Studies on the Pharmacokinetic Interaction between Meloxicam and Quercetin, a CYP2C9 and CYP3A4 Inhibiting Flavonoid, in Rabbits

By

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CERTIFICATE

K.JAYAKANTH has satisfactorily prosecuted the course of research and that the thesis entitled **"Studies on the Pharmacokinetic Interaction between Meloxicam and Quercetin, a CYP2C9 and CYP3A4 Inhibiting Flavonoid, in Rabbits"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any University.

Date: **(Dr.K.ADILAXMAMMA)**

Place: Tirupati

Major Advisor

CERTIFICATE

This is to certify that the thesis entitled **"Studies on the Pharmacokinetic Interaction between Meloxicam and Quercetin, a CYP2C9 and CYP3A4 Inhibiting Flavonoid, in Rabbits"** submitted in partial fulfillment of the requirements for the degree of **"MASTER OF VETERINARY SCIENCE"** of Sri Venkateswara Veterinary University, is a record of the bonafide research work carried out by **K.JAYAKANTH** under my guidance and supervision. The student's Advisory committee has approved the subject of the thesis**.**

No part of the thesis has been submitted for any other degree or diploma. The author of the thesis has duly acknowledged all the assistance and help received during the course of investigation.

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DECLARATION

I**, K.JAYAKANTH,** hereby declare that the thesis entitled "**Studies on the Pharmacokinetic Interaction between Meloxicam and Quercetin, a CYP2C9 and CYP3A4 Inhibiting Flavonoid, in Rabbits**" submitted to Sri Venkateswara Veterinary University for the Degree of "**MASTER OF VETERINARY SCIENCE**" is a result of original research work done by me. It is further declared that the thesis or any part thereof has not been published earlier in any manner.

Date :

Place : Tirupati.

(K.JAYAKANTH)

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ABBREVIATIONS

β	Elimination rate constant
µg	Micro gram(s)
AUC	Area under the plasma concentration – time curve
AUMC	Area under first moment curve
Cl_B	Total body clearance
C_{max}	Maximum (peak) plasma concentration
e	Base of natural logarithm
h	Hour(s)
IV	Intravenous
Kg	Kilogram(s)
K_a	First order absorption rate constant
L	Liter(s)
mg	Milligram(s)
min	Minute(s)
mL	Milliliter(s)
MRT	Mean residence time
p.o.	*Per os* (oral)
t	Time
$t_{1/2\beta}$	Elimination half life
SE	Standard error
T_{max}	Time of maximum concentration in plasma
V_{dss}	Volume of distribution at steady state

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ABSTRACT

The present study was aimed to investigate the pharmacokinetic interaction between meloxicam and quercetin, a CYP2C9 and CYP 3A4 inhibiting flavonoid, in rabbits. Fifteen male rabbits were divided into three groups with five animals in each group and the treatment was given as follows: group I (Control) received meloxicam alone @ 1.5 mg/kg b.wt orally; Group II received meloxicam @ 1.5 mg/kg b.wt orally 30 minutes after the pretreatment with quercetin orally @ 10mg/kg b.wt; group III received meloxicam @ 1.5 mg/kg b.wt orally 30 minutes after the pretreatment with quercetin orally @ 20mg/kg b.wt. Blood was collected by veinipuncture at 0, 0.25, 0.5, 1, 2, 3, 4, 8, 12 and 24 h. Plasma was separated by centrifuging the blood and was subjected to HPLC assay for estimation of meloxicam.

Important pharmacokinetic parameters after non compartmental analysis were $t_{1/2B,}$ 14.52 ± 4.4 h; $Cl_{B,}$ 0.1 ± 0.01 $L.kg^{-1}.h^{-1}$; $AUC_{0-\infty}$,16.02 ± 1.95 $\mu g.h.mL^{-1}$; $V_{dss,}$1.97 ± 0.24 $L.kg^{-1}$: and MRT, 21.89±5.32 h in group I, $t_{1/2B,}$ 11.46 ± 1.62 h; $Cl_{B,}$ 0.1± 0.01 $L.kg^{-1}.h^{-1}$; $AUC_{0-\infty}$,15.40 ± 1.18 $\mu g.h.mL^{-1}$; $V_{dss,}$1.83 ± 0.24 $L.kg^{-1}$: and MRT, 18.1 ± 1.8 h in group II and $t_{1/2B,}$ 9.93 ± 0.59 h; $Cl_{B,}$ 0.07 ± 0.01 $L.kg^{-1}.h^{-1}$; $AUC_{0-\infty}$,21.08 ± 1.36 $\mu g.h.mL^{-1}$; $V_{dss,}$1.14 ± 0.11 $L.kg^{-1}$: and MRT, 15.58 ± 0.64 h in group III.

The results in present study indicate that the quercetin pretreatment at 10 $mg.kg^{-1}$ has no significant effect on the pharmacokinetic profile of meloxicam in rabbits where as the quercetin pretreatment at 20 $mg.kg^{-1}$ has affected the pharmacokinetic parameters.

CONTENTS

INTRODUCTION

Pharmacokinetics refers to the study of the movement of drugs in the body, including the processes of absorption, distribution, localization in tissues, biotransformation and excretion. This information regarding the sojourn of the drugs in the body is essential for formulating doses and dosage regimens.

When two or more drugs are administered concomitantly or one after the other the pharmacokinetics of individual drug is bound to change and is mainly due to drug - drug interactions. These interactions can occur at any stage during absorption, distribution, metabolism and/or excretion. Most often these changes arise due to alteration in the metabolism either due to induction or inhibition of enzymes involved in the metabolism, particularly cytochrome P 450 group of enzymes, which may lead to increase or decrease in the concentration of the drug. Alteration in the pharmacokinetics would result in the altered pharmacological response and the quantification of these interactions which appear as altered concentrations is significant in formulating combination therapy.

Cytochrome P450 enzymes (CYP) represent a large family of proteins involved in the metabolism of drugs and other xenobiotics, as well as some endogenous substrates (Guengerich, 1995). Within this family CYP 2C9 isoform is considered a key enzyme and is second only to CYP 3A4 in terms of total human liver microsomal P450 content. CYP 2C9 is responsible for phase I metabolism of approximately 15% of clinically used drugs (Ali *et al*., 2009). Drug interactions can frequently arise when drugs are co-administered and one drug inhibits the metabolic clearance of the second drug by inhibition of a specific CYP enzyme (Lin and Lu, 1998).

Nonsteriodal anti-inflammatory drugs (NSAIDs) are routinely used in companion animals to provide analgesia. Meloxicam is a novel selective cyclooxygenase-2 (COX-2) inhibiting NSAID that is used extensively as an analgesic agent in humans and, more recently, in some companion animals. Unlike many other NSAIDs, meloxicam has high oral bioavailability and has a long half-life, making it an attractive analgesic for use in veterinary practice. In all species studied, meloxicam undergoes extensive hepatic metabolism into 4 inactive metabolites that are excreted in both urine and feces. (Turner *et al*., 2006). In vitro and in vivo, it is mainly metabolized to a 5'-hydroxymethyl metabolite (Fig. 1) that is further converted to a 5'-carboxy metabolite (Schmid *et al*., 1995). The 5'-hydroxylation of meloxicam is predominantly catalyzed by CYP 2C9 and with a minor contribution by CYP 3A4 (Chesne *et al*., 1998).

Flavonoids represent a group of phytochemicals that are produced by various plants in high quantities (Dixon and Steele, 1999). Quercetin as an extensive class of polyphenolic flavonoid compounds that are almost ubiquitous in plants and plant food sources is the major bioflavonoid in the human diet (NTP Technical Report, 1991). It has been reported the quercetin

can inhibit the P-gp pump efflux transporter (Scambia *et al*., 1994; Shapiro and Ling,1997) and metabolizing enzyme, CYP 3A4 in vitro (Guengerich and Kim, 1990; Miniscalco *et al*., 1992) and CYP 2C9 (Si *et al*.,2009).

With the above considerations in view, the study was taken up with the following objective:

- **To determine plasma levels and pharmacokinetic parameters of meloxicam after single oral bolus administration in rabbits.**
- **To examine the pharmacokinetic interaction between meloxicam and the flavonoid quercetin pretreatment (10 mg.kg^{-1} and 20 mg.kg^{-1}) in rabbits.**

Review of literature

2.1: Flavonoids:

Flavonoids are the most abundant polyphenols present in the human diet in vegetables, fruit and plant derivatives such as wine and tea, and provide much of the flavor and color of these products. In addition, they are the main components of many herbal products. (Zhang *et al.,* 2004).

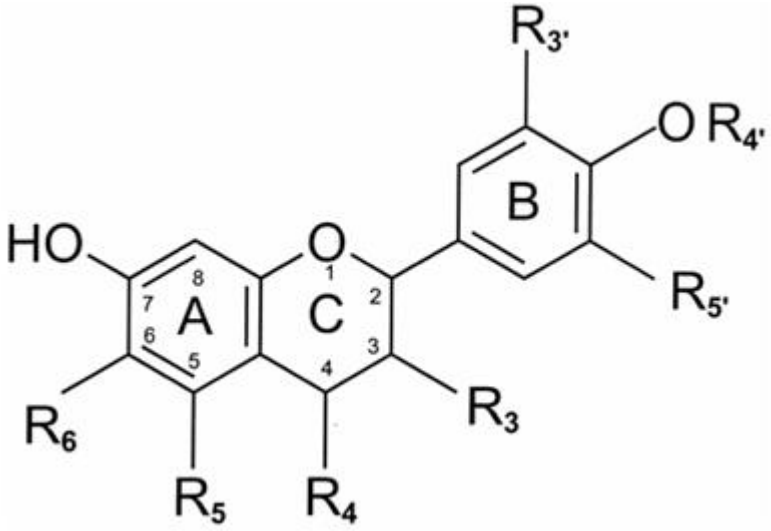


Fig 1: Structure of flavonoids.

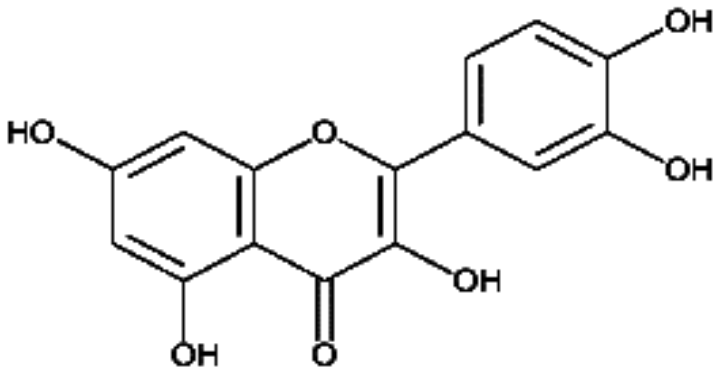
The structural formula of flavonoids contains two or more aromatic rings, each bearing at least one aromatic hydroxyl and connected with a carbon bridge. Based on

different substitutions and the oxidation status of ring C, flavonoids can be classified into several subclasses including chalcones, flavonols, flavones, procyanidins, flavan-3- ols (catechins), flavanones, and isoflavones.(Morris and Zhang., 2004). The main dietary sources of these flavonoids differ widely among subgroups. Flavonols, such as quercetin, kaempferol, and myricetin are mainly present in leafy vegetables, apples, onions, and berries and these are the most abundant flavonoids in foods.

Flavonoids have attracted much attention in recent years because of their beneficial pharmacological activities and for their additional abilities to modulate both CYP3A4 and Pgp (Miniscalco *et al.,*1992*,* Scambia *et al.,* 1994., Critchfield *et al.,*1994. and Bosch and Croot*,*1996.). Grapefruit juice was reported to increase the bioavailability of cyclosporine (Ducharme *et al.,*1993. and Ducharme *et al.,* 1995). Early efforts to identify the active CYP3A4 inhibitor in grapefruit juice focused on flavonoids such as naringenin and quercetin; quercetin was shown to be a potent inhibitor of CYP3A4 in *in vitro* studies (Miniscalco *et al.,*1992 and Guengerich and Kim.,1990).

2.2: Quercetin

Fig2: Structure of Quercetin:



Quercetin is a flavonol. It is the aglycone form of a number of other flavonoid glycosides, such as rutin and quercitrin, found in citrus fruit, buckwheat and onions. Quercetin forms the glycosides quercitrin and rutin together with rhamnose and rutinose, respectively. It is also found in many dietary supplements.

Quercetin is found to be the most active of the flavonoids in studies, and many medicinal plants owe much of their activity to their high Quercetin content. Quercetin has demonstrated significant anti-inflammatory activity because of direct inhibition of several initial processes of inflammation. For example, it inhibits both the manufacture and release of histamine and other allergic/inflammatory mediators. In addition, it exerts potent antioxidant activity and vitamin C-sparing action.

Quercetin is widely distributed mainly as glycosides in components of the daily diet such as onions, apples, berries, tea and red wine (Hertog *et al.,* 1992 and Hertog *et al.,* 1995) as well as in herbal remedy and dietary supplements available worldwide like *Sophora japonica* and *Ginkgo biloba* (Watson and Oliveira., 1999 and Hibatallah *et al.,* 1999). There has been evidence showing that orally administered Quercetin glycosides were significantly broken down to absorbable Quercetin by enterobacteria (Kuhna., 1976 and Victor and Winter., 1987).

2.3: Nonsteroidal anti-inflammatory drugs (NSAIDs)

Nonsteroidal anti-inflammatory drugs suppress one or more components of the inflammatory process and are often indicated as an adjunct to antimicrobial therapy in veterinary practice. In ruminants the use of NSAIDs is associated with the treatment of pain, mastitis, pneumonia and other inflammatory conditions (Pugh, 1991; Ziv, 1992; Deleforge *et al.,* 1994). They are widely used for the treatment of rheumatoid arthritis and osteoarthritis. However, gastrointestinal disturbances are the most frequently reported side effects of all NSAIDs.

2.3.1: Meloxicam

Meloxicam [4-hydroxy-2- methyl-N (5-methyl-2-thiazolyl)-2H-1, 2-benzothiazine- 3-carboxamide-1, 1-dioxide] is a comparatively new NSAID, with a favorable ratio of inhibition of COX-2/COX-1, giving the drug the potential to produce fewer gastric adverse effects. The chemical formula of meloxicam is C_{14} H_{13} N_3 O_4 S_2 and its molecular weight is 351.41. It is sparingly soluble in water.

It is of the oxicam or acidic enolcarboxamide class which imparts analgesic, antipyretic and anti-inflammatory activities (Davies and Skjodt., 1999). It also exhibits less gastric irritation and local tissue irritation in comparison to other NSAIDs. In clinical trials, meloxicam has been found to be effective in the treatment of rheumatoid arthritis, osteoarthritis and degenerative joint diseases (Barner., 1996, Yocum *et al.,* 2000, Schmid *et al*, 1995). In comparative clinical trials, efficacy of meloxicam was comparable to piroxicam and naproxen in patients with rheumatoid arthritis and diclofenac and piroxicam in patients with osteoarthritis. It was also found to be well

tolerated as piroxicam, diclofenac or naproxen, but had improved gastrointestinal tolerability compared to these agents (Noble and Balfour., 1996).

The cytochromes involved in the meloxicam biotransformation were clearly identified. CYP2C9 is the most important one as shown for other oxicams, however, additionally CYP3A4 was found as the minor one and this latter enzyme is known to be involved in the metabolism of a wide variety of compounds (Breimer, 1995).

2.4: General pharmacokinetics

Pharmacokinetics refers to the study of temporal variation of the concentration of drug, once it enters the body and the various processes it undergoes viz., absorption, distribution and elimination. This branch of science is of paramount importance in deciding therapeutic dosage regimen. It is more important in veterinary sciences as it helps in quantifying species difference in the bioavailability and disposition of drugs. Disease states and pharmacokinetics-based drug interaction can alter the disposition of a drug to an extent that modification of usual dosage is required for safety and efficacy of drug.

Thus, pharmacokinetics may be considered as a discipline with general objective of providing the requisite information for judicious selection of drug preparation for use in animal at dosage that will alleviate discomfort and pain, avoid undesirable drug interaction and effectively treat animal diseases.

To understand the complex phenomenon, which a drug undergoes in body, a theoretical approach of compartmentalization of body has been proposed. Lately, non-

compartmental models of pharmacokinetics are gaining more importance over compartmental models.

2.4.2: Compartmental approach

In compartmental model, body is considered to be composed of one or more compartments in which distribution of drug is studied. These compartments are only mathematical models and don't have any physiological or anatomical counterparts. An assumption associated with compartmental models is that drug elimination takes place exclusively from the central compartment and for many drugs the central compartment is blood/plasma.

One-compartment open model

In this model, whole body is considered as a single kinetically homogenous unit. Blood or plasma is the standard reference entity of this unit. Hence, the rate of change of drug concentration in plasma reflects quantitatively the change in drug concentration throughout the body. The kinetics of a drug distributing rapidly is best illustrated by this model.

Plasma concentration versus time plot on a semi-logarithmic scale yields a straight line and drug declines in accordance with equation:

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

Where,

Cp=plasma concentration of drug

t=time elapsed

β=overall elimination rate constant

B=Zero time intercept of the regression line

e=base of natural logarithm

Most of the drugs are absorbed following first order kinetics. The rate of decline in their plasma concentration is monoexponential as noted after intravenous administration. The disposition kinetics of such drugs has been reported to follow one-compartmental open model with first order absorption, where plasma concentration is expressed by the following equation:

$Cp=Be^{-\beta t} – A' e^{-Kat}$ ----------Eq2

Where,

Cp=concentration of drug in plasma at time t

A'=zero time intercept of absorption phase

B=zero time plasma intercept of absorption phase

β =first order rate constant of elimination phase

Ka =first order absorption rate constant

e = base of natural log

Two compartment model

The disposition kinetics of many drugs fit well to two-compartmental open model. In this model, the drug distributes instantaneously into the central compartment (consisting of blood and other readily accessible tissue, like liver and kidney) and then slowly into the peripheral compartment (the remainder body space). The two compartment open model also specifies that after intravenous administration, the elimination of drug takes place exclusively from the central compartment. The distribution-elimination process is assumed to follow the first order kinetics. The rate of decline in plasma concentration following intravenous administration of the drug in this model is biexponential and can be expressed by the following equation:

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

Where, Cp is the concentration of drug in plasma, A and B are zero time intercepts of the initial and terminal phases of the plasma concentration-time curve with concentration expressed as $\mu g.mL^{-1}$, α and β respectively are the distribution and elimination rate constants expressed as min^{-1} or h^{-1}, and e represents base of natural logarithm.

In a two-compartment open model, the initial steep decline in plasma drug concentration is mainly due to the distribution of drug from central to peripheral compartment. Once apparent distribution is established, the rate of decline in plasma drug concentration is determined mainly by irreversible elimination termed as β or elimination phase. The straight portion of the curve has a slope defined as β and an extrapolated zero time intercept B.

Subtraction of extrapolated values from corresponding experimental plasma values on the semi log plot yields a series of residual concentrations.

The residuals yield a second linear segment called the alpha (α) or distribution phase with a slope equal to –α/2.303 and a zero intercept A with units in concentration (Gibaldi and Wintraub, 1971). These experimental constants (A, B, α and β) are used to calculate K_{12}, K21, Kel associated with two-compartment open model.

Some drugs after extra vascular administration (oral or IM) are absorbed in a first order fashion, but their plasma concentration-time profile shows a biexponential decline during the elimination phase. The pharmacokinetics of these drugs is well fitted in a two-compartment open model, where the plasma concentration is described by equation 4 given below:

$Cp=Ae^{-\alpha t} + Be^{-\beta t} - Ae^{-Kat}$----------Eq 4.

Where, Cp is the concentration of drug in plasma at time t. A', A and B are the zero time plasma drug concentration intercepts for the absorption, distribution and elimination phases respectively. Ka, α and β are the respective first order rate constants and e is the base of natural logarithm.

Three compartmental open model

The disposition kinetics of some drugs may follow three - compartment open model, where the plasma concentration after single intravenous administration is described by a triexponential expression (equation 5):

$Cp= Ae^{-xt} + Be^{-bt} + Pe^{-\pi t}$ ----------Eq 5.

The additional constant P and π are estimated by the method of residuals. The rate constant for the drug entry into and out of the third component, i.e. K_{13} ,and K_{31} , respectively can be calculated from the above equation. (Baggot, 1977; Gibaldi and Perrier, 1982).

Baggot (1977) has stated that the overall elimination rate constant (β) is the most important pharmacokinetic parameter as it is a part of the equation used to calculate the elimination half-life ($t_{½\beta}$), volume of distribution by area method ($V_{d(area)}$) , total body clearance (Cl_B) micro constants for multi-compartment models and large interval of multiple dose regimen. Mercer *et al*., (1977) and further indicated that it might be helpful in predicting the withdrawal period for drug residues in tissue.

Gibaldi and wintraub (1971) defined the half-life as measure of the rate of drug elimination, i.e., the time required to reduce drug concentration of plasma or serum to half during the elimination phase of drug concentration-time profile. The half-life is inversely proportional to the overall elimination rate constant.

Apparent volume of distribution ($V_{d(area)}$) is hypothetical volume of body fluids that could be required to dissolve the total amount of drug at the same concentration as that found in blood. The calculated value of apparent volume of distribution ($V_{d(area)}$) is not dependent upon the method used for its calculation, if the drug distributes truly according to one compartment (Riegelmen *et al*., 1968) or multicompartment open model (Notari, 1973). Baggot (1977) stated that the apparent volume of distribution indicates the extent or magnitude of distribution of drug without providing any one clue, whether the drug is uniformly distributed or restricts to certain body tissue. This kinetic

parameter is most helpful in computing the dosage regimen that must be administered to maintain the desired plasma concentration.

Total body clearance (Cl_B) of a drug is important as it gives the sum of clearance from each elimination organ primarily liver and kidney. Unlike β and $t_{½β}$ which are dependent upon $K_{12,}$ K_{21}, and $K_{el,}$ the body clearance changes exactly in proportion to K_{el} (Jusko and Gibaldi, 1972: Rowland *et al*., 1973).

Non compartmental analysis

This method of analysis does not require the assumption of a specific compartmental model for either drug or metabolite. These can be applied to any compartmental model provided the linear pharmacokinetics is ascertained. Of late, there has been a distinct shift from the curve fitting elaboration of compartmental method towards non compartmental method of analysis (Gibaldi and Perrier, 1982). While compartmental model holds good satisfactorily explaining the drug behaviour with good curve-fitting experimental data, non compartmental approach presents a straight forward simpler approach precluding the possible misinterpretation of data due to misspecification (Martinez, 1998).

The basis of non-compartmental analysis is the statistical moment's theory (Yamaoka, *et al*., 1978). In pharmacokinetics, the three moments are described by:

$AUC = \int_0^\infty C\,dt$ $\int$ (zero moment)

$AUMC = \int_0^\infty tC\,dt / \int_0^\infty C\,dt$ (first moment)

$$VRT = \int_0^\infty t^2 C\, dt / \int_0^\infty C\, dt \qquad \text{(second moment)}$$

Where AUC is the area under the plasma concentration-time curve, MRT is the mean residence time and VRT is the variance of residence time. The moments defined above are usually calculated by the numerical integration using the trapezoidal rule. While AUC and MRT are invariably reported, the third moment is rarely used.

In non-compartmental analysis, AUC is the basic parameter which serves as the basis for comparison across dosage regimens, formulations, etc., for relating to a pharmacodynamic variable or to identify factors that may affect pharmacokinetics such as disease, food, gender, age, breed, physiological status etc.

Mean residence time (MRT) is an indicator of temporal characteristics of a dose and remains constant, regardless of dose, in the absence of saturable processes. MRT as a parameter helps to deduce the mean absorption time, mean dissolution time etc. Other important parameters such as clearance and volume of distribution can be derived from AUC/MRT which can ultimately give a complete description of a drug's pharmacokinetics.

Hodek, *et al., (*2002), studied the interaction of flavonoids with cytochromes P450 and found these interactions expressed in at least three ways:

(i) Flavonoids induce biosynthesis of several CYPs.

(ii) Enzymatic activities of CYPs are modulated (inhibited or stimulated) by these compounds.

(iii) Flavonoids are metabolized by several CYPs.

Kang, *et al., (*2009), have described that many of the natural compounds from medicinal plants have demonstrated capacity to enhance the bioavailability of co-administered drugs by inhibiting efflux pumps or oxidative metabolism, and perturbing the intestinal brush border membrane. Quercetin one among them, has shown to increase bioavailability, blood levels and efficacy of a number of drugs including diltiazem, digoxin and others. They suggested that the increased AUCs and *C*max of diltiazem by pretreatment of quercetin might have resulted from the inhibition of the P-gp efflux pump and the metabolizing enzyme, CYP3A4 in the intestinal mucosa.

It was concluded by Bailey, *et al.,* (1998), that a single glass of grapefruit juice has the potential to augment the oral bioavailability and to enhance the beneficial or adverse effects of broad range of medications. Most drugs investigated for an interaction with grapefruit juice are substrates for CYP3A4. Grapefruit juice acts by inhibiting presystemic drug metabolism by CYP3A isoforms in the small bowel. It was also studied that the grapefruit juice reduced small bowel CYP3A4 content contingent upon pretreatment levels. Individuals with highest small bowel CYP3A4 levels before the grape fruit juice has the largest reduction in CYP3A4 content.

Studies on effects of Flavonoids on cytochromes:

Kimura, *et al.,* (2010) conducted a study to systematically evaluate the inhibitory effects of 60 polyphenols and related compounds on human cytochrome P450 (CYP) 3A4 and CYP2C9 activity by in vitro assay. In addition, the kinetics of potent CYP

inhibitors was investigated by Line weaver–Burk plot analysis. Three coumarins and 12 flavonoids significantly suppressed CYP3A4 or CYP2C9 activities. Quercetin is one among the 12 flavonoids that has significant inhibitory effect on the CYP3A4 or CYP2C9 activities.

Obach., (2000), examined the Commercially available St. John's wort (*Hypericum perforatum*) extracts, preparations that are used in the treatment of depression, for the potential to inhibit human cytochrome P450 (CYP) enzyme activities, specifically CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. He also demonstrated inhibition of each of these five enzymes, CYP2D6, CYP2C9, and CYP3A4 being more sensitive than CYP1A2 and CYP2C19 by crude extracts of the plant. Extracts were fractionated by HPLC, and each of the fractions was tested for inhibition of these five CYPs to identify individual constituents with inhibitory activity. Several fractions were shown to possess inhibitory activity and quercetin was one among them.

Rashid, *et al.*, (1993) concluded that the inhibition of nifedipine metabolism by grapefruit juice is not due to quercetin but some other component, possibly naringenin. Alternatively, they postulated that vasoactive substances in grapefruit juice such as urodiolenone may have increased liver blood flow thereby reducing first-pass extraction. In addition nootkatone the immediate precursor of urodiolenone may compete with nifedipine for CYP3A4 metabolism thereby enhancing the AUC of nifedipine.

Various Pharmacokinetic studies conducted on Meloxicam in different species:

Rabbits:

Turner, *et al.,* (2006), evaluated the pharmacokinetic profile of Meloxicam (0.3 and 1.5 mg/kg) given as single and repeated (once daily for 5 d) oral doses to female rabbits (n = 5/group) to define the optimal dose and dosing interval for clinical use. They also arrived at the plasma half life, peak plasma concentration and area under curve for the same. Peak plasma concentration was 0.3 ± .09 µg/ml, Time to peak plasma concentration was 6.8 ± 0.5h, Elimination half-life was 8.39 ± 1.17h, area under concentration – time curve was found to be 5.20±1.29 $\mu g.h.mL^{-1}$.

Dogs:

Busch, *et al*., (1998) determined the disposition kinetics of Meloxicam in beagle dogs following intravenous and subcutaneous administration at the dose rate of 0.2 $mg.Kg^{-1}$. Mean values of various pharmacokinetic parameters after intravenous administration were t½β 24.0h, AUC 21.5$mg.h.L^{-1}$, MRT 34.8h and Cl_B 0.01 $L.kg^{-1}.h^{-1}$ respective values after subcutaneous administration were $t_{½\beta}$ 23.7h, AUC 24.1$mg.h.L^{-1}$, MRT 35.0h and Cl_B 0.08 $L.kg^{-1}.h^{-1}$.

Montoya, *et al*., (2004) studied the pharmacokinetics of Meloxicam after oral dosing in healthy dogs at 0.2 $mg.Kg^{-1}$ dose rate. The mean values of various pharmacokinetic parameters recoded were t½β 12.13 ± 2.15h, AUC 14.6 ± 1.3$mg.h.L^{-1}$ and Cl/F 10 ± 1.4$mL.kg^{-1}.h^{-1}$.

Sheep and goats:

Shukla, *et al*., (2007), studied the comparative pharmacokinetics of meloxicam in sheep and goat after a single intravenous dose of 0.5 $mg.kg^{-1}$. The mean ± SE values of

$t_{1/2B,}$ MRT, AUC and Cl_B in sheep were 10.85±1.21 h, 15.13±1.67 h, 31.88±2.97 µg.h.L^{-1} and 0.016±0.002 L.h^{-1}.kg^{-1} respectively, Whereas the respective values in goats were 6.73±0.58 h, 9.37±0.83 h, 19,.23±2.23 µg.h.L^{-1} and 0.03±0.01 L.h^{-1}.kg^{-1}.

Minipigs:

The pharmacokinetics of meloxicam was elucidated by Busch, *et al*., (1998) following intravenous administration in minipigs. The pharmacokinetic parameters were observed to be as follow. $t_{½β,}$ 121h; AUC, 243 mg.h.L^{-1}; Mean residence time (MRT),67.4h; $V_{dss,}$ 2.97L.Kg^{-1} and Cl_B, 0.043 mL.kg^{-1}.h^{-1}.

Rats :

Busch, *et al*., (1998) studied the pharmacokinetics of Meloxicam after intravenous dosing in rats at 1.0 mg.Kg^{-1} dose rate. The mean values of various pharmacokinetic parameters recoded were $t_{½β}$:36.8h, AUC :217. µg.h.L^{-1}, MRT: 52.6h and Cl_B 0.005 mL.kg^{-1}.h^{-1}.

Studies on effect of quercetin on pharmacokinetics of other drugs:

Pharmacokinetic parameters of diltiazem were determined by Choi and Li (2005) in rabbits after oral administration of diltiazem either co-administered or pretreated with quercetin (2, 10, 20 mg/kg). It was observed that the absolute and relative bioavailability values of diltiazem were significantly higher in rabbits co-administered with quercetin compared to the control group.

Cermak, *et al*. (2009) investigated the effect of quercetin on the bioavailability of simvastatin after administering simvastatin orally to pigs, co-administered with quercetin

and without quercetin. Results showed that the absolute bioavailability of simvastatin in the pigs treated concomitantly with quercetin was not significantly higher than the control.

Si, *et al.,* (2009) described an in-vitro investigation of inhibition of CYP 2C9 by a series of flavonoids including morin and quercetin. Among the flavonoids tested, 6-hydroxyflavone was found to be a non-competitive inhibitor of CYP2C9, where as other flavonoids were competitive inhibitors. The results of the study suggested that flavonoids can participate in interactions with drugs that act as substrates for CYP2C9.

Sudhir, *et al*. (2008) investigated and found an increase in the bioavailability of pioglitazone in rats pretreated with quercetin, and this was ascribed to inhibition of CYP3A in rat liver.

Hsiu, *et al.,* (2002), made a study aimed to measure the effect of quercetin on the absorption and disposition of cyclosporine, an immunosuppressant with a narrow therapeutic window, is a substrate for both CYP3A4 and P-glycoprotein (Pgp) in pigs and rats. It was reported that the co administration of quercetin significantly decreased cyclosporine oral bioavailability and suggested that concurrent use of quercetin or quercetin-containing dietary supplement or herbs with cyclosporine or other medications whose absorption and metabolism are mediated by Pgp and/or CYP3A4 should require close monitoring.

Chesne, *et al.,*(1998) made a study to identify the human hepatic cytochromes involved in meloxicam metabolism by three different *in vitro* models, i.e. liver

microsomes, primary hepatocytes and heterologously expressed cytochromes and found that metabolism of meloxicam in human liver involves cytochromes P4502C9 and 3A4.

Dasandi, *et al.,* (2002), developed a simple and rapid HPLC assay method for the estimation of meloxicam in plasma. This method totally eliminated the solvent extraction procedure. The plasma proteins were precipitated using perchloric acid (70%) and acetonitrile mixture n: l (v/v*)* and the supernatant was directly injected to the HPLC system.

MATERIALS AND METHODS

The experiment was designed to study the effect of pretreatment of quercetin (@ 10 & 20 mg/kg body wt) on pharmacokinetics of meloxicam in adult male rabbits. The study was conducted in the Department of Pharmacology and Toxicology, N.T.R.C.V.Sc., Gannavaram.

3.1 EXPERIMENTAL ANIMALS

The study was conducted on 18 healthy male adult rabbits aging 3months and weighing between 2.0 to 2.5 kg. The rabbits were procured from Department of Animal Genetics and Breeding, College of Veterinary Science, Rajendra nagar. The rabbits were randomly divided into three groups of six animals each and were maintained in well ventilated small animal house at N.T.R.C.V.Sc., Gannavaram. Standard feed and clean water were provided *ad libitum.*

3.2 DRUGS AND CHEMICALS

Meloxicam: Meloxicam was used as Melonex® from (M/s Intas Pharmaceuticals Ltd, Ahmadabad) 5g bolus, each bolus contains 100mg of meloxicam.

Quercetin: Quercetin dihydrate was obtained from M/s SISCO Research Laboratories Private Ltd., Mumbai.

Heparin: Heparin was obtained from M/s Loba Chemie.

Pure technical standard of meloxicam employed in HPLC study was generous gift from M/s PVS Laboratories Pvt. Ltd., Vijayawada.

Chemicals for HPLC analysis: HPLC grade acetonitrile, acetic acid, triethylamine and methanol were procured from M/s Merck. Water for HPLC analysis was prepared by filtering triple distilled water through 0.2µm filter in Millipore water purification system. All other chemicals used in the study were of analytical grade.

3.3 PREPARATION OF DRUG SOLUTIONS:

Meloxicam: Melonex® bolus was dissolved in 10 ml of distilled water by triturating with a mortar and pestle to obtain 10mg/ml meloxicam suspension.

Quercetin: 100 mg of quercetin was added to 1ml of Tween-80 and to this 9 ml of distilled water was added while triturating. The suspension thus made contained quercetin @ 10mg/ml.

3.4 PLAN OF WORK:

Adult male rabbits were divided into three groups comprising of six animals in each group. Group I served as control and received the NSAID, meloxicam alone. The remaining two groups received the flavonoid quercetin pretreatment followed by meloxicam, after 30 minutes.

The doses and schedule of the above drugs in different groups was as detailed below:

Table 1: Experimental design

GROUP	DRUG PROTOCOL
Group I	Meloxicam @ 1.5mg/Kg body weight orally

(Meloxicam control)	
Group II (Meloxicam with quercetin pretreatment)	Quercetin @ 10mg/Kg body weight orally, followed by meloxicam @ 1.5mg/kg body weight orally after 30 minutes.
Group III (Meloxicam with quercetin pretreatment)	Quercetin @ 20mg/Kg body weight orally, followed by meloxicam @ 1.5mg/kg body weight orally after 30 minutes.

3.5 ADMINISTRATION OF DRUGS:

3.5.1 QUERCETIN PRETREATMENT:

Rabbits were deprived of feed for 12 hours prior to the experimentation. Water was however made available freely. Body weights of the animals were taken just before the administration. Quercetin was given orally at the dose rate of 10mg/kg body weight and 20mg/kg body weight to group II and group III respectively.

3.5.2 MELOXICAM:

Meloxicam was administered orally at the dose rate of 1.5 mg/kg body weight in all the three groups but it was administered 30 minutes after the quercetin pretreatment in groups II and III.

3.6 COLLECTION OF BLOOD SAMPLES:

Blood was collected from the ear vein of the animal by vein puncture in heparinized vials at 0, 0.25, 0.5, 1, 2, 3, 4, 8, 12 and 24h after the oral administration of meloxicam. Plasma was separated by centrifugation at 3000 rpm for 10 minutes and the plasma samples were stored at -20ºC till analyzed for meloxicam.

3.7 ASSAY PROCEDURE:

The Concentration of meloxicam in plasma was determined by using reverse phase-High Performance Liquid Chromatography (HPLC) as described by Baert and De Backer (2003) with certain modifications. Analysis was carried out in the quality control laboratory of M/s International Health Care Ltd., Vijayawada.

3.8 PREPARATION OF STOCK SOLUTIONS FOR STANDARDS:

A stock solution of 1mg.mL^{-1} of meloxicam was prepared. 25 mg of 100% pure meloxicam was dissolved in a mixture containing HPLC grade acetonitrile & acetic acid in 1:1 ratio.

3.9 PREPARATION OF WORKING STANDARDS:

100µL of meloxicam stock solution was added to 900 µL of plasma obtained from untreated rabbits, thus giving a solution of 100 µg.mL^{-1}. From this 100 µL was added to 900 µL of drug free rabbit plasma to obtain a solution of 10µg.mL^{-1}. From this, serial

double dilutions were made using plasma from untreated rabbits to yield plasma standards of 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078 and 0.039µg.mL^{-1}.

3.10 EXTRACTION PROCEDURE FOR PLASMA:

To 0.5mL of plasma sample, 0.5mL of acetonitrile was added in the ratio of 1:1 after vortex mixing at high speed for 1 minute. The tubes were subjected to centrifugation for 10 minutes at 10000rpm. 0.5mL of clear supernatant thus obtained was transferred to a tube and 0.5 ml of HPLC-grade water was added. The aliquot was filtered through a 0.22 µm nylon membrane syringe filter and then loaded into the HPLC sampling vial.

3.11 HPLC ASSAY:

The plasma concentration of meloxicam was determined by using reverse phase-High Performance Liquid Chromatography (HPLC) method described by Baert and De Backer (2003) with certain modifications. Separation of meloxicam was achieved by using C_{18} reversed-phase column (Phenomenex, particle size 5µm, 4.6mm x 250mm) as the stationary phase. The mobile phase consisted of a mixture of acetonitrile and a buffer prepared, in the ratio 4:6 (v/v). The flow rate was adjusted to 1mL.min^{-1} with the run time of 6 min. Chromatography was performed at 35ºC with detection at 355 nm using PDA detector. Meloxicam was quantified from their respective peak heights / areas and the concentration in the plasma samples was determined by references to calibration curves based on the analysis of blank plasma samples spiked with meloxicam and analyzed as for the test samples.

3.11.1 PREPARATION OF THE BUFFER FOR MOBILE PHASE:

0.05molar sodium acetate solution was prepared using HPLC-grade water and 0.5 mL of acetic acid (HPLC grade). Triethylamine is added drop wise to adjust pH of the solution to 6.

3.12 STANDARD CALIBRATION CURVE:

Standard calibration curve for meloxicam was linear from .156µg.mL^{-1} to 2.5µg.mL^{-1} with regression coefficient of 0.999. Limit of detection was 0.078µg.mL^{-1} with the achieved recovery.

- **LOD: 0.078 µg.mL^{-1}.**
- **LOQ: 0.156 µg.mL^{-1}**
- **RECOVERY: 93%.**

Table 2: Height counts obtained by HPLC assay of various plasma standards of meloxicam.

Concentration (µg.mL^{-1})	Height counts(10^3)

0.156	0.255
0.312	0.513
0.625	1.026
1.25	2.102
2.5	4.409

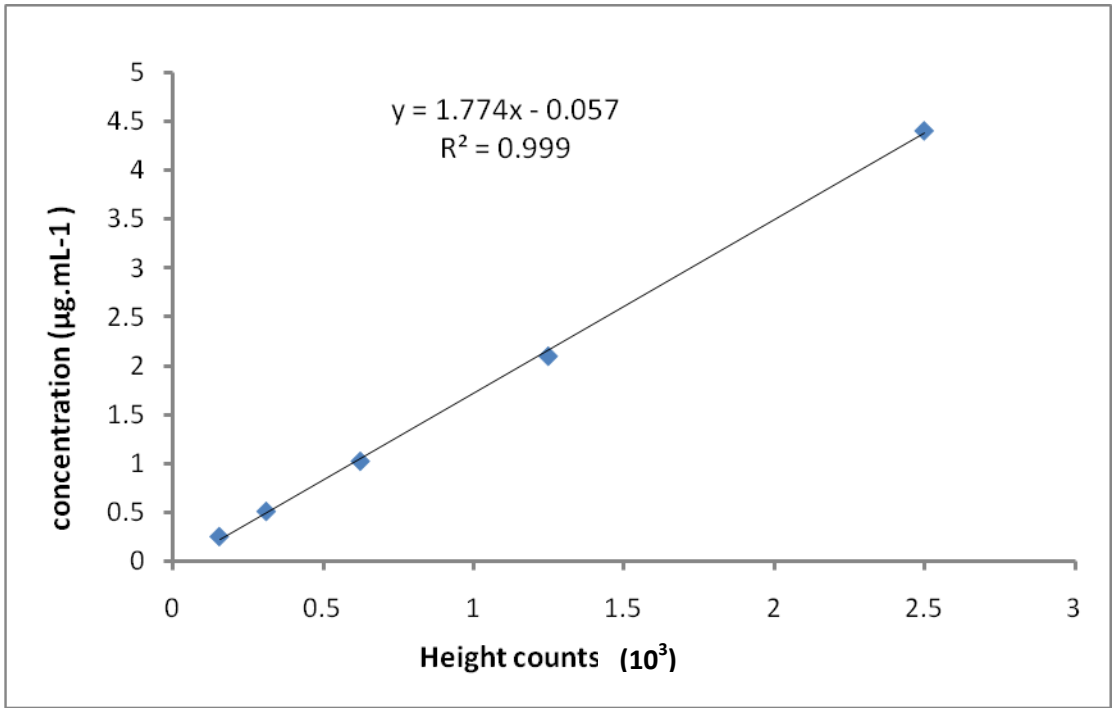


Fig 1: Standard calibration curve of Meloxicam in rabbit plasma

3.13 PHARMACOKINETIC ANALYSIS:

3.13.1 Non compartmental analysis

Plasma concentration versus time data of meloxicam obtained for three groups in the present study were utilized for calculating various pharmacokinetic parameters in rabbits with an interactive programme for personal computer software, (PK Solver, version. 2.0, 2010 by Zhang Yang).

a) $t_{½Ka,}$ absorption half-life; $t_{½\beta}$, elimination half-life were determined according to the following equation.

   (i) $t_{½Ka,} = 0.693/k_a$

   (ii) $t_{½\beta} = 0.693/\beta$

b) $AUC_{0-\infty}$ and $AUMC_{0-\infty}$, the total area under the plasma drug/metabolite concentration – time curve was calculated.

c) V_{dss} , the volume of distribution of drug at steady state is calculated according to

   $V_{dss}/F = Dose \times AUMC / (AUC)^2$

   Where, F is undetermined bioavailability

d) MRT, the mean residence time is determined according to the following equation

   MRT = AUMC / AUC

e) $Cl_{B,}$ the total body clearance is obtained by following equation.

   $Cl_B/F = Dose / AUC$

f) C_{max}, maximum plasma drug concentration and t_{max}, time to reach C_{max} are taken directly from observed values.

3.14 STATISTICAL ANALYSIS:

All data were expressed as mean ± SE. Differences in pharmacokinetic data between meloxicam alone and quercetin pretreated groups were analyzed for statistical significance using unpaired student's 't' test with Welch's correction using 'Instat' software. The level of significance was $p < 0.05$.

RESULTS

The current study was taken up with the aim of studying the pharmacokinetic interactions between meloxicam and quercetin pretreatment in adult rabbits. Both, meloxicam and quercetin were administered as single oral bolus. Meloxicam was administered at the rate of 1.5 $mg.kg^{-1}$ while quercetin was administered at two dose levels of 10 and 20 $mg.kg^{-1}$, 30 minutes before the administration of meloxicam.

Plasma levels and pharmacokinetics of meloxicam following single oral administration of meloxicam (1.5 $mg.kg^{-1}$):

The plasma concentrations of meloxicam as function of time in different animals after single oral administration are presented in table 3. Plasma concentrations (Mean ± SE) of meloxicam are presented graphically in Fig 3.

A mean peak plasma concentration of 0.70 ± 0.13 $\mu g.mL^{-1}$ was achieved at 4h and it gradually declined to 0.22 ± 0.02 $\mu g.mL^{-1}$ at 24h. When data was analyzed by non compartmental method, the elimination rate constant (β) ranged from 0.02 to 0.1 h^{-1} with the mean value of 0.07 **±** $0.01h^{-1}$. The values of C_{max} ranged from 0.67 to 1.03 $\mu g.mL^{-1}$ with a mean value of 0.84 ± 0.06 $\mu g.mL^{-1}$. Peak plasma concentrations were observed in the time range of 4-8 h with the mean t_{max} value as 6.40 **±** 0.90 h.

Non compartmental analysis of plasma drug concentrations yielded the mean values for total body clearance (Cl_β), area under plasma drug concentration curve ($AUC_{0-\infty}$), area under the first moment curve ($AUMC_{0-\infty}$), mean residence time (MRT) and volume of

distribution at steady state(V_{dss}) as **0.**10 **±** **0.**01 $L.kg^{-1}.h^{-1}$, 16.02 **±** 1.95 $\mu g.h.mL^{-1}$, 394.86 **±** 147.73 $\mu g.h^2.mL^{-1}$, 21.89 **±** 5.32h and 1.97 **± 0.**24 $L.kg^{-1}$ respectively.

Table 3: Plasma concentrations ($\mu g.mL^{-1}$) of meloxicam after single oral administration of meloxicam (1.5 $mg.kg^{-1}$) in rabbits. (n=5).

	ANIMAL NUMBER					
Time (h)	**M**	**N**	**O**	**P**	**Q**	**Mean ± SE**
0.25	0.16	0.24	0.18	0.22	0.10	0.18 ± 0.02
0.5	0.16	0.27	0.24	0.34	0.33	0.27 ± 0.03
1	0.18	0.39	0.34	0.40	0.41	0.34 ± 0.04
2	0.22	0.41	0.61	0.69	0.44	0.47 ± 0.08
3	0.32	0.46	0.66	0.83	0.52	0.56 ± 0.09
4	0.39	0.47	0.99	1.03	0.63	0.70 ± 0.13
8	0.76	0.67	0.38	0.56	0.78	0.63 ± 0.07
12	0.34	0.60	0.35	0.43	0.72	0.49 ± 0.07
24	0.14	0.23	0.27	0.25	0.21	0.22 ± 0.02

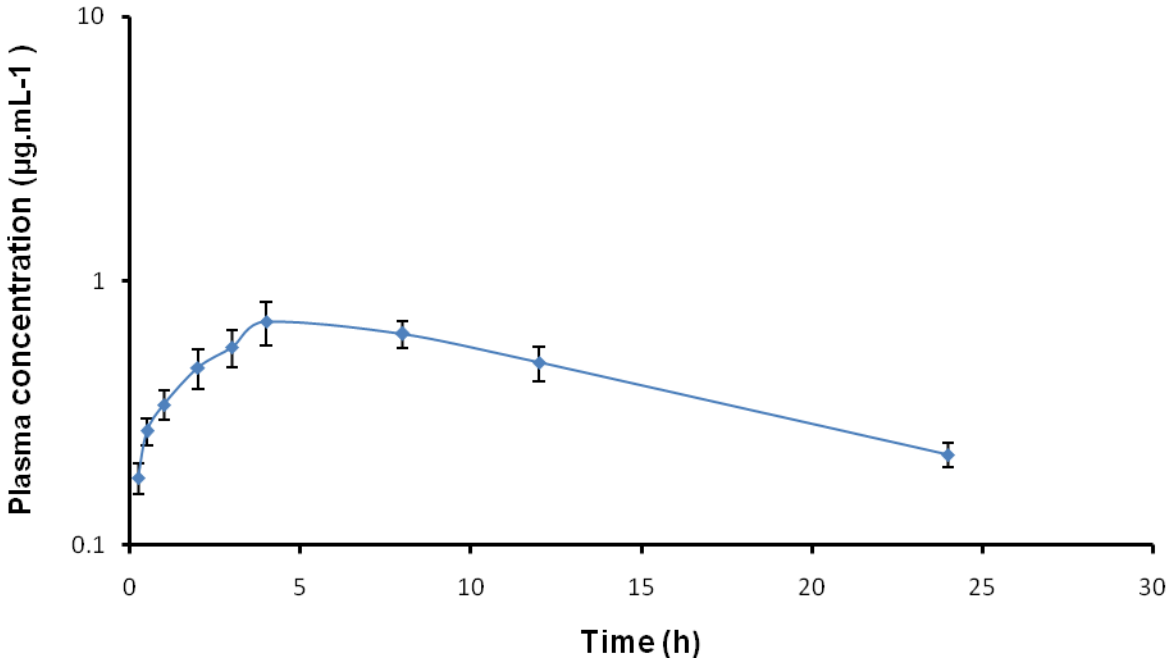


Figure 3: Semilogarithmic plot of meloxicam concentrations in plasma versus time after single oral bolus administration of meloxicam (1.5 mg.kg^{-1}) in control (Blue plot) adult male rabbits. Each point represents mean ± SE of five rabbits.

Table 4: Pharmacokinetic parameters of meloxicam after single oral administration of meloxicam (1.5mg.kg^{-1}) in rabbits. (n=5).

Parameter	Unit	A	B	C	D	E	Mean ± SE
β	h^{-1}	0.10	0.07	0.02	0.05	0.09	0.07 ± 0.01
t½β	h	7.06	10.00	33.11	14.45	7.98	14.52 ± 4.4
AUC_{0-t}	$\mu g.h.mL^{-1}$	8.34	11.36	10.04	11.80	13.14	10.94 ± 0.75
$AUC_{0-\infty}$	$\mu g.h.mL^{-1}$	9.76	14.69	22.98	17.12	15.56	16.02 ± 1.95
$AUC_{0-t}/AUC_{t-\infty}$	Per cent	14.60	22.68	56.32	31.06	15.54	28.0 ± 7.00
$AUMC_{0-t}$	$\mu g.h^2.mL^{-1}$	82.35	119.61	97.90	110.67	133.67	108.84 ± 8.06
$AUMC_{0-\infty}$	$\mu g.h^2.mL^{-1}$	131.07	247.64	1026.90	349.15	219.53	394.86 ± 147.73
MRT	h	13.42	16.85	44.68	20.39	14.11	21.89 ± 5.32
V_{dss}	$L.kg^{-1}$	2.06	1.72	2.92	1.79	1.36	1.97 ± 0.24
Cl_{β}	$L.kg^{-1}.h^{-1}$	0.15	0.10	0.07	0.09	0.10	0.10 ± 0.01
C_{max}	$\mu g.mL^{-1}$	0.76	0.67	0.99	1.03	0.78	0.84 ± 0.06
t_{max}	h	8	8	4	4	8	6.40 ± 0.90

Effect of Quercetin (10 mg.kg^{-1}) pretreatment on plasma levels and pharmacokinetics of meloxicam in adult rabbits:

The plasma concentrations of meloxicam, after single oral administration of meloxicam in quercetin (10 mg.kg^{-1}) pre-treated rabbits were shown in table 5. Mean plasma concentrations of meloxicam in quercetin (10 mg.kg^{-1}) pre-treated group were compared with those of control group in table 7. The mean time taken to achieve peak plasma concentration (t_{max}) was 4.2 ± 0.93 h as compared to 6.40 **±** 0.90 h in control group. The C_{max} was 0.83 ± 0.06 µg.mL^{-1} as against 0.84 **±** 0.06 µg.mL^{-1} in control group and they do not differ significantly.

Plasma concentration versus time curve of meloxicam in quercetin (10 mg.kg^{-1}) pretreated rabbits is graphically depicted in figure 4 , where as figure 5, compares it with the curve obtained for control group where meloxicam is administered alone. There is an apparent increase in the retention of meloxicam in pretreated group but the increase seems to be not very significant.

Pharmacokinetic parameters of meloxicam, after single oral administration of meloxicam in quercetin (10 mg.kg^{-1}) pre-treated rabbits were displayed in table 6. In table 8, pharmacokinetic parameters of meloxicam in quercetin (10 mg.kg^{-1}) pre- treated group were compared with those of control group. V_{dss} value is 1.83 ± 0.24 L.kg^{-1} compared to 1.97 ± 0.24 L.kg^{-1} in control group. The $AUMC_{0-t}$ value is 114.90 ± 14.43 µg.h^2.mL^{-1} which is higher than the control group value of 108.84 ± 8.06 µg.h^2.mL^{-1}, but the

increase is not significant. The $AUMC_{0-\infty}$ and MRT values were 275.23 ± 26.83 $\mu g.h^2.mL^{-1}$ and 18.10 ± 1.80 h respectively.

Table 5: Plasma concentrations ($\mu g.mL^{-1}$) of meloxicam after single oral administration of meloxicam (1.5 $mg.kg^{-1}$) in Quercetin (10 $mg.kg^{-1}$) pretreated rabbits (n=5).

	ANIMAL NUMBER					
Time (h)	**M**	**N**	**O**	**P**	**Q**	**Mean ± SE**
0.25	0.11	0.14	0.13	0.12	0.43	0.19 **±** 0.06
0.5	0.18	0.19	0.14	0.13	0.56	0.24 **±** 0.08
1	0.28	0.39	0.23	0.17	0.77	0.37 **±** 0.11
2	0.52	0.49	0.46	0.32	0.87	0.53 **±** 0.09
3	0.80	0.56	0.53	0.74	0.56	0.64 **±** 0.05
4	0.85	0.65	0.84	0.72	0.48	0.71 **±** 0.07
8	1.05	0.62	0.82	0.56	0.39	0.69 **±** 0.11
12	0.86	0.55	0.56	0.28	0.30	0.51 **±** 0.11
24	0.26	0.23	0.27	0.19	0.22	0.23 **±** 0.01

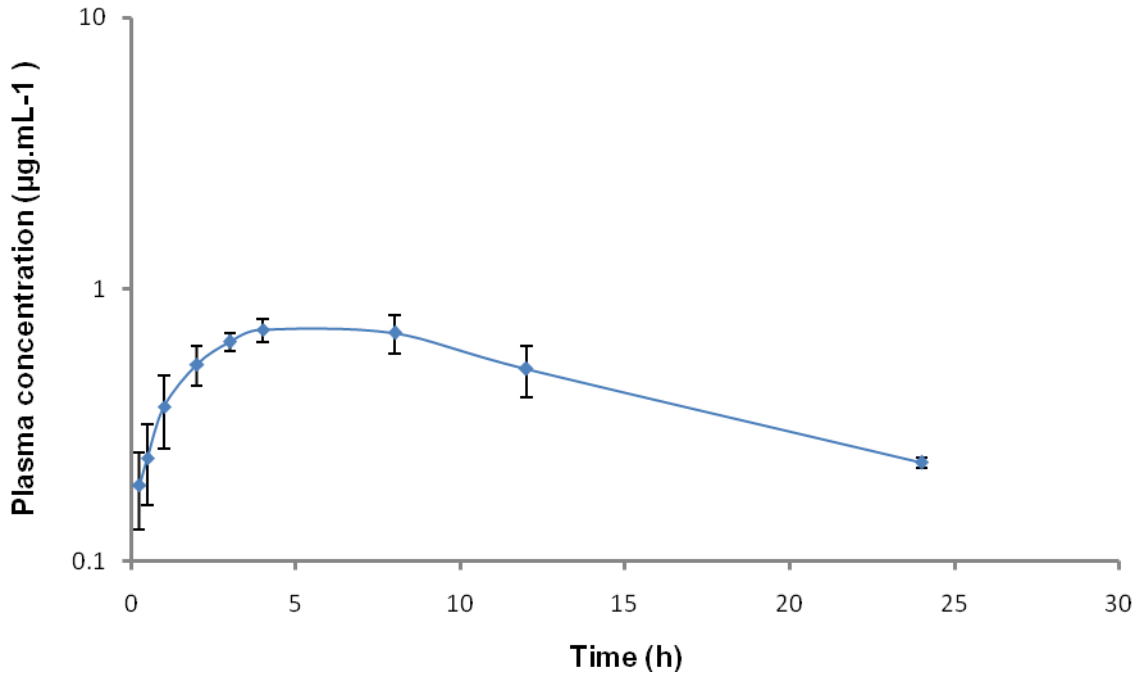


Figure 4: Semilogarithmic plot of meloxicam concentrations in plasma versus time after single oral bolus administration of meloxicam (1.5 mg.kg^{-1}) in quercetin pretreated (10 mg.kg^{-1}) (Blue plot) adult male rabbits. Each point represents mean ± SE of five rabbits.

Table 6: Pharmacokinetic parameters of meloxicam after single oral administration of meloxicam (1.5mg.kg^{-1}) in quercetin (10 mg.kg^{-1}) pretreated rabbits.

Parameter	Unit	ANIMAL NUMBER					Mean ± SE
		M	N	O	P	Q	
β	h^{-1}	0.09	0.06	0.07	0.07	0.04	0.065 ± 0.008
t½β	h	7.69	10.85	10.41	10.14	18.21	11.46 ± 1.62
AUC_{0-t}	$\mu g.h.mL^{-1}$	16.39	11.27	12.73	8.69	8.78	11.57 ± 1.30
$AUC_{0-\infty}$	$\mu g.h.mL^{-1}$	19.27	14.87	16.83	11.47	14.55	15.40 ± 1.18
$AUC_{0-t}/AUC_{t-\infty}$	Per cent	14.97	24.20	24.36	24.23	39.70	25.49 ±3.64
$AUMC_{0-t}$	$\mu g.h^2.mL^{-1}$	165.79	114.66	130.17	82.39	81.50	114.90 ± 14.43
$AUMC_{0-\infty}$	$\mu g.h^2.mL^{-1}$	267.06	257.34	290.13	189.71	371.94	275.23 ± 26.83
MRT	h	13.86	17.31	17.24	16.54	25.56	18.10 ± 1.80
V_{dss}	$L.kg^{-1}$	1.08	1.75	1.54	2.16	2.63	1.83 ± 0.24
Cl_{β}	$L.kg^{-1}.h^{-1}$	0.08	0.10	0.09	0.13	0.10	0.10 ± 0.01
C_{max}	$\mu g.mL^{-1}$	1.05	0.65	0.84	0.74	0.87	0.83 ± 0.06
t_{max}	h	8	4	4	3	2	4.2 ± 0.93

Table 7: Effect of Quercetin (10 mg.kg^{-1}) pretreatment on concentrations (µg.mL^{-1}) of meloxicam in plasma (Mean ± SE) after single oral administration of meloxicam at 1.5 mg.kg^{-1} (n=5).

Time(h)	Meloxicam alone	Quercetin (10 mg.kg^{-1})+ Meloxicam
0.25	0.18 ± 0.02	0.19 ± 0.06
0.5	0.27 ± 0.03	0.24 ± 0.08
1	0.34 ± 0.04	0.37 ± 0.11
2	0.47 ± 0.08	0.53 ± 0.09
3	0.56 ± 0.09	0.64 ± 0.05
4	0.70 ± 0.13	0.71 ± 0.07
8	0.63 ± 0.07	0.69 ± 0.11
12	0.49 ± 0.08	0.51 ± 0.11
24	0.22 ± 0.02	0.23 ± 0.01

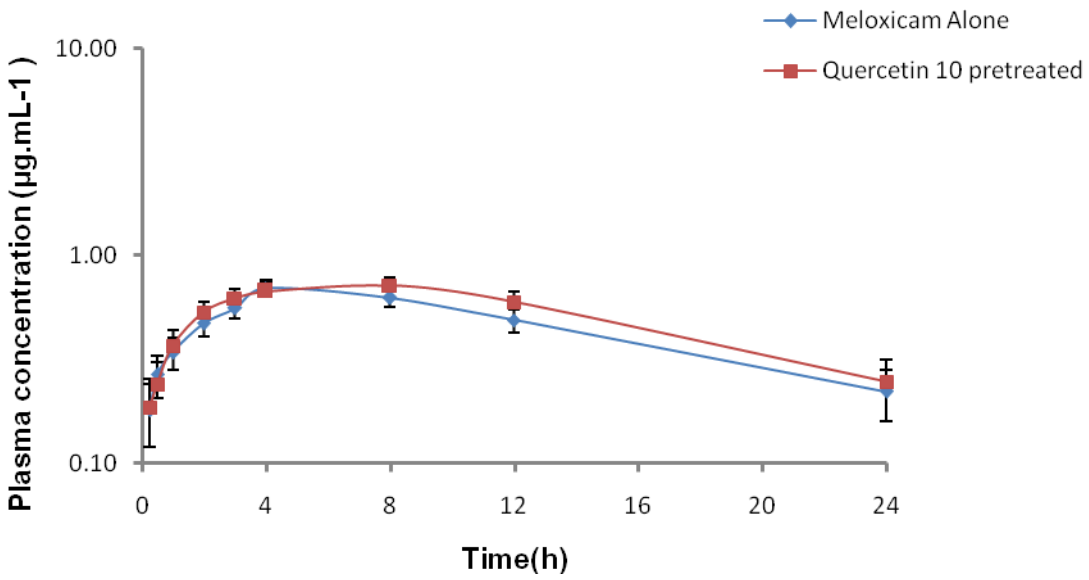


Figure 5: Semilogarithmic plot of meloxicam concentrations in plasma versus time after single oral bolus administration of meloxicam (1.5 mg.kg^{-1}) in control (Blue plot) and in quercetin pretreated (10 mg.kg^{-1}) (Red plot) adult male rabbits. Each point represents mean ± SE of five rabbits.

Table 8: Effect of quercetin (10 mg.kg^{-1}) pretreatment on pharmacokinetic parameters of meloxicam in rabbits after single oral dose administration of meloxicam (1.5 mg.kg^{-1}).

Parameter	Unit	Meloxicam alone	Quercetin(10mg/kg) + meloxicam
β	h^{-1}	0.07 ± 0.01	0.07 ± 0.01
t½β	h	14.52 ± 4.40	11.46 ± 1.62
AUC_{0-t}	$\mu g.h.mL^{-1}$	10.94 ± 0.75	11.57 ± 1.30
$AUC_{0-\infty}$	$\mu g.h.mL^{-1}$	16.02 ± 1.95	15.40 ± 1.18
$AUC_{0-t}/AUC_{t-\infty}$	Per cent	28.0 ± 7.00	25.49 ±3.64
$AUMC_{0-t}$	$\mu g.h^2.mL^{-1}$	108.84 ± 8.06	114.90 ± 14.43
$AUMC_{0-\infty}$	$\mu g.h^2.mL^{-1}$	394.86 ± 147.73	275.23 ± 26.83
MRT	h	21.89 ± 5.32	18.10 ± 1.80
V_{dss}	$L.kg^{-1}$	1.97 ± 0.24	1.83 ± 0.24
Cl_{β}	$L.kg^{-1}.h^{-1}$	0.10 ± 0.01	0.10 ± 0.01
C_{max}	$\mu g.mL^{-1}$	0.84 ± 0.06	0.83 ± 0.06
t_{max}	h	6.40 ± 0.90	4.2 ± 0.93

Effect of quercetin (20 mg.kg^{-1}) pretreatment on plasma levels and pharmacokinetics of meloxicam in adult rabbits:

Table 9, shows the plasma concentrations of meloxicam, after single oral administration of meloxicam in quercetin (20 mg.kg^{-1}) pre-treated rabbits. In table 11, mean plasma concentrations of meloxicam in quercetin (20 mg.kg^{-1}) pre-treated group were compared with those of control group. The mean time taken to achieve peak plasma concentration (t_{max}) was 4.4 **±** 0.85 h as compared to 6.40 **±** 0.90 h in control group. The C_{max} was 1.22 **±** 0.07 µg.mL^{-1} as against 0.84 **±** 0.06 µg.mL^{-1} in control group and it differs significantly from the control.

Plasma concentration versus time curve of meloxicam in quercetin (20 mg.kg^{-1}) pretreated rabbits is graphically depicted in figure 6 , where as figure 7, compares the same with the curve obtained for control group where meloxicam is administered alone. There is an apparent increase in the retention of meloxicam in pretreated group but the increase seems to be not very significant. In Figure 8, comparison is drawn between plasma concentrations of meloxicam in control group, quercetin pretreated groups at different doses.

Pharmacokinetic parameters of meloxicam, after single oral administration of meloxicam in quercetin (20 mg.kg^{-1}) pre-treated rabbits were displayed in table 10. In table 12, pharmacokinetic parameters of meloxicam in quercetin (20 mg.kg^{-1}) pre- treated group were compared with those of control group. V_{dss} value is 1.14 **±** 0.11 L.kg^{-1} compared to

1.97 ± 0.24 $L.kg^{-1}$ in control group and the difference is significant. The $AUMC_{0-t}$ value is 160.77 ± 12.76 $\mu g.h^2.mL^{-1}$ which is higher than the control group value of 108.84 ± 8.06 $\mu g.h^2.mL^{-1}$ and the increase is found to be significant. The $AUMC_{0-\infty}$ and MRT values were 326.21 **±** 19.35 $\mu g.h^2.mL^{-1}$ and 15.58 **±** 0.64 respectively.

Table 9: Plasma concentrations ($\mu g.mL^{-1}$) of meloxicam after single oral administration of meloxicam (1.5 $mg.kg^{-1}$) in quercetin (20 $mg.kg^{-1}$) pretreated rabbits (n=5).

Time (h)	Animal number					Mean ± SE
	R	S	T	U	V	
0.25	0.24	0.151	0.115	0.197	0.325	0.21 ± 0.09
0.5	0.45	0.29	0.185	0.282	0.548	0.35 ± 0.16
1	0.93	0.32	0.35	0.527	0.804	0.59 ± 0.26
2	1.13	0.434	0.75	0.588	1.147	0.81 ± 0.36
3	1.48	0.563	0.995	0.823	1.268	1.03 ± 0.46*
4	1.37	0.98	1.02	1.23	1.13	1.15 ± 0.51*
8	1.06	1.12	0.695	0.783	1.05	0.94 ± 0.42*
12	0.83	0.912	0.48	0.56	0.892	0.73 ± 0.33
24	0.29	0.31	0.258	0.272	0.373	0.30 ± 0.13*

*Significantly different ($p<0.05$) from respective values of control group.

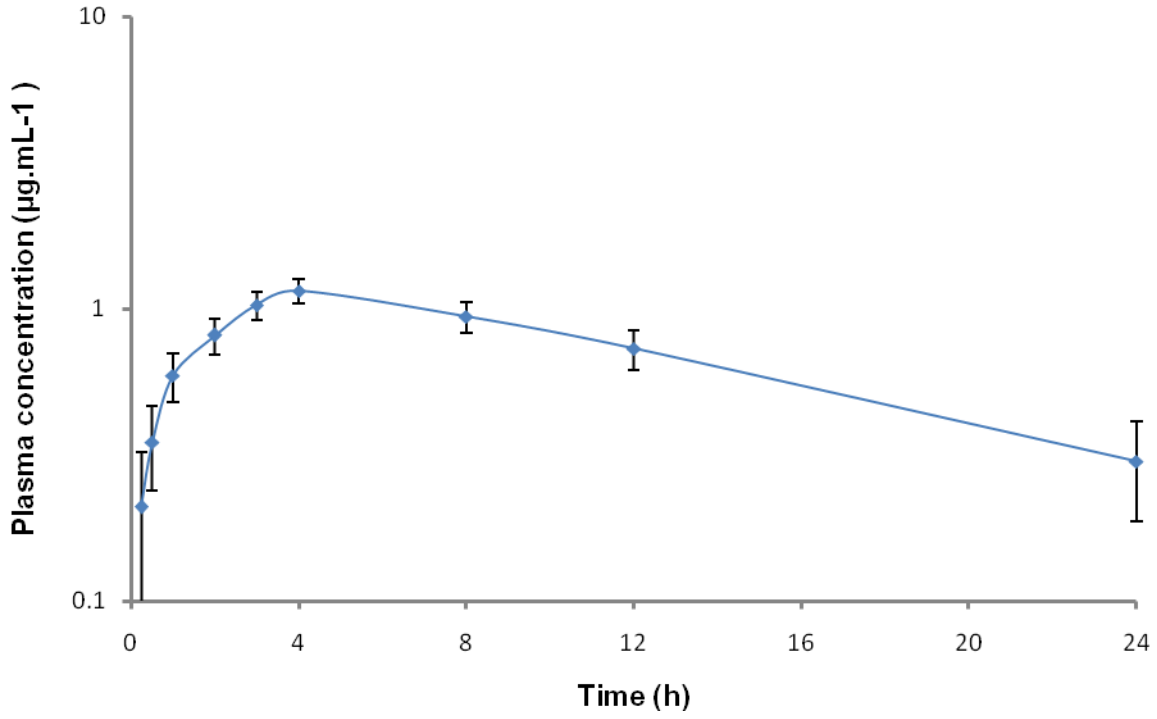


Figure 6: Semilogarithmic plot of meloxicam concentrations in plasma versus time after single oral bolus administration of meloxicam (1.5 mg.kg^{-1}) in quercetin pretreated (20 mg.kg^{-1}) (Blue plot) adult male rabbits. Each point represents mean ± SE of five rabbits.

Table 10: Pharmacokinetic parameters of meloxicam after single oral administration of meloxicam (1.5 mg.kg^{-1}) in quercetin (20 mg.kg^{-1}) pretreated rabbits (n=5).

Parameter	Unit	ANIMAL NUMBER					Mean ± SE
		M	N	O	P	Q	
β	h^{-1}	0.08	0.08	0.06	0.07	0.07	0.07 ± 0.004
t½β	h	8.47	8.40	11.63	10.71	10.42	9.93 ± 0.59
AUC_{0-t}	$\mu g.h.mL^{-1}$	19.62	17.47	12.82	14.28	19.70	16.78 ± 1.28**
$AUC_{0-\infty}$	$\mu g.h.mL^{-1}$	23.21	21.23	17.15	18.48	25.31	21.08 ± 1.36
$AUC_{0-t}/AUC_{t-\infty}$	Per cent	15.48	17.70	25.24	22.74	22.15	20.66 ± 1.62
$AUMC_{0-t}$	$\mu g.h^2.mL^{-1}$	177.26	180.69	120.46	134.39	191.07	160.77 ± 12.76*
$AUMC_{0-\infty}$	$\mu g.h^2.mL^{-1}$	307.47	316.41	297.07	300.19	409.92	326.21 ± 19.35
MRT	h	13.25	14.91	17.32	16.24	16.20	15.58 ± 0.64
V_{dss}	$L.kg^{-1}$	0.86	1.05	1.51	1.32	0.96	1.14 ± 0.11*
Cl_{β}	$L.kg^{-1}.h^{-1}$	0.07	0.07	0.09	0.08	0.06	0.07 ± 0.01
C_{max}	$\mu g.mL^{-1}$	1.48	1.12	1.02	1.23	1.27	1.22 ± 0.07**
t_{max}	h	3	8	4	4	3	4.4 ± 0.85

*Significantly different ($p<0.05$) from respective values of control group

** Significantly different ($p<0.01$) from respective values of control group

Table 11: Effect of quercetin (20 mg.kg^{-1}) pretreatment on concentrations of meloxicam in plasma (Mean ± SE) after single oral administration of meloxicam at 1.5 mg.kg^{-1} (n=5).

Time(h)	Meloxicam alone	Quercetin (20 mg.kg^{-1})+ Meloxicam
0.25	0.18 ± 0.02	0.21 ± 0.09
0.5	0.27 ± 0.03	0.35 ± 0.16
1	0.34 ± 0.04	0.59 ± 0.26
2	0.47 ± 0.08	0.81 ± 0.36
3	0.56 ± 0.09	1.03 ± 0.46
4	0.70 ± 0.13	1.15 ± 0.51
8	0.63 ± 0.07	0.94 ± 0.42
12	0.49 ± 0.08	0.73 ± 0.33
24	0.22 ± 0.02	0.30 ± 0.13

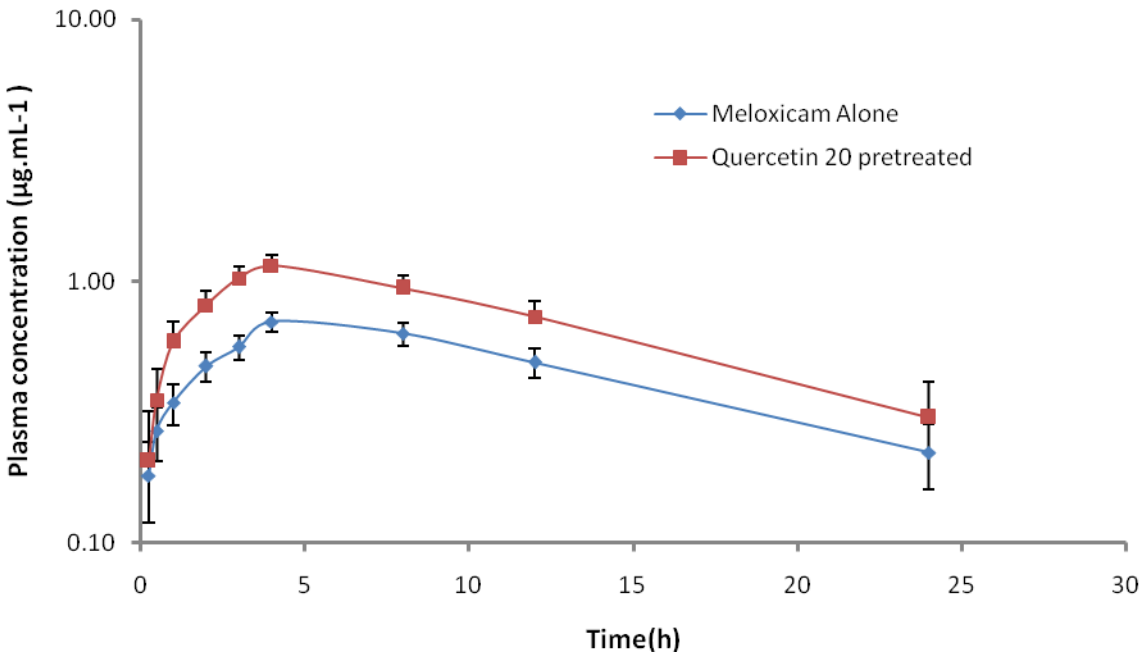


Figure 7: Semilogarithmic plot of meloxicam concentrations in plasma versus time after single oral bolus administration of meloxicam (1.5 mg.kg^{-1}) in control (Blue plot) and in quercetin pretreated (20 mg.kg^{-1}) (Red plot) adult male rabbits. Each point represents mean ± SE of five rabbits.

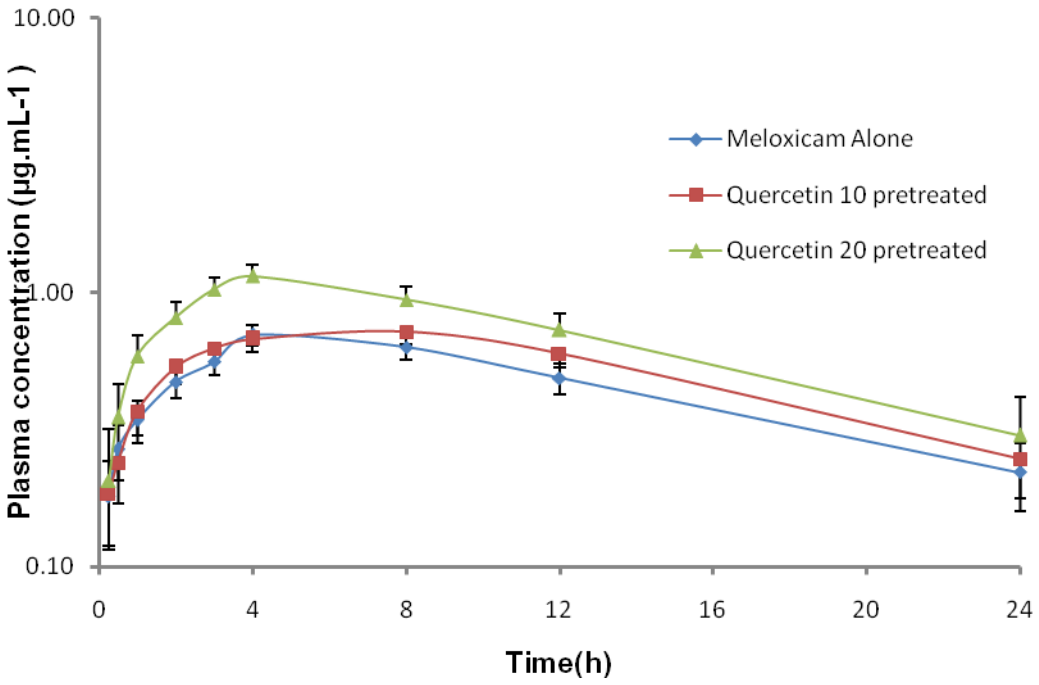


Figure 8: Semilogarithmic plot of meloxicam concentrations in plasma versus time after single oral bolus administration of meloxicam (1.5 mg.kg-1) in control (Blue plot), quercetin (10 mg.kg-1) pretreated (Red plot) and quercetin (20 mg.kg-1) pretreated (green) in adult rabbits. Each point represents the mean ± SE of five rabbits.

Table 12: Effect of quercetin (20 mg.kg^{-1}) pretreatment on pharmacokinetic parameters of meloxicam in rabbits after single oral dose administration of meloxicam (1.5 mg.kg^{-1}).

parameter	unit	Meloxicam alone	Quercetin (20 mg.kg^{-1})+ Meloxicam
β	h^{-1}	0.07 ± 0.01	0.07 ± 0.004
t½β	h	14.52 ± 4.40	9.93 ± 0.59
AUC_{0-t}	μg.h.mL^{-1}	10.94 ± 0.75	16.78 ± 1.28
$AUC_{0-\infty}$	μg.h.mL^{-1}	16.02 ± 1.95	21.08 ± 1.36
$AUC_{0-t}/AUC_{t-\infty}$	Per cent	28.0 ± 7.00	20.66 ± 1.62
$AUMC_{0-t}$	μg.h^2.mL^{-1}	108.84 ± 8.06	160.77 ± 12.76
$AUMC_{0-\infty}$	μg.h^2.mL^{-1}	394.86 ± 147.73	326.21 ± 19.35
MRT	h	21.89 ± 5.32	15.58 ± 0.64
V_{dss}	L.kg^{-1}	1.97 ± 0.24	1.14 ± 0.11
Cl_{β}	L.kg^{-1}.h^{-1}	0.10 ± 0.01	0.07 ± 0.01
C_{max}	μg.mL^{-1}	0.84 ± 0.06	1.22 ± 0.07
t_{max}	h	6.40 ± 0.90	4.4 ± 0.85

DISCUSSION

Meloxicam belongs to enolic acid class of NSAIDs having anti-inflammatory, analgesic and antipyretic activity. It preferentially inhibits cyclooxygenase-2, which is induced by inflammatory stimuli in pathophysiological conditions. Meloxicam is extensively metabolised in the liver into three inactive metabolites namely 5' carboxy (acid) metabolite, 5' hydroxyl methyl (alcohol) metabolite and the metabolite formed by the cleavage of side chain. (Schmid*, et al.,* 1995). Pharmacokinetic behavior of meloxicam was well established in different species of animals, like rats, mice, mini-pigs, beagle dogs, rabbits, sheep and goats (Busch *et al.,* 1998; Turner. ., 2006; Shukla, *et al.,* 2007). Drug interaction occurs when two or more drugs are administered together and one of them alters the absorption, distribution, metabolism or elimination of the other, such that the amount of drug reaching the site of action or it's presence at the site may be altered.

Meloxicam undergoes extensive metabolism, primarily by cytochrome P450 isozyme CYP2C9 and to a minor extent by CYP3A4 (Chesné., 1998*).* Further it was also reported that voricanazole a known CYP2C9 and CYP3A4 inhibitor increased the

plasma meloxicam concentrations while itraconazole a CYP3A4 inhibitor decreased the same in humans (Hynninen, *et al.,* 2009) and this decrease was attributed to some unknown mechanism by which itraconazole inhibited the gut absorption of meloxicam.

Recently, Kimura*, (*2010) reported that flavonoid quercetin has strong inhibitory effect on cytochrome P450 (CYP) 3A4 and CYP2C9 activity. Drug interactions are bound to arise when quercetin is given along with other drugs which are substrates for CYP2C9 and CYP3A4, because of its inhibitory effect on them. Many people have reported that quercetin has increased the oral bioavailability of various drugs that have been substrates for CYP3A4 like pioglitazone and diltiazem (Choi and Li., 2005; Sudhir, *et al*., 2008).

Hence, the present study was taken up with the aim of investigating the effect of quercetin pretreatment at two doses (i.e.) 10 and 20 $mg.kg^{-1}$ on the plasma concentrations and pharmacokinetic parameters of meloxicam in rabbits when administered orally at the rate of 1.5 $mg.kg^{-1}$ in rabbits**.**

5.1 Plasma levels and pharmacokinetics of meloxicam in rabbits after single oral administration of meloxicam alone at 1.5 $mg.kg^{-1}$ body weight:

In the present study, meloxicam was administered *per os* in adult male rabbits with an average weight of 2.0-2.5 kg at the dose rate of 1.5 $mg.kg^{-1}$ to study pharmacokinetic behavior of meloxicam. Turner, *et al.,* (2006) also investigated the pharmacokinetic profile of meloxicam in rabbits after single oral bolus administration at the dose rate of 0.3 $mg.kg^{-1}$ and 1.5 $mg.kg^{-1}$. The pharmacokinetic profile of meloxicam

was also investigated in a number of animal species including mini-pigs at dose rate used in rabbits of the present study (Busch, *et al.,* 1998).

Initial plasma concentration of meloxicam at 0.25 h was found to be 0.18 ± 0.02 $\mu g.mL^{-1}$ after single oral administration of meloxicam at the dose rate of 1.5 $mg.kg^{-1}$ and peak plasma concentration of 0.7 ± 0.13 $\mu g.mL^{-1}$ was attained at 4 h. Lower peak plasma concentrations (0.3±0.09 $\mu g.mL^{-1}$) were obtained in rabbits when meloxicam was given orally at 1.5 $mg.kg^{-1}$ by Turner, *et al*. (2006). Lower peak plasma (0.464 $\mu g.mL^{-1}$) levels of meloxicam were also noticed in beagle dogs administered meloxicam at 0.2 $mg.kg^{-1}$(Busch, *et al.,* 1998).

Elimination half life ($t_{1/2\beta}$) obtained for meloxicam after its oral administration in rabbits was 14.52 ± 4.4 h. Almost similar ($t_{1/2\beta}$) was observed in rats (15.5 h) after oral administration of meloxicam whereas a longer $t_{1/2\beta}$ of 23.7 h was reported in beagle dogs which is longer compared to the value obtained in rabbits of the present study (Busch *et al.,* 1998). Much shorter ($t_{1/2\beta}$) values of 8.16 ± 2.19 h and 8.39 ± 1.17 h were obtained in female rabbits at the dose rates of 0.3 $mg.kg^{-1}$ and 1.5 $mg.kg^{-1}$ respectively. The difference in elimination half-lives within rabbits for meloxicam may be due to gender difference as gender differences in rabbits for half-lives do exist for various drugs.

Time taken to attain peak plasma concentration of meloxicam (t_{max}) in rabbits was 6.4 ± 0.9 h. Turner*, et al.,* (2006) reported t_{max} values (6.4 ± 0.8 h; 0.3 $mg.kg^{-1}$ and 6.8 ± 0.5 h; 1.5 $mg.kg^{-1}$) that were in agreement with t_{max} obtained in rabbits after oral administration of meloxicam in the present study. A higher t_{max} value of 7.5 h was

obtained in beagle dogs after oral dosing at the rate of 0.2 $mg.kg^{-1}$ of meloxicam and a lower value of 3.0 h in mini-pigs after oral dosing at the dose rate of 10 $mg.kg^{-1}$ (Busch, *et al*., 1998).

Mean residence time (MRT) is an important pharmacokinetic parameter, which gives indication about the time for which the drug persists in the body after administration. The MRT value was found to be 21.89 ± 5.32 h in the present study. Much shorter values of 3.89 and 4.48 h were observed in male and female mice after oral administration of meloxicam at 10 $mg.kg^{-1}$ and longer values of 31.8 h were reported after oral dosing of meloxicam at 1.0 $mg.kg^{-1}$ in albino rats and 40 h in beagle dogs after administering at the rate of 0.2 $mg.kg^{-1}$ (Busch, *et al*., 1998).

Volume of distribution at steady state (V_{dss}) value was observed to be 1.97 ± 0.24 $L.kg^{-1}$ in the present study, where as a lower value of 1.46 ± 0.48 $L.kg^{-1}$ and a higher value of 4.14 ± 1.03 $L.kg^{-1}$ were attained in rabbits after oral dosing at the rate of 0.3 and 1.5 $mg.kg^{-1}$ respectively (Turner, *et al.,* 2006).

Area under concentration time curve (AUC) is an important parameter and is needed for calculating the bioavailability of a drug and to determine the clearance of a drug from the body. In the present study, the value of $AUC_{0-\infty}$ obtained was 16.02 ± 1.95 $\mu g.h.mL^{-1}$. For the same parameter, lower values were recorded by Turner, *et al*. (2006) in rabbits after dosing at the rate of 0.3 and 1.5 $mg.kg^{-1}$ and the values were 2.57 ± 0.21 and 5.2 ± 1.29 $\mu g.h.mL^{-1}$ respectively. The value in case of beagle dogs was found to be 22.9 $\mu g.h.mL^{-1}$ after oral dosing of meloxicam at 0.3 $mg.kg^{-1}$ (Busch, *et al.,* 1998).

Clearance (Cl_B) in the present study was obtained as 0.1± 0.01 $L.kg^{-1}.h^{-1}$ and the value is higher compared to the values of 0.009 $L.kg^{-1}.h^{-1}$ in case of beagle dogs and 0.007 $L.kg^{-1}.h^{-1}$ in rats reported by Busch, *et al.,* (1998).

5.2 Effect of quercetin pretreatment (10mg.kg^{-1}) on the plasma concentration and pharmacokinetic parameters of meloxicam:

Quercetin pretreatment was given at the rate of 10$mg.kg^{-1}$ orally followed by meloxicam after 30 minutes. The initial plasma concentration of meloxicam was found to be 0.19 ± 0.06 $\mu g.mL^{-1}$ at 0.25 h and this value is not significantly different from the value obtained in control group where meloxicam was given alone. The peak plasma concentration was obtained as 0.83±0.06 $\mu g.mL^{-1}$ which is almost similar to the value in control group.

Clearance (Cl_B) is same as that of the control group indicating that the quercetin pretreatment at 10$mg.kg^{-1}$ is not having significant effect on the clearance of meloxicam. $AUC_{0-\infty}$ in the present study was found to be 15.4±1.18 $\mu g.h.mL^{-1}$ which was not significantly different from the control group value. Though, other pharmacokinetic parameters like elimination half life ($t_{1/2\beta}$), $V_{dss,}$ MRT were different from the corresponding values in control group the difference is not of significance.

In the present study, the pharmacokinetic parameters of meloxicam in control group were similar to the values obtained in quercetin pretreated group at the rate of 10

mg.kg^{-1} and no noticeable differences were observed. It indicates that quercetin pretreatment at 10 mg.kg^{-1} was not effective in altering the pharmacokinetic profile of meloxicam and is needed in higher dose.

5.3 Effect of quercetin pretreatment (20 mg.kg^{-1}) on the plasma concentration and pharmacokinetic parameters of meloxicam:

Quercetin pretreatment was given at the rate of 20 mg.kg^{-1} orally followed by meloxicam after 30 minutes. The initial plasma concentration at 0.25 h was found to be 0.21 ± 0.09 µg.mL^{-1} and is not significantly different from the value in control group. The plasma concentrations at 3, 4, 8 and 12 h were 1.03 ± 0.46, 1.15 ± 0.51, 0.94 ± 0.42 and 0.3 ± 0.13 µg.mL^{-1} respectively and these values were significantly different from corresponding values in control group.

The value of C_{max} in the present study was 1.22 ± 0.07 µg.mL^{-1} and is significantly ($p<0.01$) higher than the value in control group (0.84± 0.06 µg.mL^{-1}). The value of AUC_{0-t} (16.78 ± 1.28 µg.h.mL^{-1}) was significantly higher than the corresponding value (10.94 ± 0.75 µg.h.mL^{-1}) in control. The $AUMC_{0-t}$ and V_{dss} values were 160.77 ± 12.76 µg.h^2.mL^{-1} and 1.14 ± 0.11L.kg^{-1} which differed significantly from those of the control group ($p<0.05$).

The values obtained for quercetin pretreatment 20 $mg.kg^{-1}$ group were different from those of the control group. AUC_{0-t} increased by 53% compared to the control group in which meloxicam was administered alone.

Results in the present study reveal that there is an interaction between meloxicam and quercetin which is evident from the apparent differences noticed in various pharmacokinetic parameters of meloxicam in quercetin pretreatment group (20 $mg.kg^{-1}$). Further studies are needed to establish the appropriate dose at which the interactions are prominent and to establish the gender differences in the pharmacokinetic profile of meloxicam.

Flavonoids exhibit a wide range of beneficial biological activities including antioxidant, radical scavenging, and anti-inflammatory properties (Nijveldt, *et al.,* 2001). Besides altering the pharmacokinetic profile of meloxicam, this combination is thought to bring about additive effect to the anti-inflammatory effect of meloxicam in clinical situations as flavonoids were reported to have anti inflammatory activity. To establish the clinical efficacy of this combination, further studies may be required.

Summary

In the present study, pharmacokinetic profile of meloxicam was studied when administered alone as single oral bolus and also when given 30 minutes after the quercetin pretreatment at dose rates of 10 and 20 $mg.kg^{-1}$. Meloxicam concentrations in plasma were detected by HPLC assay.

In phase I, meloxicam was administered alone as a single oral bolus at the rate of 1.5 $mg.kg^{-1}$. Important pharmacokinetic parameters after non compartmental analysis were $t_{1/2B,}$ 14.52 ± 4.4 h; $Cl_{B,}$ 0.1 ± 0.01 $L.kg^{-1}.h^{-1}$; $AUC_{0-\infty}$,16.02 ± 1.95 $\mu g.h.mL^{-1}$; $V_{dss,}$1.97 ± 0.24 $L.kg^{-1}$: and MRT, 21.89±5.32 h.

In phase II of the study, meloxicam (1.5 $mg.kg^{-1}$) was administered orally, 30 minutes after the quercetin pretreatment at the rate of 10 $mg.kg^{-1}$. Important pharmacokinetic parameters after non compartmental analysis were $t_{1/2B,}$ 11.46 ± 1.62 h; $Cl_{B,}$ 0.1± 0.01 $L.kg^{-1}.h^{-1}$; $AUC_{0-\infty}$,15.40 ± 1.18 $\mu g.h.mL^{-1}$; $V_{dss,}$1.83 ± 0.24 $L.kg^{-1}$: and MRT, 18.1 ± 1.8 h. The parameters in quercetin (10 $mg.kg^{-1}$) pretreatment group were similar to the values of control group.

In phase III of the study, meloxicam (1.5 $mg.kg^{-1}$) was administered orally, 30 minutes after the quercetin pretreatment at the rate of 20 $mg.kg^{-1}$. Important pharmacokinetic parameters after non compartmental analysis were $t_{1/2B,}$ 9.93 ± 0.59 h; $Cl_{B,}$ 0.07 ± 0.01 $L.kg^{-1}.h^{-1}$; $AUC_{0-\infty}$,21.08 ± 1.36 $\mu g.h.mL^{-1}$; $V_{dss,}$1.14 ± 0.11 $L.kg^{-1}$: and MRT, 15.58 ± 0.64 h. The results obtained in this phase differed markedly from the values in control group. AUC_{0-t} is 53% higher than the control. $AUMC_{0-t,}$ V_{dss}, and C_{max} also differed significantly from the corresponding values in control.

From the above results it is concluded that quercetin (10$mg.kg^{-1}$) pretreatment has no significant effect on the pharmacokinetic parameters of meloxicam. But, quercetin (20$mg.kg^{-1}$) pretreatment affected the pharmacokinetic profile of meloxicam. Further studies may be required to establish the dose of quercetin at which interaction would be clinically significant and to know the gender influence.

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