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Influence of Co-Administration of Piperine on Pharmacokinetic Profile of Gatifloxacin in Layer Birds

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Abstract: Piperine (1-piperoyl piperdine) is a major component of the Piper species, used widely as spices and in various systems of Indian medicine. Pepper (*Piper nigrum*) is used alone or along with long pepper and ginger to enhance the therapeutic efficacy of the concurrently administered drugs. The present study investigated the influence of co-administration of piperine on pharmacokinetic profile of Gatifloxacin in Layer birds. Gatifloxacin(GFX) is a fourth generation, 8-methoxy fluoroquinolone. The pharmacokinetic profile of gatifloxacin (10 mg/kg body weight) along with piperine coadministration (15 mg/kg) via single oral dose in layer birds shown half life ($t_{1/2}$ β), Maximum drug concentration (C_{max}) and AUC of 4.03±0.097 hour, 2.14±0.019 µg/ml and 17.54 ± 0.204 µg.h/ml, respectively, which is found significantly higher than gatifloxacin alone (3.74 ± 0.073 h, 1.74 ± 0.023 µg/ml and 15.25 ± 0.219 µg.h/ml respectively). This study reveals that piperine has significant effect on the pharmacokinetics of the Gatifloxacin. There is enhancement in bioavailability (F) from 74.52±1.021% (gatifloxacin alone treated group) to 85.74 ± 0.956 % (piperine coadministration with gatifloxacin treated group). The results obtained are the combined effect of piperine on the absorption kinetics and the inhibition of the metabolism of Gatifloxacin.

Key words: Coadministration · Piperine · Gatifloxacin · Pharmacokinetics · Layer Birds

INTRODUCTION

Piperine has shown bioenhancing property on various antibiotics like β-lactam antibiotics, ciprofloxacin, oxytetracycline, norfloxacin and ampicilin [1-4]. It is major active pungent constituent in various Piper species (*Piperaceae*) [5,6]. The piperine contents in (black pepper) *P. nigrum Linn* and *P. longum Linn (*long pepper*)* are 3-9% and 3-5% (on dry weight basis), respectively [7]. Piperine play a role in metabolism via inhibition of hepatic monooxygenase and UDP-glucouronyl transferase and intestinal glucouronidation [8-10], inhibition of CYP3A4 and p-glycoprotein (PGP) [11]. Along with these inhibitory effect of piperine on drug metabolizing enzymes (Cytochrome P450 family) and various metabolism processes like arylhydrocarbon hydroxylation, Ehtylmorphin-N-Demethylation, 7-eyhoxycoumarin-o-dethylaation and 3-hydroxy benzo (a) pyrene gluourodination in non competitive manner imparts it to enhance the bioavailability [9 ,10].

Gatifloxacin (GFX), a synthetic broad-spectrum 8-methoxyfluoroquinolone antibacterial agent for oral or intravenous administration. Gatifloxacin has shown bactericidal activity by binding to bacterial topoisomerases II (DNA gyrase) and IV and interfere with DNA replication, repair and transcription, resulting in bacterial death [12].The present study was planned to study the influence of co-administration of piperine on pharmacokinetic profile of Gatifloxacin.

MATERIALS AND METHODS

Piperine Extract: Piperin*e* (95 % *Piper nigrum* extract), a yellowish creamy powder was received as a gift sample from M/s, Sami Laboratories Ltd, Banglore. It was dissolved in 10% ethanol and used for oral administration at a dose rate of 15 mg/kg of body weight in layer birds. 10 % Ethanol was administered to control group birds as vehicle.

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Gatifloxacin: Gatifloxacin sesquihydrate technical grade powder was received as a gift sample from Sun Pharmaceutical Ltd, Vadodara, Gujarat. Gatifloxacin infusion (20 mg/ ml) was made from technical grade powder in 0.1 N Hydro Chloric Acid. Gatifloxacin tablet (200 mg; Gatispan®, Lupin Ltd. Mumbai) was dissolved in water and used for oral administration.

Chemicals for Hplc Bioassay: Acetonitrile, methanol, ethanol, triethylamine, perchloric acid (about 70%), ortho-phosphoric acid (min. 58%, analytical grade) and deionised water of HPLC grade were purchased from Merck Limited, Mumbai.

Experimental Animals: The study was conducted on 24 layer birds of 6-8 weeks weighing between 1.5 and 1.8 kilograms. The birds were maintained at Central Poultry Research Station (CPRS), College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, India. Birds were kept under observation for two weeks prior to commencement of experiment. During this period they were subjected to clinical examination in order to exclude the possibility of any disease. The birds were kept in clean cages and were provided standard layer ration. Water was provided *ad libitum.* Standard managemental practices were followed to keep the birds free from stress. The experimental protocol was approved by the institutional animal ethics committee (IAEC).

Experimental Design: The experiment was conducted as following study design (Table 1).

Blood samples (2 mL) were collected using IV catheter (Venflon, 22G x 25mm) fixed into the contra lateral wing vein and transferred to clean sterilized heparinized test tubes. Following IV administration blood samples were collected at 0 time (before drug administration) and at 0.033 (2 minutes), 0.083 (5 minutes), 0.166 (10 minutes), 0.25 (15 minutes), 0.5 (30 minutes), 0.75 (45 minutes), 1, 2, 4, 8, 12, 18 and 24 hours. Following oral administration blood samples were collected at 0 time (before drug administration) and at 0.083 (5 minutes), 0.166 (10 minutes), 0.25 (15 minutes), 0.5 (30 minutes), 0.75 (45 minutes), 1, 2, 4, 8, 12, 18, 24 and 36 hours. Plasma was harvested soon after collection by centrifugation (3000 g at 4°C for 10 minutes) and stored at-40°C and assayed within 24 hours.

Gatifloxacin Assay: Gatifloxacin was assayed in plasma by reverse-phase C18 column (Thermo, 5 µ ODS; 250 X 4.6 mm ID) high performance liquid chromatography (HPLC) at room temperature, using a reported assay with minor modifications [13, 14]. Data integration was performed using software Clarity (Version 2.4.0.190). The mobile phase consists of a mixture of water containing 1% triethylamine, acetonitrile and metahanol (85:15:15 v/v) adjusted to pH 3.0 with ortho-phosphoric acid. Mobile phase was filtered through 0.45 µ filter and pumped into column at a flow rate of 1.0 mL. min^{-1} at ambient temperature. The effluent was monitored by UV detection at 290 nm. Sample was prepared by taking 500 µl of plasma samples in 2 mL clean micro-centrifuge tube. 5 µg of ciprofloxacin was added as an internal standard in each sample. Perchloric acid (50 µl) was added to precipitate plasma proteins. The mixture was shaken on a vortex mixer for 1 minute and centrifuged at 3000 g at 4 °C for 10 minute. The clean supernatant was collected and an aliquot of 20 µl of the supernatant was injected into the loop of HPLC system through manual injector.

Gatifloxacin standardization in plasma was done by preparing range of concentrations from 0.1 to 50 $\mu g.mL^{-1}$. The calibration curve was prepared by using these standards and was rejected unless it had a R^2 value > 0.99. The lower limit of quantification (LOQ) was 0.1 $\mu g.mL^{-1}$. The assay was sensitive and reproducible with good

Table 1: Study Design for Experiment

	Phase I (Pharmacokinetic study)		Phase II (Bioenhancing study)	
Group	A	B	Control	Test
No. of Birds	6	6	6	6
Dose and Treatment	GFX 10 mg/kg B.W. Single dose	GFX 10 mg/kg B.W. Single dose	Vehicle coadministration (1 ml/kg B.W. single dose) + GFX (10 mg/kg B.W. Single dose)	Piperine coadministration (15 mg/kg B.W. single dose) + GFX (10 mg/kg B.W. Single dose)
Bird No. and Route of Administration	L 1-6 (IV)	L 7-12 (PO)	L 13-18 (PO)	L 18-24(PO)

linearity between 0.1 to 50 µg.mL^{-1}. Precision and accuracy were determined with known concentrations of 0.25, 5.0 and 50 µg. mL^{-1} in plasma (5 replicates each/day). The intraday and interday coefficients of variation for 5 samples were satisfactory, with relative standard deviations (RSD) less than 8%. Intraday and interday variations were under acceptable limits. The retention time of the drug was 7.2 min. Recovery of the drug from plasma was found to be more than 95%.

Pharmacokinetic Analysis: Pharmacokinetic parameters were determined for each bird by non-compartmental analysis with commercial software (PK solution 2.0, USA). Following oral administration of the drug, maximum concentration (C_{max}) and time to reach the maximum concentration (T_{max}) were determined from the concentrations-time curve.

The area under the plasma concentration vs. time curve and area under concentration-time Vs time curve from time zero to the last sampling time (AUC_{0_t} and $AUMC_{0\_t}$) were calculated using the trapezoidal rule. The first-order elimination rate constant (β) was obtained as the slope of the logarithmic concentration-time curve using the concentrations in the elimination phase. The area from the last sampling time to infinity was estimated using and the last measured concentration based on the following formulae:

$$AUC_{\infty} = AUC_{(0-t)} + \frac{C_n}{\lambda_z}$$

Where, C_n is the last concentration

$$MRT = \frac{AUMC_{\infty}}{AUC_{\infty}}$$

The mean residence time (MRT) was calculated based on the equation

The volume of distribution (Vd area) was calculated as

$$V = \frac{FD}{AUC_{\infty}\lambda_{\infty}}$$

The total body clearance (Cl_B) was found to be

$$CL = \frac{FD}{AUC_{\infty}}$$

Bioavailability was calculated using the following formula [15] .

$$F\% = (AUC_{oral}\, X\, Dose_{i.v} / AUC_{i.v}\, X\, Dose_{oral})\, X\, 100$$

(Because the same birds were not used for oral and i.v. studies, mean AUC values were used for the calculation of bioavailability.)

RESULTS

In the present study, significant changes in the plasma concentration-time profile and various pharmacokinetic parameters of gatifloxacin have been occur while co administration with piperine in layer birds which is shown in Table 2 and 3. The piperine treated group showed a significant increase in plasma concentration at 0.5 (30 minutes), 0.75 (45 minutes), 1, 4 and 8 hours in comparison with control and vehicle control group. There were significant increases in elimination half life, Cmax, area under curve, volume of distribution and bioavailability. Figure 1 shows the comparative plasma concentration Vs time curve.

Table 2: Comparison of plasma concentrations (µg/mL) of gatifloxacin in gatifloxacin alone, vehicle control and piperine treated birds after single oral administration (10 mg/kg).

Time (hr)	Plasma concentrations after GFX alone administration. (n = 6)	Plasma concentrations after administration of GFX along with vehicle. (n = 6)	Plasma concentrations after administration of GFX along with piperine. (n = 6)
0.5	0.48 ± 0.013	0.5 ± 0.006	0.53 ± 0.009**
0.75	1 ± 0.014	1.02 ± 0.005	1.16± 0.029**
1	1.35 ± 0.016	1.39 ± 0.008	1.46 ± 0.021*
2	1.74 ± 0.023	1.81 ± 0.013	2.14 ± 0.020
4	1.35 ± 0.02	1.39 ± 0.011	1.47 ± 0.034*
8	0.97 ± 0.009	0.89 ± 0.004	1.08 ± 0.019**
12	0.46 ± 0.01	0.41 ± 0.005	0.54 ± 0.008
18	ND		

ND: Not Detected; *Significant at p<0.05, **Highly significant at p<0.01

Table 3: Comparison of pharmacokinetic parameters (Mean ± S.E.) of gatifloxacin in gatifloxacin alone, vehicle control and piperine treated layer birds after single oral administration (10 mg/kg)

Pharmacokinetic parameters	Unit	Gatifloxacin Alone	Gatifloxacin Co- administration along with Vehicle	Gatifloxacin Co- administration along with piperine
A	µg/ml	2.18 ± 0.142	1.99 ± 0.073	1.3 ± 0.266*
B	µg/ml	4.32 ±0.12	4.21 ± 0.058	4.34 ± 0.212
β	per hour	0.19 ± 0.004	0.19 ± 0.002	0.17 ± 0.003*
$t_{1/2\ (Ka)}$	Hour	2.45 ± 0.083	2.14 ± 0.012	3.3 ± 0.039*
$t_{1/2}\ \beta$	Hour	3.74 ± 0.073	3.58 ± 0.033	4.03 ± 0.097*
C_{max}	µg/ml	1.74 ± 0.023	1.81 ± 0.013	2.14 ± 0.019*
T_{max}	Hour	2 ± 0.0	2 ± 0.00	2 ± 0.0
AUC	µg.h/ml	15.25 ± 0.219	14.73 ± 0.123	17.54 ± 0.204*
AUMC	µg.h^2/ml	108.34 ± 2.53	98.28 ± 1.17	128.91 ± 2.48
$Vd_{(area)}$	l/kg	3.54 ± 0.038	3.51 ± 0.012	3.33 ± 0.083*
$Vd_{(ss)}$	l/kg	6.93 ± 0.291	6.61 ± 0.055	6.26 ± 0.332
$Cl_{(B)}$	l/hr/kg	0.66 ± 0.009	0.68 ± 0.006	0.57 ± 0.006
MRT	Hour	7.1 ± 0.077	6.67 ± 0.025	7.34 ± 0.096*
MAT (MRT_{oral}- $MRT_{i.v.}$)	Hour	2.66 ± 0.081	2.2 ± 0.060	2.91 ± 0.085*
F	%	74.52 ± 1.021	72.24 ± 0.853	85.74 ± 0.956*

**Highly significant at p<0.01, *Significant at p<0.05

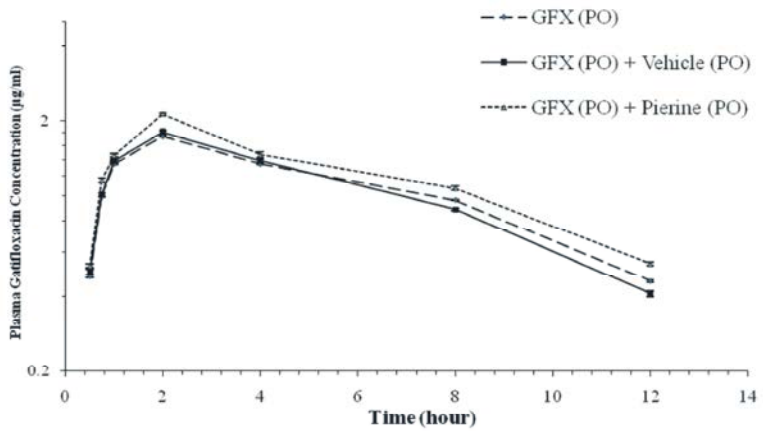

Fig. 1: Semi logarithmic plot of plasma concentrations of gatifloxacin in gatifloxacin alone, vehicle control and piperine treated birds after via oral administration (10 mg/kg). Each point represents mean ± S.E of twelve layer birds.

DISCUSSION

In the present study, the significant higher peak plasma levels of gatifloxacin were 2.14 ± 0.020 µg/ml in piperine treated group, as compared to 1.81 ± 0.013 µg/ml in vehicle treated group and 1.74 ± 0.023 µg/ml in gatifloxacin alone group at 2 hours, respectively. Similar types of results were obtained by Singh *et al.* [3] in case of oxytetracycline in hen, Janakiraman and Manavalan [4] in case of norfloxacin in rabbits. There is significant increase in plasma concentration of gatifloxacin at 1 hour (1.46 ± 0.021 µg/ml), 4 hours (1.47 ± 0.034 µg/ml) and 8 hours (1.08±0.019 µg/ml) in piperine treated group compared gatifloxacin alone treated group. These variations might be due to effect of piperine to increase permeability to gastro intestinal tract epithelial cell membrane, inhibition of drug metabolizing enzymes and suppression of first pass metabolism [16].

There were significant increase in the elimination half-life (4.03±0.097 hrs) in piperine treated group layer birds than gatifloxacin alone treated group (3.74 ± 0.073 hrs) and vehicle treated group (3.58 ± 0.033 hrs). These increase in the elimination half life were observed by Janakiraman and Manavalan [4] after administration of norfloxacin with piperine co administered group, Bhise and Pore [2] after

administration of ciprofloxacin alone and with piperine coadministration. This increase in half life might be due to inhibitory effect of piperine on renal elimination which leads to decrease in renal clearance [17]. As a result of increased half life, the mean residence time in piperine treated group (7.34 ± 0.096 hrs) has been found significantly higher than gatifloxacin alone treated group (7.1 ± 0.077 hrs) and vehicle treated group (6.67 ± 0.025 hrs). The apparent volume of distribution of gatifloxacin in piperine co administered layer birds was 3.33 ± 0.083 l/kg which is significantly lower than gatifloxacin alone treated group (3.54 ± 0.038 l/kg) and vehicle treated group (3.51 ± 0.012 l/kg) might be due to inhibitory activity of piperine on renal elimination.

The values of AUC in piperine treated layer birds was 17.54 ± 0.204 µg.h/ml which is significantly higher than gatifloxacin alone treated group (15.25 ± 0.219 µg•h/ml) and vehicle treated group (14.73 ± 0.123µg.h/ml). Bhise and Pore [2] and Janakiraman and Manavalan [4] were observed similar results when give piperine along with norfloxacin and ciprofloxacin respectively. Increased AUC in piperine treated group may be because of inhibition of enzymes responsible for metabolism of gatifloxacin in liver [9] that leads to more free drug concentration for longer duration in body [18]. There were significantly lower total body clearance of gatifloxacin in piperine treated layer birds (0.57 ± 0.006 l/hr/kg) compared to gatifloxacin alone treated group (0.66 ± 0.009 l/hr/kg) and vehicle treated group (0.68 ± 0.006 l/hr/kg). The reason of lower clearance of gatifloxacin in birds may be due to inhibition of renal excretion as an effect of piperine on renal system [17].

The bioavailability of gatifloxacin in piperine treated layer birds was 85.74±0.956 % which is significantly higher than gatifloxacin alone treated group (74.52 ±1.021 %) and vehicle treated group (72.24 ± 0.853 %). The enhancement of bioavailability in present study in piperine treated group might be due to interaction of piperine with membrane, which in turn increases absorption area resulting in efficient permeation through membrane as explained by Khajuria *et al.* [19]. Piperine produce concentration related site cytochrome PIAI in the small intestine as well as in the liver which may modulate xenobiotic metabolizing system at the site of primary portal of entry to systemic circulation [9, 10].

CONCLUSION

Piperine (15 mg/kg b.wt.) enhances the bioavailability of gatifloxacin when it was co-administered along with gatifloxacin (10 mg/kg b. wt.). The significant increase was noticed in peak plasma concentration (C_{max}), elimination half life ($t_{1/2}\beta$), volume of distribution (Vd), area under the curve (AUC) and bioavailability (F) of gatifloxacin in piperine co-administered layer birds than gatifloxacin alone treated layer birds. There was significant decrease in absorption half life ($t_{1/2(Ka)}$) and clearance (Cl) of the drug in piperine treated group birds compared to plane gatifloxacin treated layer birds. The changes between these two groups has been observed because of effect of piperine to decrease permeability to gastro intestinal tract epithelial cell membrane, suppression of first pass metabolism and inhibition of enzymes responsible for metabolism of gatifloxacin in liver.

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