated to dryness in a water bath at 60^0C.

4. The residue is reconstituted in 500 µl HPLC eluent (mobile phase)

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

6. From various concentrations of standards versus area, standard curve was plotted in a graph paper for meloxicam.

7. Using the standard graph and the data obtained from test plasma and urine samples collected at various time intervals, the concentrations were estimated in test plasma and test urine samples separately.

Calculation of kinetic parameters

The following pharmacokinetic parameters of meloxicam was calculated after its i.v. administration from semilog plot of plasma drug concentration *versus* time curve. The experimental data was analysed using two compartment (for i.v. route) open model as described by Gibaldi and Perrier (1975) and Notari (1980).

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

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

where , Cp = drug concentration of the drug in plasma at time 't'

e = base of natural logarithm.

The description of different kinetic parameters are as follows:

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

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

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

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

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

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

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

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

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

h. AUC, the total area under plasma drug concentration time curve ($mg/L.h^2$)

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

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

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

j. MRT, mean residential time (h)

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

II. TOXICITY STUDIES

For toxicity analysis, the following parameters were estimated.

A) Haematological parameters :

i. Hemoglobin (gm/dl)

ii. Total leucocyte count (TLC)

iii. Differential leucocyte count (DLC)

B) Biochemical parameters :

i. Blood sugar

ii. Serum cholesterol

iii. Blood urea nitrogen (BUN)

iv. Total protein and albumin

v. Serum glutamate oxaloacetate trausaminase (SGOT)

vi Serum glutamate pyruvate transaminase (SGPT)

A. **Hematological parameters** :

(i) ***Haemoglobin (gm/dl)***

Haemoglobin content was estimated by Acid Haematin method using Hellige-sahli haemoglobinometer as per procedure described by Kolmer *et al.* (1969).

Principle :- When blood is added to 0.1 NHCl, Hb is converted to brown coloured acid haematin and the colour is matched with standard.

Procedure :- Five drops of N/10 hydrochloric acid (HCL) was taken in a measuring tube. Fresh blood drawn from jugular vein was taken in a tube containing EDTA as anticoagulant. In the special pipette, the blood containing the above noted anticoagulant was drawn upto the mark 20. Extra blood using fingertip was wiped out. The pipette was placed in the tube containing HCL and the blood was transferred into the acid. Acid was sucked into the pipette and the acid washed out all the contents into the tube. The tube was left for 5 min and distilled water was added drop by drop with a dropper and mixed by stirring with a rod to match the colour of the standard. When the colour matches, reading of the scale on tube was noted, which is the value of haemoglobin gram per 100 ml of blood.

II) Total leucocyte count (TLC) :

Total leucocyte count of blood was done using improved Neubauer chamber by the method as described by Schalm *et al.* 1975).

Principle : Acid diluting fluid does not destroy WBCs stain added to diluting fluids makes nuclei visible in a counting chamber under the microscope.

Procedure : The total leucocyte count was performed by hemocytometer using a WBC diluting pepette. The blood was sucked into the pipette upto 0.5 mark and then WBC diluting fluid was drawn upto 11 mark. Then the rubber tubing was removed, keeping the pipette in horizontal position. The content of pipette was thoroughly mixed by rotating the pipette and the first few drops were discarded. Later, the space in between cover slip was filled in the counting Neubauer's chamber. A period of 1-2 min for setting of the cells was allowed. The counting chamber was placed under a microscope and the cells in four large corner squares were counted with the ruled area under low power. The total leucocyte per cu.mm. of blood was thus calculated by multiplying the total number of cells counted in the four square with 50.

III) Differential leucocyte count (DLC) :

The differential leucocytic count of blood was done by the method as advocated by Schalm *et al.* (1975).

Procedure :- For differential leucocyte count, a thin blood smear was prepared on a glass slide. A drop of fresh blood was placed on one corner end of slide and spreaded as smear with the help of another slide using its thin edge at an angle of 45^0. Smear should be uniform and thin smear was dried in air and thick portion of smear was marked with identification number. Smear was stained with Geimsa stain diluted 1:10 in distilled water for 30 min. The slide was washed, air dried and examined under oil immersion of the microscope. At least 100 cells was counted by battlement / Zig-Zag method. Counted cells were neutrophils, lymphocytes, eosinophils, monocytes and basophils. The cells counted were presented in percentage (%).

B. Biochemical parameters :

Ready to use kits were used for biochemical tests. (NICE® and SPAN®)

(i) Blood glucose

Method – Folin and Wu's method.

The method was described by Frankel *et al.* (1970).

Principle :- In this method, proteins from the blood are precipitated by tungstic acid. The proteins free filtrate is heated with an alkaline cupric solution under standard conditions. The glucose produces a precipitate of cuprous oxide, which in turn is dissolved by and reduces phosphomolybdic solution to a blue colour, which is compared colorimetrically with the one similarly treated from a standard glucose solution.

Reagents :

Solution No. 1 (2/3 N sulphuric acid)

Solution No. 2 (10% sodium tungstate)

Solution No. 3 (Alkaline copper tartarate)(Folin and Wu)

Solution No. 4 (Phosphomolybdic) (Folin and Wu)

Solution No. 5 (Standard glucose solution). (1 ml = 10 mg of glucose)

1 ml of solution was diluted to 100 ml with distilled water before use. It was prepared freshly every time (1ml = 0.1 mg)

Preparation of protein free blood :

In a 10 ml test tube, the followings are added.

Distilled water	3.5 ml
Blood	0.1 ml

solution No 2. (10% sodiumtungustate) 0.2 ml

solution No. 3 (2/3 sulphuric acid) 0.2 ml

Mixed and allow to stand for 10 min. Then centrifuged to get a protein free filtrate.

Procedure :

1. Three Folin and Wu tubes were taken and marked them B (Blank), T (Test) and S (Standard).
2. The following agents were added:
    i. 2ml distilled water to B
    ii. 2ml protein free blood filtrate to T
    iii. 1 ml of glucose standard (working standard) and 1 ml distilled water to S.
3. To each of the above tube, 2 ml of solutions 3 (alkaline copper tartarte) was added. Then the contents of each tube was mixed well and placed in a boiling water bath for exactly 8 min.
4. The tubes were cooled without shaking and to each tube 2 ml solution No. 4 was added and mixed well.
5. The tubes were allowed to stand for 5 min, diluted to 12.5 ml with distilled water and mixed well.
6. Readings in colorimeter was done at 440 nm against blank set at zero.

Calculation

Mg glucose in 100 ml blood (mg%)

$$= \frac{0.D \text{ of } T}{0.D \text{ of } S} \times 0.1 \times \frac{100}{0.05} \qquad \text{i.e.} \frac{0.D \text{ of } T}{0.D \text{ of } S} \times 200$$

II) Serum cholesterol

Method :- Ferric chloride

The method was described in detail by Wootton (1964).

Principle :

Cholesterol reacts with ferric chloride in the presence of acetic acid and sulfuric acid. The red colour thus produced is measured colorimetrically.

Reagents

Solution No. 1	Acetic acid (aldehyde Free)
Solution No. 2	Stock Ferric chloride
Solution No. 3	Cholesterol standard [1ml = 1 mg]
Solution No. 4	Sulphuric acid [36 N]

Procedure:

1. 0.1 ml of serum was taken.
2. Preparation of working reagent.

0.5 ml solution No.2 [Ferric chloride] was diluted to 50 ml with solution No. 1 [Acetic acid aldehyde free]

3. 9.9 ml of working reagent was pipetted out into a centrifuge tube. 0.1 ml of serum was added to it and mixed well. The centrifuge tubes was allowed to stand for 15 min and then centrifuged. Thus, protein free solution was prepared.

3. Take Three test tubes and marked them as :-

B [Blank], S[Standard] and T[Test]

Then, the following steps were done.

Sl.No.	Name of Reagent	Blank [B]	Standard [S]	Test [T]
1.	Protein free solution	-	-	5.0 ml
2.	Working reagent	5.0 ml	4.9 ml	-
3.	Cholesterol standard [Solution No. 3]	-	0.1 ml	
4.	Sulphuric acid [SolutionNo. 4]	3.0 ml	3.0 ml	3.0 ml

5. The tubes were mixed well and allowed to stand for 30 min in dark.

7. Optical density at 560 nm was measured against distilled water at zero.

Calculation

a. Serum cholesterol in mg per 100 ml (mg %)

$$= \frac{\text{O.D. of T} - \text{O.D. of B}}{\text{O.D. of S} - \text{O.D. of B}} \times 200$$

III) Blood urea nitrogen

Method : Diacetyl monoxime

The method was explained in detail by Wootton (1964).

Principle

Urea reacts with diacetylmonoxime (DAM) in the presence of an activator to form a pink coloured derivative. This is measured colorimetrically.

Reagents

Urea Reagent 1 – Acid Reagent

Urea Reagent 2 – Colour Reagent (DAM)

Urea Reagent 3 – Standard 40 mg %

Specimen dilution :

Dilution of serum and standard 1-20 was done. Three tubes were taken and marked as blank [B], test [T] and standard [S] and the following procedure was adopted.

Name of Reagent	**Blank [B]**	**Test[T]**	**Standards [S]**
Urea reagent 1 (Acid reagent)	2.0 ml	2.0 ml	2.0 ml
Distilled water	0.2 ml	-	-
Diluted sample [1-20]	-	0.2 ml	-
Diluted standard [1-20]	-	-	0.2ml
Urea Reagent 2 [Colour reagent]	2.0 ml	2.0 ml	2.0 ml

The content of the tubes were mixed, boiled in a water bath for ten min and then cooled. Reading of OD was done at 540 nm against blank.

Calculation

$$\frac{0.D \text{ of } T}{0.D \text{ of } S} \times 40mg\%$$

i.e., mg of urea in 100 ml samples.

Blood urea nitrogen in mg% = urea × 0.47.

IV. Total Protein and Albumin

Method : Biuret and Bromocresol method

The detail of the method was explained of Reinhold [1953].

Principle : The peptide linkages of aminoacids in protein reacts with biuret reagent to form a violet coloured complex.

Albumin reacts with bromocresol green solution at p^H 4.1 to form a green colour derivative.

Reagents supplied.

1. Protein reagent . 1 - Biuret reagents
2. Albumin Reagent 2 – Bromocresol green solution
3. Reagents 3
   (a) Protein standard
   (b) Albumin standard.

Procedure :

Three tubes were labeled as blank [B], test [T] and standard [S]. Then the following procedure was adopted.

Sl. No.	Reagent	Blank [B]	Standard [S]	Test [T]
1.	Protein Reagent (Biuret Reagent)	5.0 ml	5.0 ml	5.0 ml
2.	Distil water	0.1 ml	-	-
3.	Serum	-	0.1 ml	-
4.	Protein standard (Reagent 3)	-	-	0.1 ml

The contents of the tubes were mixed well and kept at 37^0C for 10 min. Optical density (O.D) was measured at 530 – 560 nm against blank.

Calculation

Serum protein in gm per 100 ml of serum (gm %)

$$= \frac{\text{O.D of T}}{\text{O.D of S}} \times \text{S}$$

ALBUMIN :

Dilution of Specimen :

Serum and standard were diluted 1;10 with distilled water (i.e., dilute 0.1 ml serum and standard with 0.9 ml distilled water).

Procedure :

Three test tubes were labeled as blank [B], test [T] and Standard [S]. Then the following reagents were added as noted below.

Sl. No.	Reagent	Blank [B]	Standard [S]	Test [T]
1.	Albumin Reagent (Bromocresol green sol.)	4.0 ml	4.0 ml	4.0 ml
2.	Distilled water	0.2 ml	-	-
3.	Diluted serum	-	0.2 ml	-
4.	Diluted standard	-	-	0.2 ml

The contents were mixed well and kept for 5 min. Optical density was measured at 620 – 640 nm against blank.

Calculation

$$\text{Albumin in gm per 100 ml of serum (gm \%)} = \frac{T}{S} \times 5$$

GLOBULIN :

It is obtained by subtracting the value of albumin from the value of total protein.

Albumin : Globulin ratio

$$\text{A : G ratio} = \frac{\text{Albumin(gm\%)}}{\text{Globulin(gm\%)}}$$

V. Serum Glutamate Oxaloacetate Transaminase (SGOT) or Aspartate Transaminase (AST) :

Method : *Reitman and Frankel :*

The method was described in detail Reitman and Frankel (1957).

Principle : α-ketoglutarate + L-Aspartate ⇔ L-Glutamate+Oxaloacetate.

Oxaloacetate so formed is coupled with 2, 4 – Dinitrophenyl hydrzaine (2, 4 – DNPH) to give the corresponding hydrazone, which gives brown colour in alkaline medium and this is measured colorimetrically.

Reagents :

Reagent 1 : Buffered Aspartate α - keto glutarate substrate, p^H - 7.4

Reagent 2 : DNPH colour Reagent

Reagent 3 : Sodium hydroxide, 4 N

Reagent 4 : Working pyruvate standard, 2mM.

Preparation of working solutions :

Dilution of solution 1 : 1 ml of reagent 3 was diluted to 10 ml with purified water. Reagents 1, 2 and 4 are available as ready to use.

Procedure

Standard curve

As the reaction proceeds with time more amount of products are formed and since the end products inhibit the enzyme, there is more of inhibition. This is the major problem with colorimetric methods for the estimation of this enzyme. On the other hand, in kinetic methods, since the enzyme activity is measured during the initial few minutes, the amount of products formed during the short time are negligible to cause any inhibition.

Because of the above problem, it is necessary to standardize any colorimetric method against a standard kinetic method. In this kit method, this standardization is done against the standard international unit assay (Kinetic) and this is extrapolated to different amounts of pyruvate and this has been throughly rechecked. At this point, it is important to note that the standard graph of enzyme activity (in IU/L) on x-axis versus OD in y. y - axis is not a linear one, which shows that O.D. increases with increase in enzyme activity at a decreasing rate.

For colorimeter

Tube No.	1	2	3	4	5
Enzyme activity (IU/L)	0	11.5	29.3	54.7	91.2
Reagents 1 : Buffered Alanine p^H – 7.4	0.5	0.45	0.4	0.35	0.3
Reagent 4: Working pyruvate standard, 2mM	-	0.05	0.1	0.15	0.2
Purified water (ml)	0.1	0.1	0.1	0.1	0.1
Reagent 2:DNPH color reagent (ml)	0.5	0.5	0.5	0.5	0.5
The contents were mixed well and allowed to stand at R. T. for 20 min.					
Solution : 1	5.0	5.0	5.0	5.0	5.0

All tubes were mixed well by inversion and allowed to stand at room temperature (R.T) for 10 min and measurement of the O. D. of all the five tubes against purified water on a colorimeter at 505 nm were done.

Standard graph was plotted by taking enzyme activity on x-axis and O.D. on y-axis.

	Test [T]
Reagent 1 : Buffered alamine, P^H.-7.4 :	0.25 ml
Tubes incubated at 37°C for 5 min	
Serum :	0.05 ml
Mixed well and incubated at 37° for 60 min	
Reagent 2 : DNPH colour reagent :	0.25 ml
Mixed well and allowed to stand R. T for 20 min :	
Solution 1 :	2.5 ml

Mixed well and allowed to stand room temperature for 10 min. and O.D. was noted against purified water as blank on a colorimeter at 505 nm.

Calculation :

From the O.D. of Test on the y-axis of the standard curve and extrapolated it to the corresponding enzyme activity on x-axis, the concentration of test serum can be obtained on enzyme activity (IU/L).

VI. Serum glutamate pyruvate transaminase (SGPT) or Alanine Transaminase (ALT)

Method :- Reitman and Frankel

The method was described in detail by Reitman and Frankel (1957)

Principle : SGPT (ALT – alanine transaminase) catalyses the following reaction

α - ketoglutarate + L – Alanine ⇔ L – Glutamate + Pyruvate

Pyruvate so formed is coupled with 2, 4 – Dinitrophenyl hydrazine (2, 4 – DNPH) to give the corresponding hydrzaone, which gives brown colour in alkaline medium which is finally measured colorimetrically.

Reagents :

Reagent 1 : Buffered Alanine α - keto glutarate substrate, p^H – 7.4

Reagent 2 : DNPH colour reagent

Reagent 3 : Sodium Hydroxide, 4 N

Reagent 4 : Working pyruvate standard, 2 mM.

Preparation of working solutions.

Solution 1 : 1 ml of Reagent 3 was diluted to 10 ml with purified water. Reagents 1, 2 and 4 are available as ready for use.

Procedure:

As the reaction proceeds with time, more amounts of products are formed and since the end products inhibit the enzyme, there is more of inhibition. This is the major problem with colorimetric methods for the estimation of enzyme. On the other hand in kinetic methods, since the enzyme activity is measured during the initial few minutes, the amount of products formed during that short time are negligible to cause any inhibition.

Because of the above problem, it is necessary to standardize any colorimetric method against the standard international unit assay (Kinetic) and this is extrapolated to different amounts of pyruvate and this has been throughly rechecked. At this point, it is important to note that the standard graph of enzyme activity (in IU/L) on x-axis versus O.D. Y- axis is not linear one, which shows that O.D. increases with increase in enzymes activity at a decreasing rate.

For colorimeter.

Tube No.	*1*	*2*	*3*	*4*	*5*
Enzyme activity (IU / L)	0.0	13.4	27.4	46.6	72.0
Reagents 1 : Buffered Alanine (ml) p^H - 7.4	0.5	0.45	0.4	0.35	0.3
Reagent 4: Working pyruvate standard, 2mM	-	0.05	0.1	0.15	0.2
Purified water (ml)	0.1	0.1	0.1	0.1	0.1
Reagent 2:DNPH color reagent (ml)	0.5	0.5	0.5	0.5	0.5
All tubes were mixed well and allowed to stand at room temperature (R.T.) for 20 min.					
Solution 1	5.0	5.0	5.0	5.0	5.0

Contents of all tubes were mixed well by inversion. They were allowed to stand at room temperature (R.T.) for 10 min and O.D. of all the five tubes was noted against purified water on a colorimeter at 505 nm.

TEST

	Test [T]
Reagent 1 : Buffered alamine, P^H.-7.4 :	0.25 ml
Tubes incubated at 37°C for 5 min	
Serum :	0.05 ml
Mixed well and incubated at 37° for 30 min	
Reagent 2 : DNPH colour reagent :	0.25 ml
Mixed well and allowed to stand R. T for 20 min :	
Solution 1 :	2.5 ml

Mixed well and allowed to stand at room temperature (R. T.) for 10 min and O.D. was noted against purified water as blank on a colorimeter at 505 nm.

Calculations

From the O.D. of test on the y–axis of the standard curve and extrapolated it to the corresponding enzyme activity on x-axis, the concentration of test serum can be obtained on enzyme activity (IU/L).

❑❑❑❑❑

Chapter - 4

Results

RESULTS

I. PHARMACOKINETICS STUDY OF MELOXICAM

Pharmacokinetic study of meloxicam was conducted in five healthy female goats following single intravenous (i.v.) dose of 0.5 mg/kg and the results are presented below: -

1. Plasma levels

Concentrations of meloxicam in plasma at various time intervals following its single i.v. administration at the dose rate of 0.5 mg/kg have been shown in Table 1 and Fig. 1. The mean peak plasma concentration of 68.23 ± 6.80 μg/ml was attained at 0.042 h. The drug was detectable upto 24 h in all animals with the mean of 0.14 ± 0.02 μg/ml.

2. Urine levels

Table 2 and Fig. 2 reveal urine concentrations of meloxicam after its single i.v. administration (0.5 mg/kg). The drug appeared in urine of 2 out of 5 animals at 0.042 h with a mean of 0.04±0.03 h but appeared all animals at 0.083 h with a mean of 0.39 ± 0.04 μg/ml. The mean peak urine drug concentration of

Table - 1

Plasma concentrations of meloxicam (μg/ml) in female healthy goats after its single intravenous administration (0.5 mg/kg)

Time (h)	Animal Number					Mean ± S.E.M
	1	2	3	4	5	
0.042	81.78	67.88	72.20	76.65	42.64	68.23 ± 6.80
0.083	61.42	50.17	41.24	46.84	21.25	44.18 ± 6.61
0.167	6.27	24.31	8.00	12.50	10.40	12.30 ± 3.18
0.25	4.04	6.77	4.85	8.20	7.80	6.32 ± 0.81
0.333	2.54	5.80	3.76	5.22	4.62	4.38 ± 0.57
0.5	2.45	4.06	3.40	4.15	4.00	3.61 ± 0.31
0.75	2.36	3.96	2.55	3.54	2.72	3.02 ± 0.30
1	1.75	3.19	2.10	2.60	1.75	2.27 ± 0.27
1.5	1.69	1.68	1.50	2.00	1.58	1.69 ± 0.08
2	1.35	1.35	1.12	1.45	1.02	1.25 ± 0.08
3	0.59	0.82	0.68	0.98	0.94	0.76 ± 0.07
4	0.35	0.57	0.62	0.60	0.85	0.59 ± 0.07
6	0.30	0.49	0.56	0.54	0.78	0.53 ± 0.07
8	0.27	0.41	0.50	0.48	0.60	0.45 ± 0.05
10	0.21	0.34	0.42	0.38	0.54	0.37 ± 0.05
12	0.15	0.18	0.26	0.32	0.50	0.28 ± 0.06
24	0.12	0.10	0.14	0.16	0.22	0.14 ± 0.02

Table - 2

Urine concentrations of meloxicam (μg/ml) in female healthy goats after its single intravenous administration (0.5 mg/kg)

Time (h)	Animal Number					Mean ± S.E.M
	1	2	3	4	5	
0.042	N.D.	N.D.	N.D.	0.12	0.10	0.04 ± 0.03
0.083	0.28	0.52	0.35	0.40	0.38	0. 39 ± 0.04
0.167	40.25	45.50	42.22	36.45	42.15	41.31 ± 1.48
0.25	51.28	56.40	50.45	45.65	56.80	52.12 ± 2.06
0.333	28.68	32.24	54.24	56.24	34.15	41.11 ± 5.84
0.5	19.96	24.50	26.08	28.12	22.45	24.22 ± 1.41
0.75	16.12	18.54	19.12	20.10	17.85	18.35 ± 0.67
1	14.19	16.24	15.40	16.86	14.02	15.34 ± 0.56
1.5	12.88	13.55	13.02	12.98	12.96	13.08 ± 0.12
2	10.85	11.12	1.00	10.92	10.68	10.91 ± 0.74
3	9.22	10.08	9.88	8.88	9.15	9.44 ± 0.23
4	6.12	5.88	6.04	6.42	5.82	6.06 ± 0.10
6	5.06	4.92	4.98	5.12	4.45	4.91 ± 0.12
8	4.58	4.12	4.24	4.65	4.02	4.32 ± 0.12
10	3.93	3.25	3.85	4.00	3.14	3.63 ± 0.18
12	3.24	2.86	3.02	3.56	2.54	2.54 ± 0.17
24	1.86	1.22	1.82	1.92	1.55	1.67 ± 0.13
30	0.79	0.54	0.68	0.70	0.52	0.65 ± 0.05
36	0.39	0.22	0.35	0.40	0.20	0.31 ± 0.04
48	0.14	N.D.	0.12	0.22	N.D.	0.10 ± 0.04

N.D. = Non detectable

52.12 ± 2.06 μg/ml was achieved at 0.25 h. The drug was detectable upto 36 h in all animals with a mean of 0.31 ± 0.4 μg/ml. The drug was detectable only in 3 out of 5 animals at 48 h with a mean of 0.10 ± 0.04 μg/ml.

3. *Kinetic parameters*

Plasma drug concentration versus time profile has confirmed a two compartment open model for meloxicam 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 concentration during distribution phase (A) elimination (B) and theoretical zero time concentration $\left(C_p^o\right)$ were noted to be 16.61 ± 1.75, 0.82 ± 0.10 and 17.43 ± 1.81 μg/ml, respectively. The distribution rate constant (α) ranged from 1.504 to 2.761 h^{-1} with a mean of 2.010 ± 0.21 h^{-1} while its elimination rate constant (β) ranged from 0.067 to 0.097 h^{-1} with a mean value of 0.075 ± 0.006 h. The mean distribution half life ($t_{1/2}\,\alpha$) and elimination half life ($t_{1/2}\,\beta$) were noted to be 0.36 ± 0.04 h^{-1} and 9.37 ± 0.61 h, respectively. The mean area under curve (AUC) of 19.47 ± 1.52 mg/L.h, area under first moment curve (AUMC) of

Table – 3

Kinetic parameters of meloxicam in healthy female goats (calculated by 2-compartment open model) after its single intravenous dose (0.5 mg/kg)

Kinetic Parameter	Unit	Animal Number					Mean ± S.E.M
		1	2	3	4	5	
A	µg/ml	12.58	22.69	14.08	15.91	17.81	16.61 ± 1.75
B	µg/ml	0.47	0.87	0.85	0.78	1.13	0.82 ± 0.10
C_p^o	µg/ml	13.05	23.56	14.93	16.69	18.94	17.43 ± 1.81
α	h^{-1}	1.704	2.103	1.980	1.504	2.761	2.010 ± 0.21
β	h^{-1}	0.067	0.097	0.077	0.067	0.069	0.075 ± 0.006
$t_{1/2}\alpha$	h	0.41	0.33	0.35	0.46	0.25	0.36 ± 0.04
$t_{1/2}\beta$	h	10.34	7.14	9.00	10.34	10.04	9.37 ± 0.61
AUC	mg/L.h	14.40	19.76	18.15	22.22	22.82	19.47 ± 1.52
AUMC	$mg/L.h^2$	109.00	97.60	146.95	180.79	239.68	154.80 ± 25.80
MRT	H	7.57	4.94	8.04	8.13	10.50	7.84 ± 0.88
K_{12}	h^{-1}	0.739	0.836	1.048	0.685	1.770	1.016 ± 0.198
K_{21}	h^{-1}	0.126	0.171	0.185	0.134	0.229	0.169 ± 0.019
Kel	h^{-1}	0.906	1.193	0.824	0.752	0.831	0.901 ± 0.077
Fc	-	0.074	0.081	0.093	0.089	0.083	0.084 ± 0.003
T ≈ P	-	12.53	11.29	9.20	10.22	11.06	10.86 ± 0.55
Vdc	L/kg	0.038	0.021	0.033	0.029	0.026	0.029 ± 0.003
Vd_B	L/kg	1.06	0.57	0.58	0.64	0.44	0.66 ± 0.10
Vd_{area}	L/kg	0.52	0.26	0.35	0.33	0.38	0.37 ± 0.04
Vd_{ss}	L/kg	0.26	0.12	0.21	0.17	0.22	0.20 ± 0.02
Cl_B	ml/kg/min	0.58	0.42	0.43	0.36	0.43	0.44 ± 0.04

154.8 ± 25.80 mg/L.h^2 and mean residential time MRT of 7.84±0.88 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 1.016 ± 0.198 h^{-1}, 0.169 ± 0.019 h^{-1}, 0.901 ± 0.77 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 0.084 ± 0.003 and 10.86 ± 0.55. Various values of volume distribution obtained by different methods are shown in Table 2. A mean Vd_{area} of 0.37 ± 0.04 L/kg was noted. The total body clearance (Cl_B) value ranged from 0.36 to 0.58 with a mean of 0.44 ± 0.04 ml/kg/min.

II. TOXICITY STUDY OF MELOXICAM

Toxicity study of meloxicam was conducted in each of five healthy goats after i.m. administration of the drug at the dose rate 1 mg/kg body weight daily for 7 days. The blood samples were collected on 0 (before administration of the drug) 2, 4 and 8 days post drug administration. The following parameters were studied.

A. *HAEMATOLOGICAL PARAMETERS*

(i) Haemoglobin

Different values of haemoglobin (gm/dl) noted on different days (0, 2, 4 and 8) after daily i.m. injections of meloxicam

(1 mg/kg) for seven days are shown in Table 4 and Fig. 4. The values of haemoglobin in healthy goats before drug administration varied from minimum of 8.5 gm/dl to a maximum of 10.0 gm/dl with a mean value of 9.26 ± 0.28 gm/dl. The value slightly increased to 9.28 ± 0.25 g m/dl on day 2 and thereafter slightly decreased to 9.08 ± 0.27 gm/dl on day 4 and then increased to 9.16 ± 0.24 gm/dl on day 8. However, the analysis of data showed non significant differences between days post i.m. injection of meloxicam (Table 4A). This shows that meloxicam has no effect on haemoglobin.

(ii) Total leucocyte count (TLC)

Effect of meloxicam after daily i.m. injection at the dose rate of 1 mg/kg for 7 days on TLC are shown in Table 5 and Fig. 5. On day 0, the mean ± S.E.M. of TLC in healthy goats was observed to be 9860 ± 107.7 which significantly increased to 10410 ± 165.4 on day 2. Subsequently, TLC was noted to be increased on successive days (i.e., on day 4 and 8). On statistical analysis, the data shows significant difference between days. Thus, meloxicam has effect on TLC.

(iii) Differential leucocyte count (DLC)

Differential leucocyte count noted on different days (0, 2, 4 and 8) after daily i.m. injection of meloxicam (1 mg/kg) for 7 days

Table - 4

Effect of meloxicam (1 mg/kg i.m. daily for 7 days) on haemoglobin

Animal Number	HAEMOGLOBIN (gm/dl)			
	Day			
	0	2	4	8
1	8.5	8.6	8.4	8.5
2	9.2	9.1	9.0	9.1
3	10.0	9. 8	9.8	9.7
4	9.8	10.0	9.6	9.7
5	8.8	9.0	8.6	8.8
Mean ± S.E.M	9.26 ± 0.28	9.28 ± 0.25	9.08 ± 0.27	9.16 ± 0.24

No superscript denotes non-significant effect of meloxicam on haemoglobin.

Table - 4A

Showing analysis of data by single factor ANOVA

Parameter	Sources of variation	Degree of freedom	C.S.S.	Mean square	F-value
Haemoglobin	Between days	3	0.15	0.5	$1.42^{N.S.}$
	Error	16	5.63	0.35	

N.S. = Non significant

Table – 5

Effect of meloxicam (1mg/kg, i.m. daily for 7 days) on total leucocyte count (TLC)

Animal No.	Total Leucocyte count			
	Day			
	0	2	4	8
1	10,200	10,700	11,350	11,775
2	9,700	10,750	11,225	11,775
3	9,800	10,250	10,800	11,000
4	9,600	9,850	10,000	10,450
5	10,000	10,500	11,250	11,450
Mean ± S.E.M.	$9,860^{a} \pm 107.7$	$10,410^{b} \pm 165.4$	$10,925^{c} \pm 249.7$	$11,290^{d} \pm 253.5$

Different superscripts denote significant effect of meloxicam on TLC.

Table - 5A

Showing analysis of data by single facto ANOVA

Parameter	Sources of variation	Degree of freedom	C.S.S.	Mean square	F-value
Total leucocyte Count (TLC)	Between days	3	5.818	1.939	9.367**
	Error	16	3.312	0.207	

** $P < 0.01$

are presented in Table 6 and Fig. 6. Under the parenthesis the original values converted to arcsine values are presented for proper statistical analysis. Single factor ANOVA test reveals that there is no significant effect of meloxicam on DLC namely neutrophil, lymphocyte, monocyte, eosinophil and basophil.

B. BIOCHEMICAL PARAMETERS

(i) Blood sugar

Mean ± S.E.M of blood sugar values noted on different days after daily i.m. injection of meloxicam (1 mg/kg) for 7 days are presented in Table 7 and Fig. 7. The blood sugar value on day 0 (before injection of meloxicam) varied from 57.10 to 68.00 mg/dl with a mean of 62.14 ± 1.77 mg/dl. On i.m. administration, on day 2 the mean value was noted to be 59.22 ± 2.36 mg/dl. Subsequently, slightly increased values were noted on day 4 (64.82 ± 2.25 mg/dl) and on day 8 (64.39 ± 1.56 mg/dl). On statistical analysis, these values do not differ significantly from day 0 and between them also. Thus, it is clearly shown that meloxicam has no effect on sugar metabolism.

Table - 6

Effect of meloxicam (1 mg/kg i.m. daily for 7 days) on differential leucocyte count (DLC) in %

Animal Number	Day 0					Day 2					Day 4					Day 8				
	N	L	M	E	B	N	L	M	E	B	N	L	M	E	B	N	L	M	E	B
1	36 (36.87)	54 (47.29)	5 (12.92)	4 (11.54)	1 (5.74)	35 (36.27)	56 (48.45)	4 (11.54)	5 (12.92)	0 (0.00)	37 (37.47)	55 (47.87)	4 (11.54)	3 (9.98)	1 (5.74)	36 (36.87)	55 (47.87)	5 (12.92)	3 (9.98)	1 (5.74)
2	39 (38.65)	50 (45.00)	5 (12.92)	5 (12.92)	1 (5.74)	40 (39.23)	50 (45.00)	5 (12.92)	4 (11.54)	1 (5.74)	38 (38.06)	52 (46.15)	5 (12.92)	4 (11.54)	1 (5.74)	41 (39.82)	51 (45.57)	5 (12.92)	3 (9.98)	0 (0.000)
3	35 (36.27)	55 (47.87)	5 (12.92)	5 (12.92)	0 (0.00)	36 (36.87)	54 (47.29)	5 (12.92)	4 (11.54)	1 (5.74)	39 (38.65)	52 (46.15)	5 (12.92)	4 (11.54)	0 (0.00)	40 (39.23)	52 46.15)	4 (11.54)	4 (11.54)	0 (0.00)
4	40 (39.23)	50 (45.00)	4 (11.54)	5 (12.92)	1 (5.74)	39 (38.65)	51 (45.57)	5 (12.92)	4 (11.54)	1 (5.74)	41 (39.82)	49 (44.93)	5 (12.92)	5 (12.92)	0 (0.00)	37 (37.47)	52 (46.15)	5 (12.92)	5 (12.92)	1 (5.74)
5	38 (38.06)	52 (46.15)	5 (12.92)	5 (12.92)	0 (0.00)	39 (38.65)	53 (46.72)	4 (11.54)	4 (11.54)	0 (0.00)	40 (39.23)	52 (46.15)	4 (11.54)	4 (11.54)	0 (0.00)	36 (36.87)	54 (47.29)	5 (12.92)	4 (11.54)	1 (5.74)
G. M.	37.55	52.16	4.78	4.78	0.00	37.75	52.76	4.57	4.18	0.00	38.97	51.96	4.57	3.95	0.00	37.94	59.82	4.78	3.73	0.00
Mean ± S.E.M	(37.82 ± 0.55)	(46.26 ± 0.58)	(12.64 ± 0.28)	(12.64 ± 0.28)	(3.44 ± 1.41)	(37.93 ± 0.57)	(46.61 ± 0.61)	(12.36 ± 0.34)	(11.82 ± 0.28)	(3.44 ± 1.41)	(38.65 ± 0.41)	(46.25 ± 0.47)	(12.37 ± 0.34)	(11.50 ±0.46)	(2.30 ± 1.41)	(38.05 ± 0.62)	(46.61 ± 0.42)	(12.64 ±0.28)	(11.20 ± 0.55)	(3.44 ± 1.41)

Note : N = Neutrophil, L = Lymphocyte, M = Monocyte, E = Eosinophil, B = Basophil

Values within the parenthesis denote arcsine value.

Analysis of data was done on the basis of arcsine value which in the table 6.

No superscript denotes non significant effect of meloxicam on DLC.

Table - 6A

Showing analysis of data by single factor ANOVA.

Parameters	Source of variation	Degree of freedom	C.S.S.	Mean square	F-value
Neutrophil	Between days	3	2.01	0.67	0.44^{NS}
	Error	16	23.81	1.49	
Lympocyte	Between days	3	0.62	0.21	0.15^{NS}
	Error	16	22.32	1.39	
Monocyte	Between days	3	0.38	0.13	0.32^{NS}
	Error	16	6.62	0.41	
Eosinophil	Between days	3	9.57	3.19	0.67^{NS}
	Error	16	75.43.	4.71	
Basophil	Between days	3	4.94	1.65	0.16^{NS}
	Error	16	158.15	9.89	

Table - 7

Effect of meloxicam (1mg/kg i.m. daily for 7 days) on blood sugar

Animal No.	Blood sugar (mg/dl)			
	Day			
	0	2	4	8
1	60.87	54.54	68.57	63.15
2	57.10	56.83	69.61	63.63
3	61.87	56.76	67.15	64.75
4	62.87	64.75	59.25	65.73
5	68.00	66.23	59.53	62.56
Mean ± S.E.M	62.14 ± 1.77	59.22 ±2.36	64.82±2.25	64.39±1.56

No superscript denotes non-significant effect of meloxicam on blood sugar.

Table - 7A

Showing analysis of data by single factor ANOVA

Parameter	Source of variation	Degree of freedom	C.S.S.	Mean square	F-value
Blood sugar	Between days	3	73.47	24.49	2.50^{NS}
	Error	16	156.16	9.76	

NS = non-significant

(ii) Serum cholesterol

Table 8 Fig. 8 present the values of serum cholesterol before (0 days) and after daily i.m. injection of meloxicam (1 mg/kg) in each of five female goats. On day 0, the value of serum cholesterol ranged from 65.00 to 80.42 mg/dl with a mean ± S. E. M. of 73.66 ± 2.95 mg/dl. The value slightly increased on day 2 (77.46 ± 2.71 mg/dl) and subsequently decreased on day 4 (74.76 ± 2.96 mg/dl) and day 8 (74.74 ± 2.69 mg/dl). On statistical analysis these values do not differ significantly from day 0 and between them also. Thus, it can be assumed that meloxicam has no effect on serum cholesterol.

(iii) Blood urea nitrogen (BUN)

Table 9 and Fig. 9 depict the value of blood urea nitrogen (BUN) before (day 0) and after daily i.m. injection of meloxicam (1mg/kg) in each of five female goats. On day 0, mean ± S.E.M. of BUN in the healthy goats was observed to the 10.70 ± 0.22 mg/dl which is significantly increased to 12.24 ± 0.21 mg/dl on day 2. Subsequently, BUN was observed to be increased on successive days (i.e. on day 4 and 8). On statistical analysis (Table 9A), the data shows significant difference between days. Thus, meloxicam has a significant effect on BUN.

Table - 8

Effect of meloxicam (1 mg/kg i.m. daily for 7 day) on serum cholesterol

Animal No.	Serum cholesterol (mg/dl)			
	Day			
	0	2	4	8
1	65.00	69.36	66.54	65.80
2	68.70	77.19	73.13	75.05
3	80.42	85.65	84.25	82.15
4	75.40	74.85	72.25	73.14
5	78.80	80.25	77.62	77.54
Mean ± S.E.M	73.66±2.95	77.46±2.71	74.76±2.96	74.74±2.69

No superscript denotes non-significant effect of meloxicam on serum cholesterol.

Table - 8A

Showing analysis of data by single factor ANOVA.

Parameter	Source of variation	Degree of freedom	C.S.S.	Mean square	F-value
Serum Cholesterol	Between days	3	39.34	13.11	0.33^{NS}
	Error	16	642.19	40.13	

NS = non-significant

Table - 9

Effect of meloxicam (1 mg/kg i.m. daily for 7 days) on blood urea nitrogen (BUN)

Animal No.	Blood urea nitrogen (mg/dl)			
	Day			
	0	2	4	8
1	10.50	12.22	14.10	15.20
2	10.75	13.00	14.50	15.75
3	11.00	12.00	13.10	14.75
4	10.00	11.75	13.69	15.00
5	11.25	12.25	13.70	15.50
Mean± S.E.M	$10.70^{a}\pm0.22$	$12.24^{b}\pm0.21$	$13.82^{c}\pm0.23$	$15.24^{d}\pm0.18$

Different superscripts denote significant effect of meloxicam on blood urea nitrogen (BUN)

Table - 9A

Showing analysis of data by single factor ANOVA

Parameter	Source of variation	Degree of freedom	C.S.S.	Mean square	F-value
Blood urea Nitrogen (BUN)	Between days	3	57.74	19.25	87.5**
	Error	16	3.52	0.22	

** $p < 0.01$

(iv) ***Total protein :***

Table 10 and Fig. 10 present the value of total protein before (day 0) and after i.m. daily injection of meloxicam (1mg/kg) in each of five female goats. On day 0, mean ± S.E.M. value of total protein in the healthy goat was observed to be 6.02 ± 0.006 gm/dl that significantly increased to 6.62 ± 0.071 gm/dl and 7.02 ± 0.010 gm/dl, respectively on day 2 and day 4. On day 8, mean ± S.E.M. value of total protein was found to be slightly decreased to 6.82 ± 0.012 gm/dl from the value on day 4. On statistical analysis (Table 10A), the data shows significant difference between days. Thus, meloxciam has a significant role on protein metabolism.

Mean ± S.E.M. value of albumin noted on different days after i.m. injection of meloxicam (1 mg/kg) for 7 days are present in Table 10 and and Fig. 8. On day 0, Mean ± S.E.M. value of albumin in healthy goat was observed to the 2.34 ± 0.007 gm/dl which is significantly increased to 2.57 ± 0.014 gm/dl on day 2. Subsequently, albumin was observed to increase on successive days (ie., on day 4 and 8). On statistical analysis the data shows significant difference between days. Thus, meloxicam may have effect on albumin.

Table - 10

Effect of meloxicam (1mg/kg i.m daily for 7 days) on total protein, albumin, globulin and A/G ratio.

Animal No.	Total protein (gm/dl)				Albumin (gm/dl)				Globulin (gm/dl)				A/G Ratio			
	Day				Day				Day				Day			
	0	2	4	8	0	2	4	8	0	2	4	8	0	2	4	8
1	6.01	6.61	7.01	6.85	2.34	2.56	2.98	3.14	3.69	4.05	4.03	3.71	0.63 (52.53)	0.63 (52.53)	0.74 (59.34)	0.85 (67.21)
2	6.03	6.67	7.04	6.87	2.37	2.63	2.99	3.18	3.66	4.04	4.05	3.69	0.65 (53.73)	0.65 (53.73)	0.74 (59.34)	0.86 (68.03)
3	6.00	6.59	6.99	6.78	2.34	2.55	2.90	3.05	3.66	4.04	4.09	3.73	0.64 (53.13)	0.63 (52.53)	0.71 (57.42)	0.82 (67.90)
4	6.03	6.62	7.03	6.84	2.36	2.56	2.93	3.07	3.67	4.06	4.10	3.77	0.64 (53.13)	0.63 (52.53)	0.71 (57.42)	0.81 (64.16)
5	6.02	6.63	7.05	6.80	2.33	2.59	2.96	3.18	3.68	4.04	4.09	3.62	0.63 (52.53)	0.64 (53.13)	0.72 (58.05)	0.88 (69.73)
Mean ± S.E.M	6.02^{a} ±0.006	6.62^{b} ± 0.13	7.02^{c}± 0.01	6.82^{d} ± 0.12	2.34^{a} ± 0.007	2.57^{b} ± 0.014	2.95^{c} ± 0.016	3.12^{d} ± 0.061	3.67^{a} ± 0.005	4.04^{b} ± 0.004	4.07^{b} ± 0.013	3.70^{a}± 0.025	-	- -	-	-
												G. M.	0.64	0.64	0.72	0.84
												Mean ± S.E.M	(52.97^{a} ± 0.25)	(52.89^{a}± 0.24)	(58.31^{b} ± 0.43)	(68.81^{c} ± 1.02)

G. M. = Geometric mean,

Value within parenthesis denote arcsine value

Different superscript denotes significant effect of meloxicam on total protein, albumin, globulin and A/G ratio while same superscript denote non significant effect of meloxicam.

Table - 10A

Showing analysis of data by single factor ANOVA

Parameters	Sources of variation	Degree of freedom	C.S.S.	Mean square	F-value
Total protein (gm/dl)	Between days	3	2.84	0.95	7.60**
	Error	16	0.02	0.00125	
Albumin (gm/dl)	Between days	3	1.86	0.62	330.67**
	Error	16	0.03	.001875	
Globulin (gm/dl)	Between days	3	0.69	0.23	36.8**
	Error	16	.01	0.00625	
A/G ratio	Between days	3	642.08	214.03	128**
	Error	16	26.78	1.67	

$p < 0.01$

Mean ± S.E.M. value of globulin noted on different days after i.m. injection of meloxicam are shown in Table 10 and Fig. 10. On day 0, mean ± S.E.M. value of globulin in healthy goat is noted to be 3.67 ± 0.005 gm/dl which is significantly increased to 4.04 ± 0.004 gm/dl on day 2 and 4.07 ± 0.013 gm/dl on day 4. On day 8, significant decrease is noted but there is no significant difference as compared to day 0. The values of globulin do not differ significantly between day 2 and day 4. The data shows that meloxicam has some effect on globulin that too for a short period.

Mean ± S.E.M. value of albumin globulin ratio (A : G) noted on different days post injection of meloxicam are presented in Table 10 and Fig. 8. Under parenthesis the original values converted to arcsine values are presented after converting the ratio into percentage for proper statistical analysis. No significant difference is found between day 0 and 2 and other days value differ significantly between them.

(v) Serum glutamate oxaloacetate transaminase (SGOT) or Alanine transaminase (ALT)

Table 11 and Fig. 11 present the value of SGOT before (0 day) and after daily i.m. injection of meloxicam (1 mg/kg) in each of five healthy goats. On day 0, mean ± S.E.M. value of SGOT is noted

Table - 11

Effect of meloxicam (1mg / kg i.m daily for 7 days) on serum glutamate oxaloacetate transaminase (SGOT)

Animal No.	SGOT (IU/L) Day			
	Day			
	0	2	4	8
1	49.0	52.9	56.5	59.5
2	48.8	52.3	56.1	59.2
3	48.9	52.0	56.3	59.6
4	48.7	52.3	56.4	59.4
5	49.1	52.4	56.3	59.4
Mean ± S.E.M.	$48.90^{a}\pm0.07$	$52.38^{b}\pm0.15$	$56.36^{c}\pm0.07$	$59.42^{d}\pm0.67$

Different superscripts denote significant effect of meloxicam on SGOT

Table - 11A

Showing analysis of data by single factor ANOVA.

Parameter	Source of variation	Degree of freedom	C.S.S.	Mean square	F-value
SGOT	Between days	3	316.50	105.50	439.58**
	Error	16	0.73	0.24	

** $p < 0.01$

Table - 11

Effect of meloxicam (1mg / kg i.m daily for 7 days) on serum glutamate oxaloacetate transaminase (SGOT)

Animal No.	SGOT (IU/L) Day			
	Day			
	0	2	4	8
1	49.0	52.9	56.5	59.5
2	48.8	52.3	56.1	59.2
3	48.9	52.0	56.3	59.6
4	48.7	52.3	56.4	59.4
5	49.1	52.4	56.3	59.4
Mean ± S.E.M.	$48.90^{a}\pm0.07$	$52.38^{b}\pm0.15$	$56.36^{c}\pm0.07$	$59.42^{d}\pm0.67$

Different superscripts denote significant effect of meloxicam on SGOT

Table - 11A

Showing analysis of data by single factor ANOVA.

Parameter	Source of variation	Degree of freedom	C.S.S.	Mean square	F-value
SGOT	Between days	3	316.50	105.50	439.58**
	Error	16	0.73	0.24	

** $p < 0.01$

Table - 12

Effect of meloxicam (1 mg/kg i.m. daily for 7 days) on serum glutamate pyruvate transaminase (SGPT)

Animal No.	SGPT (IU/L)			
	Day			
	0	2	4	8
1	7.2	10.1	15.1	17.4
2	7.4	10.2	15.3	17.1
3	7.3	10.3	15.6	17.6
4	7.5	10.1	15.4	17.2
5	7.4	10.4	15.6	17.5
Mean ± S.E.M	$7.36^{a} \pm 0.50$	$10.22^{b} \pm 0.58$	$15.38^{c} \pm 0.86$	$17.36^{d} \pm 0.93$

Different superscripts denote significant effect of meloxciam on SGPT.

Table -12A

Showing analysis of data by single factor ANOVA

Parameter	Source of variation	Degree of freedom	C.S.S.	Mean square	F-value
SGPT	Between days	3	65.932	21.1	767.27**
	Error	16	0.44	0.0275	

** $p < 0.01$

to be 48.90 ± 0.07 IU/L which is significantly increased to 52.38 ± 0.15 IU/L on day 2. Subsequently, SGOT values increased on successive days (i.e. on day 4 and 8). On statistical analysis (Table 12A) the data shows significant difference between days. Thus, melxociam has significant effect on myocardial tissues.

(vi) Serum glutamate pyruvate transaminase (SGPT) or aspartate transaminase (AST)

Table 12 and Fig. 12 present the values of SGPT before (0 day) and after daily injection of meloxicam (1 mg/kg, i.m.) in each of five female goats. On day 0, 7.36 ± 0.50 IU/L is noted to be the mean ± S.E.M. value of SGPT which is significantly increased to 10.22 ± 0.58 IU/L on day 2. Subsequently, SGPT is noted to be increased on successive days (ie., on day 4 and 8). On statistical analysis (Table 12A) the data shows significant difference between days. Thus, meloxicam may have a significant effect on liver function.

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Chapter - 5

Discussion

DISCUSSION

Meloxicam, a newly introduced novel non-steroidal anti-inflammatory drug (NSAID) is being preferred clinically over older groups of NSAIDs against various inflammatory conditions. It is well known that older groups of NSAIDs mainly act on cyclooxygenase-1 (COX-1), due to which these drugs have narrow margin of safety and have more adverse effects, particularly on gastrointestinal and renal systems with antithrombogenic activity (Vane and Botting, 1996). In contrast, meloxicam mainly acts on COX-2 (Barner, 1996) and has a high intrinsic activity with a low ulcerogenic potential with a high therapeutic index (Engelhardt *et al.*, 1996 b). Though meloxicam has been introduced in veterinary practice during the last few years. kinetic studies of meloxicam were conducted mainly in humans and experimental animals like rate, mice, minipig, baboon etc. But, only a few studies have been conducted in food producing animals and kinetic studies on goats are lacking.

Anti-inflammatory agents are required to be administered for a longer of time and hence, the adverse effects on prolonged administration are required to be studied so that the assessment of therapeutic effects of the drug in comparison to its

adverse effects is to be studied thoroughly for its effective use in therapy.

With the above mentioned aims and objectives the present study has been undertaken in goats.

KINETIC STUDY

(a) Distribution in biological fluids

Meloxicam was present with a mean plasma concentration of 68.23 ± 6.80 μg/ml at 0.042 h and the drug declined with time and was traced upto 24 h (0.14 ± 0.02 μg/ml) in all animals on its i.v. administration @ 0.5 mg/kg (Table 1). In case of urine, the drug was present only in 2 out of 5 animals with a mean concentration of 0.04 ± 0.03 μg/ml at 0.042 h while it was detectable in all animals at 0.083 h (0.39 ± 0.04 μg/ml). Mean peak urine concentration of 52.12 ± 2.06 μg/ml was obtained at 0.25 h. Thereafter, the drug was found to decline with time and it was detectable in all animals upto 36 h post administration. At 48 h, the drug was detectable in urine of 3 out of 5 animals with the mean of 0.10 ± 0.04 μg/ml (Table 2).

(b) Kinetic parameters

In the present study, the high mean value of 16.16 ± 1.75 μg/ml for zero time concentration during distribution phase (A)

while low mean of 0.82 ± 0.10 μg/ml (A) for zero time concentration during elimination phase (B) and mean value of 17.43 ± 1.81 μg/ml for theoretical zero time concentration ($C_p^o = A + B$) were obtained after its single i.v. administration (0.5 mg/kg). A moderately high value for distribution rate constant (α) of 2.010 ± 0.21 h^{-1} and moderately low distribution half life ($t_{1/2}$ α) of 0.36 ± 0.04 h denote that the drug is distributed to peripheral tissues at moderate rate. The elimination half ($t_{1/2}$ β) of 9.37 ± 0.61h obtained in the present study denotes that the drug is comparatively removed at a slower rate. This has lead to a longer mean residential time (MRT) of 7.84 ± 0.88 in goat. A comparatively lower $t_{1/2}$ β of 6.12 h in mice while higher $t_{1/2}$ β of 13.4 h in male rats, 24.0 h in dogs and very high $t_{1/2}$ β of 121 h in mini-pigs were noted (Busch *et al.*, 1998). As compared to other NSAIDs, the elimination half life ($t_{1/2}$ β) of meloxicam was found to be higher in ruminants. An elimination half life ($t_{1/2}$ β) of diclofenac was noted to be 1.15 h in man (Kurowski, 1988) 2.4 h in pig (Oberle *et al.*, 1994) and 4.06 ± 0.59 h in buffalo calf (Nitesh kumar *et al.*, 2003). Similarly, paracetamol also showed lower $t_{1/2}$ β in many ruminants. An elimination half life ($t_{1/2}$ β) of paracetamol was noted to be 8.69 ± 0.83 h in buffalo calf (Sindhu *et al.*, 1993), 4.84 ± 1.26 h in cross bred calf (Sharma *et al.*, 1995), 0.53 h in black Bengal goat (Manna *et al.*, 1994) and 3.56 ± 0.13 in goat (Sudha Kumari, 1998).

The above facts clearly indicate that meloxicam is remained in the body of ruminants for a longer period as compared to older groups of NSAIDs. The higher $t_{1/2}\beta$ obtained with meloxicam led to a higher mean residential time (MRT) of 7.84 ± 0.88 h in the present study in goat.

A moderate value of volume of distribution of 0.37 ± 0.04 L/kg and a high value of tissue to plasma concentration ratio (T ≈ P) of 10.86 ± 0.55 indicate that the drug may be distributed to greater extent in peripheral tissues, which may be beneficial in the treatment arthritis and other inflammatory conditions in goats.

TOXICITY STUDY

(a) Haematological parameters

In the present study, the effects of meloxicam on hematological parameters namely hemoglobin, total leucocyte count (TLC), differential leucocyte count (DLC) after its i.m. injection (1 mg/kg) daily for 7 days were carried out. The study revealed that there was no significant change in haemoglobin (gm/dl) and the value varied from 9.08 ± 0.27 gm/dl to 9.28 ± 0.25 gm/dl from 0 to 8 days. Similarly there was no significant effect on DLC, and they differed only non significantly from 0 to 8 days. In contrast, the TLC values

while low mean of 0.82 ± 0.10 μg/ml (A) for zero time concentration during elimination phase (B) and mean value of 17.43 ± 1.81 μg/ml for theoretical zero time concentration ($C_p^o = A + B$) were obtained after its single i.v. administration (0.5 mg/kg). A moderately high value for distribution rate constant (α) of 2.010 ± 0.21 h^{-1} and moderately low distribution half life ($t_{1/2}$ α) of 0.36 ± 0.04 h denote that the drug is distributed to peripheral tissues at moderate rate. The elimination half ($t_{1/2}$ β) of 9.37 ± 0.61h obtained in the present study denotes that the drug is comparatively removed at a slower rate. This has lead to a longer mean residential time (MRT) of 7.84 ± 0.88 in goat. A comparatively lower $t_{1/2}$ β of 6.12 h in mice while higher $t_{1/2}$ β of 13.4 h in male rats, 24.0 h in dogs and very high $t_{1/2}$ β of 121 h in mini-pigs were noted (Busch *et al.*, 1998). As compared to other NSAIDs, the elimination half life ($t_{1/2}$ β) of meloxicam was found to be higher in ruminants. An elimination half life ($t_{1/2}$ β) of diclofenac was noted to be 1.15 h in man (Kurowski, 1988) 2.4 h in pig (Oberle *et al.*, 1994) and 4.06 ± 0.59 h in buffalo calf (Nitesh kumar *et al.*, 2003). Similarly, paracetamol also showed lower $t_{1/2}$ β in many ruminants. An elimination half life ($t_{1/2}$ β) of paracetamol was noted to be 8.69 ± 0.83 h in buffalo calf (Sindhu *et al.*, 1993), 4.84 ± 1.26 h in cross bred calf (Sharma *et al.*, 1995), 0.53 h in black Bengal goat (Manna *et al.*, 1994) and 3.56 ± 0.13 in goat (Sudha Kumari, 1998).

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A moderate value of volume of distribution of 0.37 ± 0.04 L/kg and a high value of tissue to plasma concentration ratio (T ≈ P) of 10.86 ± 0.55 indicate that the drug may be distributed to greater extent in peripheral tissues, which may be beneficial in the treatment arthritis and other inflammatory conditions in goats.

TOXICITY STUDY

(a) Haematological parameters

In the present study, the effects of meloxicam on hematological parameters namely hemoglobin, total leucocyte count (TLC), differential leucocyte count (DLC) after its i.m. injection (1 mg/kg) daily for 7 days were carried out. The study revealed that there was no significant change in haemoglobin (gm/dl) and the value varied from 9.08 ± 0.27 gm/dl to 9.28 ± 0.25 gm/dl from 0 to 8 days. Similarly there was no significant effect on DLC, and they differed only non significantly from 0 to 8 days. In contrast, the TLC values

significantly increased from day 0 (9860 ± 107.7) to day 2 (10410 ± 165.4), day 4 (10925 ± 249.7) and day 8 (11290 ± 253.5). Though marked significant increasing trend was noted with increasing days, the TLC values remained within the normal range of 8000-12000 as noted for goats (Dukes, 1970). Similarly, Magarwadiya *et al.* (2002) showed no significant change in haemoglobin and differential leucocyte count in buffalo calves after administering meloxicam for 5 consecutive days @ 0.15 mg/kg body weight by i.m. route.

(b) Biochemical parameters

The present study reveals that the value of blood sugar noted before the injection of meloxicam (day 0) was found to be 62.14 ± 1.77 mg/dl, which differed non significantly on day 2 (59.22 ± 2.36 mg/dl), on day 4 (64.82 ± 2.25) and on day 8 (64.39 ± 1.56 mg/dl). This clearly indicates that meloxicam has no effect on blood sugar metabolism. Similarly, meloxicam was found to have no significant effect on serum cholesterol and the values on day 2, day 4 and day 8 did not differ significantly from the value noted before injection of meloxicam (73.66 ± 2.95 mg/dl). However, significant effect of meloxicam on blood urea nitrogen (BUN) was observed. The value noted on day 0 (10.70 ± 0.22 mg/dl) increased significantly on day 2

(12.24 ± 0.21 mg/dl), day 4 (13.82 ± 0.23 mg/dl) and day 8 (15.24 ± 0.18). Though there is some effect of meloxicam on kidney as noted in the value of BUN, but the increased values are within normal range. The normal value reported in goat for BUN varies from 10 to 20 mg/dl (Kaneko *et al.*, 1997).

In the present study, the values of total protein as well as albumin increased significantly with increase in days after daily i.m. administration (1 mg/kg). In case of globulin, the value significantly differed on day 2 and 4 while non significant difference was noted on day 8 as compared to day 0 and also there is no significant difference between day 2 and 4 (Table 10). In case of A/G ratio, the values on day 2 did not differ significantly with day 0 while significant difference was noted on day 4 and day 8 from day 0 value. An increase in total protein was reported on 48 h (2 day) 120 h (5 day) and 144 h (6 day) while increase in albumin values on 48 h (2 day), 96 h (4 day) and 144 h (6 day) were noted by Magarwadiya *et al.* (2002), after i.m. injection of meloxicam for 5 consecutive day @ 0.15 mg/kg in buffalo calves. The reason for increase in value is difficult to be interpreted but may possibly be due to increase in assimilation of protein in the body.

The serum glutamate oxaloacetate transaminase (SGOT) before injection of meloxicam (0 day) was noted to be 48.90 ± 0.07 IU/L, which increased subsequently on day 2 (52.38 ± 0.15 IU/L), on day 4 (56.36 ± 0.07 IU/L) and on day 8 (59.42 ± 0.67 IU/L). This shows that meloxicam may have some minor effects on myocardial muscle. After withdrawal of the drug it may come to normal but it has not been undertaken in the present study. Similar trend was noted with serum glutamate pyruvate transaminase (SGPT). The value of SGPT was noted to be 7.36 ± 0.50 IU/L on day 0 (before injection of meloxicam) which increased to 10.22 ± 0.58 IU/L on day 2, 15.38 ± 0.86 IU/L on day 4 and 17.36 ± 0.93 IU/L on day 8 after daily i.m. injection of meloxicam (Table 12). A report in human female patient with rheumatoid arthritis developed acute cytolytic hepatitis due to meloxicam, which may demonstrate the potential of this drug to induce hepatic damage (Stacrkel and Hormane, 1999).

Much toxicity studies have not been conducted in domestic animals, particularly in goat. However, Cherian *et al.* (2001) had demonstrated meloxicam given i.v. (0.25 and 7.5 mg/kg) daily for 14 days did not show any significant changes in the haematological and biochemical parameters in 24 male and female rats.

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Chapter - 6

Summary

SUMMARY

Pharmacokinetics and toxicological studies of meloxicam were conducted in five healthy goats of non-descript breed weighing between 20-22 kg. Concentrations of meloxicam in plasma and urine as well as kinetic parameters were estimated in health goats after i.v. administration (0.5 mg/kg). Further, toxicological study was conducted after i.m injection (1 mg/kg) of meloxicam once daily for 7 days. Various haematological (haemoglobin, total leucocyte count, differential leucocyte count) and biochemical (blood sugar, serum cholesterol, blood urea nitrogen, total protein, serum glutamate oxalo-acetate transaminase and serum glutamate pyruvate transaminase) parameters were estimated on 0, 2, 4 and 8 days post i.m. injection. The salient findings of the present study were as follows.

KINETIC STUDY

i. Following single i.v administration, the mean peak plasma concentration of 68.23 ± 6.80 μg/ml and mean peak urine concentration of 52.12 ± 2.06 μg/ml were found at 0.083 and 0.25 h, respectively.

ii. The drug was detectable in all animals upto 24 h in plasma and upto 36 h in urine.

The mean extrapolated zero time concentration during distribution phase (A), elimination (B) and theoretical zero time concentration $\left(C_p^o\right)$ were noted to be 16.61 ± 1.75, 0.82 ± 0.10 and 17.43 ± 1.81 μg/ml, respectively.

iii. A moderate high value for distribution rate constant (α) of 2.010 ± 0.21 h^{-1} and moderate value of distribution half life ($t_{1/2}$ α) of 0.36 ± 0.04 h denote that the drug is expected to be distributed to peripheral tissues at moderate rate.

iv. The elimination half life ($t_{1/2}$ β) of 9.37 ± 0.61 h obtained in the present study denotes that the drug is removed at a slower rate as compared to older groups of NSAIDs. This has led to high value of mean residential time (MRT) of 7.81 ± 0.88 h. The lower rate of elimination is further supported by lower value of total body clearance (Cl_B) of 0.44 ± 0.04 ml/kg/min.

v. A moderate value of volume of distribution of 0.37 ± 0.04 L/kg and a high value of tissue to plasma concentration ratio (T ≈ P) of 10.86 ± 0.55 denote better distribution of the drug in different body fluids and tissues of goats.

TOXICITY STUDY

I) *Haematological parameters* :-

i. Haemoglobin values between 0 day (pre-treatment) and 2, 4, 8 days after drug administration did not show any significant change.

ii. The total leucocyte count (TLC) before pretreatment was noted to be 9860 ± 107.7, which showed significant increase with increasing days (2, 4 and 8 days post treatment). However, no significant difference was noted between pretreatment (0 day) and post treatment of meloxicam on differential leucocyte count (DLC).

*II) **Biochemical Parameters :-***

i. Blood sugar value of 62.14 ± 1.77 mg/dl was noted as pretreatment value, which did not differ significantly from day 2, 4 and 8 post treatment values.

ii. In case of serum cholesterol also, the values noted on pretreatment (0 day) did not differ significantly from those of day 2, 4 and 8 post treatment.

iii. A mean value of 10.70 ± 0.22 mg/dl was noted for blood urea nitrogen (BUN) in healthy goats as pretreatment value, which increased steadily to 12.24 ± 0.21, 13.82 ± 0.23 and 15.24 ± 0.18 mg/dl on day 2, 4 and 8, respectively.

iv. The study of the effect of meloxicam on serum protein revealed that the total protein value noted on day 0 (6.02 ± 0.06 g/dl) increased significantly on day 2 (6.62 ± 0.07 g/dl), which further increased on day 4 (7.02 ± 0.01 g/dl) but reduced

significantly on day 8 (6.82 ± 0.01 g/dl) as compared to day 4 but significantly higher than that of day 0. In case of albumin, the values increased significantly steadily with progressing days of treatment of meloxicam as compared to pretreatment value. However, globulin values differ significantly on day 2 and 4 from pretreatment value but on day 8 it returned to pretreatment level. A:G ratio value did not differ significantly on day 2 from day 0 value but it increase significantly on day 4 and further higher on day 8.

The value of serum glutamate oxaloacetate transaminase (SGOT) noted on day 0 (48.90 ± .07 IU/L) increased significantly on subsequent days of post treatment of meloxicam and the values were noted to be 52.38 ± 0.15 I.U/L, 56.36 ± 0.07 IU/L and 59.42 ± 0.67 IU/L on day 2, 4 and 8 post treatment, respectively.

As noted for SGOT, the values of serum glutamate pyruvate transaminase (SGPT) increased significantly with increasing days of post treatment as compared to pre treatment (0 day) value. The mean values of 7.36 ± 0.50, 10.22 ± 0.58, 15.38 ± 0.86 and 17.36 ± 0.93 IU/L were noted on day 0, 2, 4 and 8, respectively.

From the above noted observations of the present study, it can be summarized that a moderate distribution half life (0.36 ± 0.04h) and longer elimination half life (9.37 ± 0.61 h) denote comparatively faster distribution but slower elimination of meloxicam as compared to many NSAIDs. This longer acting effect combined with the moderate value of Vd_{area} of 0.37 ± 0.04 L/kg and a high value of tissue to plasma concentration ratio (T ≈ P) of 10.86 ± 0.55 may denote greater distribution of meloxicam in peripheral tissues and body fluids, which in turn may be highly beneficial in the treatment of arthritis and other inflammatory conditions.

The toxicological study on meloxicam did not show any significant difference in hemoglobin values post i.m. injection (1 mg/kg daily for 7 days) from pretreatment value. This fact indicates that even in higher doses (recommended therapeutic dose = 0.20 mg/kg), it seems that the drug may not affect the blood forming system and hence may not induce anaemia. However, significant rise in TLC was noted on all post treatment days. The maximum value was noted on day 8 (11290 ± 253.5), which is within the normal value. The value reported for TLC in goat ranged from 8000 to 12000 (Dukes, 1970). It is reported that various physiological factors such as excitement, estrus, digestion etc. may effect the TLC values

(Chauhan, 1995) Hence, apart from the effect of the drug, the above noted factors may also contribute towards the increase in TLC, which is within the physiological limit.

There is no significant effect of drug on blood sugar and serum cholesterol levels as compared to its pretreatment value (Table 7 and 8). Blood urea nitrogen (BUN) increased significantly with increase in days and the maximum value of 15.24 ± 0.18 mg/dl was noted on day 8 as compared to pretreatment value of 10.70 ± 0.22 mg/dl. The normal value reported in goat for BUN varies between 10-20 mg/dl (Kaneko *et al.*, 1997). From the above fact, it can be assumed that the drug may not have much toxicity on kidney but it should be used carefully, when the animals suffer from kidney diseases.

Significant increase in total protein and albumin was noted on all post treatment days as compared to pretreatment day. In case of globulin, the values showed significant increase on day 2 and 4, while the value on day 8 is similar to pretreatment value (Table 10). In case of A/G ratio also the value on day 2 is similar to pretreatment value but increased A/G ratio was noted on day 4 and 8. The values of total protein, albumin, globulin and A/G ratio ranged from 6.4-7.0 gm/dl, 2.7-3.9 gm/dl, 2.7-4.1 gm/dl and 0.63-1.26, respectively (Kaneko

et al., 1997). The increased value noted for the above after meloxicam injection is within the normal physiological value and hence it can be concluded, the drug may not affect much protein metabolism.

There is significant rise in level of SGOT and SGPT values post i.m. injection of meloxicam as compared to pretreatment value. It is noted that SGOT increases 10,000 times in myocardial cells and 500 times in liver cells in myocardial infraction and liver diseases whereas SGPT increases 3000 times more in liver and 400 times more in heart in disease condition than in healthy condition (Vasudevan and Kumari, 1998). The significant increase in SGOT and SGPT level noted in the present study after injection of meloxicam in within the physiological limit. The normal value in goat for SGOT and SGPT varies 43-132 IU/L and 7- 24 IU/L, respectively (Kaneko, 1974). Hence, the drug should be used carefully in cases of liver and muscular disorders.

By taking into account of various advantages and its least toxicity as noted above, meloxicam can be used safely in animals, particularly on goat. However, precaution may be taken before prescribing this drug in animals, which have the history of kidney, liver and heart diseases.

□□□□□

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Appendix

APPENDIX

Calculation of kinetic parameters

Kinetic parameters were calculated from log plasma concentration versus time profile. An example is noted below from the data of goat no 4 obtained after single i.v. injection of meloxicam (0.5 mg/kg). The data showed a biphasic curve and hence, fits well into a two compartment open model.

Sl. No.	Time (h)	x^2	Plasma drug concentration µg/ml	Log Y	XY
1	4	16	0.60	-0.2218	-0.8872
2	6	36	0.54	-0.2676	-1.6056
3	8	64	0.48	-0.3187	-2.5496
4	10	100	0.38	-0.4202	-4.2020
5	12	144	0.32	-0.4948	-5.9376
6	24	576	0.16	0.7958	-19.0992
$\Sigma n=6$	$\Sigma x=64$ $\overline{X} = 10.6666$	$\Sigma x^2 = 936$		$\Sigma \log y =$ -2.5189 $\overline{Y} = -0.4198$	$\Sigma xy =$ -34.2812

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

$= \frac{6(6x - 10.7173) - 64 \times (-2.5189)}{(6 \times 936) - (64 \times 64)}$

$= \frac{-205.6872 + 161.2096}{5616 - 4096}$

$= \frac{-44.4776}{1520} = -0.0292$

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

$= -0.0292 \times -2.303$

$= 0.067\ h^{-1}$

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

where $\bar{y}$ = mean during drug concentration

$\bar{x}$ = mean time

b = slope of line

a = zero time concentration

Therefore,

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

$= \log -0.4198 - (-0.0292 \times 10.6666)$

$= \log - 0.10833528$

Zero time concentration (B) = antilog of log 0.10833528 = 0.78 μg/ml

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

Subtracting the theoretical values from observed values, a series of residual concentrations were obtained and slope of line in natural log (distribution rate constant, α) and the zero time intercept (zero time concentration during distribution phases, A) can be calculated as per method adopted for calculation of B and β. The value of A is 15.91 μg/ml and α is 1.504 h^{-1}. C_p^0, the theoretical plasma concentration at time zero during distribution phase.

C_p^0 = A + B

= 15.91 + 0.78

= 16.69 μg/ml

t ½ α = distribution half life

$$t\ ½\ \alpha = \frac{0.693}{\alpha} = \frac{0.693}{1.504} = 0.46h$$

$$t\ ½\ \beta = \frac{0.693}{\beta} = \frac{0.693}{0.067} = 10..34h$$

AUC, area under curve

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta} = \frac{15.91}{1.504} + \frac{0.78}{0.067} = 22.22 \text{ mg / L.h}$$

AUMC, area under first moment curve

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

$$= \frac{15.91}{(1.504)^2} + \frac{0.78}{(0.067)^2}$$

$$= \frac{15.91}{2.262016} + \frac{0.78}{.004489}$$

$$= 7.03 + 173.76$$

$$= 180.79 \text{ mg/L.h}^2$$

MRT, Mean residential time

$$MRT = \frac{AUMC}{AUC} = \frac{180.79}{22.22} = 8.13h$$

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

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

$$= \frac{15.91 \times 0.067 + 0.78 \times 1.504}{16.69}$$

$$= 0.134 \text{ h}^{-1}$$

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

$$Kel = \frac{\alpha.\beta}{K_{21}} = \frac{1.504 \times 0.067}{0.134} = 0.752\ h^{-1}$$

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

$$K_{12} = a + b - K_{21} - Kel$$

$$= 1.504 + 0.067 - 0.134 - 0.752$$

$$= 0.685\ h^{-1}$$

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

$$Fc = \frac{\beta}{Kel} = \frac{0.067}{0.752} = 0.089$$

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

$$T \approx P = \frac{K_{12}}{K_{21} - \beta} = \frac{0.685}{0.134 - 0.067}$$

$$= \frac{0.685}{0.067} = 10.22$$

Vdc, volume of distribution based on both distribution and elimination.

$$Vdc = \frac{D}{C_p^o} = \frac{0.5}{16.91} = 0.029\ L./kg$$

where, D = Dose (0.5 mg/kg)

Vd_B, the volume of distribution based on elimination

$$Vd_B = \frac{D}{B} = \frac{0.5}{0.78} = 0.64 \text{ L/kg}$$

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

$$Vd_{area} = \frac{D}{AUC.\beta} = \frac{0.5}{22.22 \times 0.067} = \frac{0.5}{1.48} = 0.33 \text{ L/kg}$$

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

$$Vdss = \frac{K_{12} + K_{21}}{K_{21}} \times Vd_c$$

$$= \frac{0.685 + 0.134}{0.134} \times 0.029$$

$= 0.17$ L/kg

Cl_B, the total body clearance

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

$= 0.33 \times 0.067$

$= 0.022$ L/kg/h