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Research Article

EFFECT OF LICORICE ON PHARMACOKINETICS OF MELOXICAM IN BIRDS

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ABSTRACT

The study was aimed to investigate the pharmacokinetic interaction between the commonly used NSAID, Meloxicam, a CYP2C9 substrate and licorice which contains Glycyrrhizin, a CYP2C9 inhibiting flavonoid in birds. The broiler chicken weighing about 2 kgs were randomly divided into two groups consisting 8 birds in each. Birds in group I (meloxicam control) received single oral bolus of meloxicam (2mg.Kg^{-1} B.W) whereas birds in group II received single oral bolus of meloxicam (2mg.Kg^{-1} B.W) 01 h after pre-treatment with licorice (500 mg.Kg^{-1}B.W, orally). Blood samples (0.5 ml) were collected from tarsal vein at pre-determined time intervals prior to meloxicam administration and at 0.166, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h post-administration of meloxicam. Plasma was separated and analyzed for meloxicam by HPLC assay. Based on plasma concentrations of meloxicam, the pharmacokinetic parameters were determined by non-compartmental methods. The pharmacokinetic parameters such as $AUC_{0-\infty}$, Cl_B, $t_{1/2}\beta$, Vd_{ss} and MRT were not significantly different when compared between group I and II birds. There were no significant differences in either plasma concentrations or pharmacokinetic parameters of meloxicam in both the groups. It is concluded that licorice which contains a flavonoid glycyrrhizin known to be putative inhibitor of CYP2C9 and CYP3A4 has no role on the activity of these enzymes in birds, as the plasma concentrations and pharmacokinetics parameters of meloxicam were found unaltered.

Keywords: CYP2C9, Glycyrrhizin, Licorice, Meloxicam

Pain is the most negative experience possible with physical and emotional effects with debilitating factor in healing. In birds, relief of pain has demonstrated a quicker return to recovery (Forbes, 1999). Bumble foot, cloacal prolapse, fractures, certain inflammatory diseases and also numerous routine practices (such as de-beaking or de-spurring) in poultry elicit significant pain and the inflammatory response similar to mammals. The commonly used NSAIDS *viz*., meloxicam and carprofen are proven to be effective in birds (Malik and Valentine, 2018).

Meloxicam produces anti-inflammatory, analgesic and antipyretic properties through preferential inhibition of COX2 enzyme (Engelhardt *et al*., 1995) and primarily metabolized by CYP2C9 isoform of cytochrome p450 drug metabolizing enzymes (Chesne *et al*., 1998).

Licorice is derived from the sweet root of various species of Glycyrriza that contains bio active components such as flavonoids and glycyrrhizin with pharmacological properties and medicinal applications. The licorice extract has been found to show immunologic and antioxidant activities that might improve the growth performance, feed efficiency, carcass traits and blood biochemical indices of the poultry birds and also potential solution for solving respiratory, digestive and immune problems in poultry (Alagawany *et al*., 2019). Glycyrrhizin is a well known putative CYP2C9 enzyme inhibitor (Kent *et al*., 2002). Currently, healthcare professionals are deeply concerned about drug-herb interactions and pharmacokinetics of drug and reduction of side effects of NSAIDS (Kennedy and Seely, 2010).

Hence, the present study was designed to know the pharmacokinetic interaction of licorice with meloxicam and the effect of licorice, a CYP2C9 inhibitor on pharmacokinetics of meloxicam.

MATERIALS AND METHODS

Experimental animals: Sixteen adult birds weighing 2kg were procured from M/s Srinivasa Hatcheries, Vijayawada, India. They were maintained under standard management and husbandry conditions with free access to feed and water. The experiments were approved by Institutional Animal Ethics Committee (IAEC), N.T.R College of Veterinary Science, Gannavaram, Andhra Pradesh, India.

Drugs and chemicals: Oral bolus formulation of meloxicam (Melonex®) was used for oral administration and pure technical grade powder of meloxicam used as external standard in HPLC assay. Heparin 20,000 IU/vial was obtained from M/s Loba Chemie, Mumbai, India. Acetonitrile and other chemicals of HPLC grade used in the experiment were procured from M/s Merck, Mumbai, India. Water for HPLC was obtained by Millipore water purification system and was filtered through 0.2 µm filter prior to use. All other chemicals used in the study were of analytical grade.

Licorice (*Glycyrrhiza glabra*) roots were obtained

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Table 1

Pharmacokinetic parameters (Mean ± SE) of meloxicam in different groups of birds (n = 8)

Parameter (Unit)	Group I (Meloxicam)	Group II (Licorice extract pre-treatment + Meloxicam)
β (h^{-1})	0.14±0.03	0.19±0.03
$t_{1/2}\beta$ (h)	4.97±0.93	4.43±0.99
AUC_{0-t} ($\mu g.h.ml^{-1}$)	40.42±6.08	44.63±6.65
$AUC_{0-\infty}$ ($\mu g.h.ml^{-1}$)	68.35±13.20	49.09±9.20
$AUC_{0-t}/AUC_{0-\infty}$ (Per cent)	75.14±11.56	94.76±3.64
$AUMC_{0-t}$ ($\mu g.h^2.ml^{-1}$)	378.22±66.01	438.33±82.86

commercially from crude herbs traders. The roots were washed in distilled water, shade dried and ground to course powder and stored at room temperature until further use.

Licorice extract was prepared by dissolving 200 grams of powder in 2000 ml of 1:1 acetone and distilled water. The conical flask was subjected to constant stirring using an orbital shaker overnight at room temperature with a speed of 100 rpm. The extract prepared was filtered using Whatman's filter paper (MN 640md) and the filtrate was subjected to slow evaporation.

Experimental design: Sixteen adult birds of both sexes were divided into 2 groups consisting eight birds in each. Meloxicam was administered as single oral bolus dose of 2 $mg.Kg^{-1}$ in both the groups whereas birds in group II were pretreated with licorice extract (500$mg.Kg^{-1}$, orally) 60 min prior to administration of meloxicam. Meloxicam dose selected basing on basic analgesia dose in birds prescribed by Malik and Valentine (2018). The content of glycerrhizin in licorice varies from 2-25% depending on species and prolonged consumption leads to adverse effects like hypokalemia (Omar *et al.*, 2012). No observed effect level for purified glycyrrhizin was 2mg/kg/day and the oral bioavailability is poor when administered as licorice extract, which has hampered attempts to establish clear dose-effect levels in animals and humans (Isbrucker and Burdock, 2006). In our study, licorice extract dose of 500 $mg.Kg^{-1}$ was selected to induce maximum effect on pharmacokinetic interaction, if any and no adverse effects were observed at this dose.

Blood samples (0.5 ml) were collected in heparinized tubes by venipuncture (left and right veins of tarsal and jugular vein) prior to meloxicam administration and at 0.166, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h time interval after meloxicam administration. They were centrifuged at 3000 rpm for 5 min and plasma was harvested and stored at -20 °C till analyzed for meloxicam by high performance liquid chromatography (HPLC).

Assay of meloxicam: Meloxicam concentrations in plasma were determined by high performance liquid chromatography as described by Baert and De Backer (2003) with slight modifications. In brief, acetonitrile was added to plasma sample in the ratio of 1:1 (0.2 mL each). After vortex mixing at high speed for 20 s, the tubes were subjected to centrifugation for 10 min at 9000g. The clear supernatant thus obtained was transferred to a tube and equal volume of HPLC grade water was added to the supernatant. The aliquot was filtered through a 0.2 µm nylon membrane filter and 20 µL of filtrate was injected into the HPLC system.

The HPLC system (Shimadzu Corporation, Kyoto, Japan) comprised of LC-20AD quaternary gradient pump, Rheodyne manual loop injector with a 20 µL loop, SPD-20 AV UV-Vis detector, column oven CTO-10ASVP and work station software Lab_solutions version 4-0512-039 was used for data analysis. Separation of meloxicam was performed by using a C18 reverse phase column (4.6 x 250 mm, 5µm particle size) as stationary phase and a mixture of 40 parts of acetonitrile and 60 parts of 0.05 M sodium acetate buffer (pH 6.0) as mobile phase at a wavelength of 355 nm . The flow rate was adjusted at 0.8 mL min^{-1}. There were no interfering substances in the plasma at the retention time of meloxicam as evident by the chromatograms obtained for plasma blank and spiked plasma standards (Fig. 1). Peak heights were taken for the quantification of meloxicam in plasma from calibration curves obtained on analysis of blank plasma samples spiked with meloxicam (external standards) and analyzed as described for the experimental samples. The limit of quantification was 0.03125$\mu g.ml^{-1}$. The method was found to be linear and reproducible in the concentration range of 0.3- 10 $\mu g.ml^{-1}$ for meloxicam.

Pharmacokinetic analysis: Plasma concentration versus

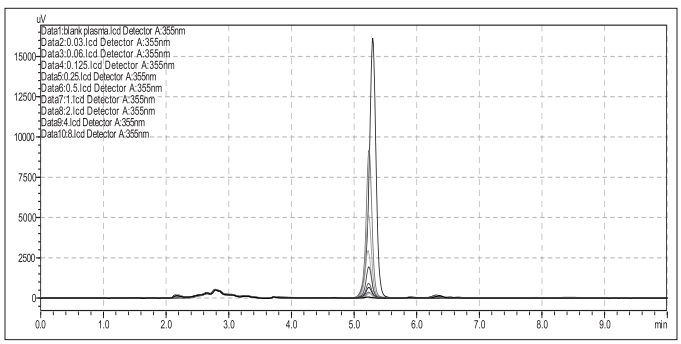

Fig. 1. Chromatogram of meloxicam standard in plasma

time data of meloxicam obtained in birds of both groups was utilized for calculating various pharmacokinetic parameters with an interactive least squares linear regression by computer software (PK Solver Version 2.0, 2010 by Zhang *et al.*). Best-fit model was chosen by using minimal Akaike information criteria estimation (Yamaoka and Nakagawa, 1978). Peak plasma concentration (C_{max}) and time to reach peak concentration (t_{max}) were calculated from the actual plasma data of each bird.

Statistical analysis: The plasma concentrations and pharmacokinetic variables of meloxicam are expressed as Mean±S.E. Differences in plasma concentrations and pharmacokinetic data between the two groups were analyzed for statistical significance using two samples t-test assuming unequal variances using 'Instat' software. AUC, $t_{1/2}\beta$, C_{max} values were log transformed prior to the analysis. The $P<0.05$ values were considered statistically significant.

RESULTS AND DISCUSSION

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 its presence at the site may be altered.

The mean plasma levels of meloxicam at different time intervals after single oral administration of meloxicam (2 $mg.Kg^{-1}$) to both control group and licorice pretreated group birds are presented graphically in Fig. 2. Various pharmacokinetic parameters estimated by non compartmental analysis of plasma concentrations of meloxicam in both groups of birds are summarized in Table 1. The initial mean meloxicam plasma concentration of 0.09±0.08 $\mu g.ml^{-1}$ was observed at 0.166 h. Mean peak plasma levels (C_{max}) of meloxicam observed in this study was 3.44±0.50 $\mu g.ml^{-1}$ at t_{max} of 6.50±0.47 h after oral administration of

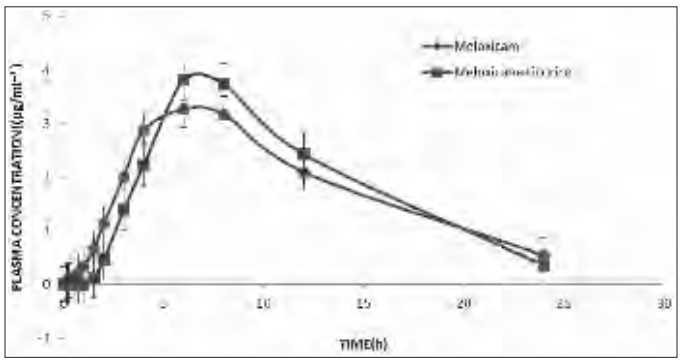

Fig. 2. Semi logarithmic plot of meloxicam concentration in plasma versus time after single oral administration of meloxicam in different groups of birds (n = 8)

meloxicam. The pharmacokinetic profile of meloxicam was also investigated in number of species including vultures at the dose rate used in birds of the present study (Naidoo *et al*., 2008) and C_{max} observed in vulture was 5.25 $\mu g.ml^{-1}$ at t_{max} of 0.47 h. Variations in the C_{max} and t_{max} of meloxicam may be due to the species difference.

The various pharmacokinetic parameters of meloxicam observed were elimination rate constant (β) (0.14±0.03 h^{-1}), elimination half-life $t_{1/2}\beta$ (4.97±0.93 h). Almost similar elimination half-life of 3.29 h of meloxicam in chicken and a shorter half-life of 0.52 h in ostriches was reported (Baert and Backer, 2003). Variation in the elimination half-lives of meloxicam may be due to species difference.

The area under the concentration-time curve (AUC) in pharmacokinetics forms the basis for calculation of some kinetic parameters like MRT, Cl_B, and Vd_{ss}, etc. Mean±SE value of $AUC_{0-\infty}$ obtained in the present study for meloxicam was 68.35±13.20 $\mu g.h.ml^{-1}$ after single oral administration of meloxicam (2 $mg.kg^{-1}$). Comparatively lower AUC value of 0.73 $\mu g.h.ml^{-1}$ for meloxicam when given at a dose rate of 0.5 $mg.kg^{-1}$ was reported in ostriches (Baert and Backer, 2003). AUC varies with the administered dose that may be the reason for the difference in the AUC of meloxicam.

Vd_{ss} provides an estimate of drug distribution that is independent of elimination process. It is a function of drug's ability to reach various peripheral tissues in the body after absorption. In the present study, the Vd_{ss} obtained for meloxicam after single oral administration was 0.54±0.10 $L.kg^{-1}$. A lower Vd_{ss} of 0.065 $L.kg^{-1}$ was reported in Muscovy ducks (Baert and Backer, 2003). Clearance (Cl_B) is a characteristic of the drug and indicates volume of plasma cleared of the drug by various elimination processes per unit time. The clearance obtained in the present study was 0.06±0.01 $L.Kg^{-1}.h^{-1}$. Mean residence time (MRT) is the mean time required for a drug molecule to transverse through the body and thus reflects time associated with absorption, distribution and elimination. In the present study, MRT values obtained for meloxicam was 9.24±0.95 h.

Effect of licorice pretreatment on the plasma levels and pharmacokinetics of meloxicam oral administration (2 $mg.Kg^{-1}$) was observed. The initial meloxicam plasma concentration of 0.00±0.0 $\mu g.ml^{-1}$ was observed at 0.166 h, which is non-significantly lower compared to the initial plasma concentration of 0.09±0.08 $\mu g.ml^{-1}$ obtained in

group I. The peak plasma concentration (C_{max}) of meloxicam was 4.26±0.55 $\mu g.ml^{-1}$ which is non-significantly higher than that of the value obtained in the meloxicam alone group 3.44±0.50 $\mu g.ml^{-1}$. Similarly reports are there where meloxicam C_{max} increased from 0.84 to 1.22 $\mu g.ml^{-1}$ when pre-treated with quercetin in rabbits and this may be attributed to the inhibition of CYP2C9, which metabolizes meloxicam (Jayakanth, 2010). Grape juice containing flavonoids decreased C_{max} from 2.4 to 1.1$\mu g.ml^{-1}$ when given concurrently with phenacetin in humans and this was due to the enhancement of first pass metabolism of phenacetin by grape juice (Dong *et al*. 1999).

It appears that licorice pretreatment has non-significantly increased the meloxicam concentration and hence the higher C_{max} for meloxicam was noticed in the licorice pretreated birds. This may be due to interference of licorice to the enzyme CYP2C9.

There were no significant differences in elimination half-lives ($t_{1/2\beta}$), area under curve ($AUC_{0-\infty}$), mean residence time (MRT), volume of distribution at steady state (Vd_{ss}) and the total body clearance (Cl_B) of meloxicam alone and meloxicam in licorice pretreated group. The results obtained suggest that there were no pharmacokinetic interaction between licorice and meloxicam in birds when licorice was given 60 min prior to the administration of meloxicam as single dose. A recent study investigated the interaction of licorice with CYP3A4 in rats and their study demonstrated that glycyrrhetinic acid, a glycyrrhizin metabolite, activated the CYP3A4 function and subsequently reduced the oral bioavailability of cyclosporine A (Hou *et al*., 2012; Huang *et al*., 2008).

Licorice, a CYP2C9 and CYP3A4 inhibitor (Kent *et al.,* 2002), did not significantly alter the important pharmacokinetic parameters like elimination half-life and clearance of both meloxicam. However, there are reports that glycyrrhetinic acid and glycyrrhizin altered the pharmacokinetic parameters of other CYP3A4 substrates like cyclosporine A (Hou *et al*., 2012; Huang *et al*., 2008). Probably the experimental design made in the present study had no scope to detect such alterations because of administration of licorice in single dose at 60 min prior to the administration of meloxicam.

CONCLUSION

The present study revealed that there is no significant difference in pharmacokinetics of meloxicam between licorice extract single dose pretreated meloxicam group and meloxicam alone treated group. Hence, licorice extract in a single dose has no effect on pharmacokinetics of meloxicam in broiler chicken. Further studies are required with prolonged administration of licorice to assess the pharmacokinetic interaction between meloxicam and licorice.

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REFERENCES

Alagawany, M., Elnesr, S.S., Farag, M.R., Abd El-Hack, M.E., Khafaga, A.F., Taha, A.E., Tiwari, R., Yatoo, M.I., Bhatt, P., Marappan, G. and Dhama, K. (2019). Use of licorice (*Glycyrrhiza glabra*) herb as a feed additive in poultry: Current knowledge and prospects. *Animals (Basel)*. **9 (8)**: 536.

Baert, K. and Backer, P.D. (2003). Comparative pharmacokinetics of three non-steroidal anti-inflammatory drugs in five bird species. *Comp. Biochem. Physiol. C.* **134:** 25-33.

Chesne, C., Guyomard, C., Guillouzo, A., Schmid, J., Ludwig, E. and Sauter, T. (1998). Metabolism of meloxicam in human liver involves cytochromes P4502C9 and 3A4. *Xenobiotica.* **28**: 1-3.

Dong, S.X., Zhi Ping, Z., Zhong Xiao, W., Chong Shu, C., Fattore, C., Gatti, G., D'Urso, S. and Perucca, E. (1999). Possible enhancement of the first-pass metabolism of phenacetin by ingestion of grape juice in Chinese subjects. *Br. J. Clin. Pharmacol.* **48**: 638-640.

Engelhardt, G., Homma, D., Schlegel, K., Utzlnann, R. and Schnitzler, C. (1995). Anti-inflammatory, analgesic, antipyretic, and related properties of meloxicam, a new non-steroidal anti-inflammatory agent with favourable gastrointestinal tolerance. *Inflamm. Res.* **44**: 423-433.

Forbes, N. (1999). Birds, in Anaesthesia and Alalgesia of Exotic Species (Ch. 27). In: BSAVA Manual of Small Animal Anaesthesia and Analgesia. Seymour,

C. and Gleed, R (Edts.), BSAVA, Cheltenham. pp. 283-293.

Hou, Y.C., Lin, S.P. and Chao, P.D. (2012). Liquorice reduced cyclosporine bioavailability by activating P-glycoprotein and CYP 3A. *Food Chem.* **135**: 2307-2312.

Huang, B.B., Li, G.F., Ren, F., Tang, Z.K., Ma, H.F., Sun, Y.B., Chen, L.J. and Yang, L. (2008). Effect of *Glycyrrhiza inflata* and *Daphne genkwa* on permeabilities of rhodamine 123, a P-glycoprotein substrate across rat jejunum membranes *in vitro*. *China J. Chinese Materia Medica.* **33**: 2521-2526.

Ibrucker, R.A. and Burdock, G.A. (2006). Risk and safety assessment on the consumption of licorice root (*Glycyrrhiza glabra*), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin. *Regul. Toxicol. Pharmacol.* **46(3)**: 167-192.

Jayakanth, K. (2010). Studies on the pharmacokinetic interaction between meloxicam and quercetin, a CYP2C9 and CYP3A4 inhibiting flavonoid, in rabbits. Ph.D. thesis submitted to Sri Venkateswara Veterinary University, Tirupathi – 517 502, Andhra Pradesh, India.

Kennedy, D.A. and Seely, D. (2010). Clinically based evidence of drug–herb interactions: a systematic review. *Expert Opini Drug Saf.* **9**: 79-124.

Kent, U.M., Aviram, M., Rosenblat, M. and Hollenberg, P.F. (2002). The licorice root derived isoflavan glabridin inhibits the activities of human cytochrome P450S 3A4, 2B6, and 2C9. *Drug Metab. Dispos.* **30**: 709-715.

Malik, A. and Valentine, A. (2018). Pain in birds: A review for veterinary nurses. *Vet. Nurs. J.* **33(1)**: 11-25.

Naidoo, V., Wolter, K., Cromarty, A.D., Bartels, P., Bekker, L., McGaw, L., Taggart, M.A., Cuthbert, R. and Swan, G.E. (2008). The pharmacokinetics of meloxicam in vultures. *J. Vet. Pharmacol. Ther.* **31**: 128–134.

Omar, H.R., Komarova, I., El-Ghonemi, M., Fathy, A., Rashad, R., Abdelmalak, H.D., Yerramadha, M.R., Ali, Y., Helal, E. and Camporesi, E.M. (2012). Licorice abuse: Time to send a warning message. *Ther. Adv. Endocrinol. Metab.* **3(4)**: 125-138.

Yamaoka, K. and Nakagawa, T. (1978). Statistical moments in pharmacokinetics. *J. Pharmacokinet. Biopharm.* **6**: 547-557.

Zhang, Y., Huo, M., Zhou, J. and Xie, S. (2010). PK_ Solver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. *Comput. Meth. Prog. Bio.* **99(3)**: 306-314.