

Safety and Tissue Residue Determination of Gatifloxacin in Broiler Chicken

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Abstract

Gatifloxacin is a fluoroquinolone having broad-spectrum activity and good antibacterial activity at low plasma/tissue concentration. The present study was designed to investigate safety of gatifloxacin (10 mg/kg body weight) after repeated oral administration at 12 hours interval for 14 days in broiler chickens and to determine tissue concentration of the drug following oral administration (10 mg/kg body weight) for 5 days. Repeated oral administration of gatifloxacin in broiler chickens was found safe based on evaluation of hematological (Hb, PCV and TLC), biochemical (AST, ALT, ALP, LDH, Serum uric acid, Serum Creatinine, Blood glucose and Total bilirubin) and histopathology of liver, kidney, heart and joint cartilage. Drug concentration in tissue was determined using High Performance Liquid Chromatography (HPLC). The concentration of gatifloxacin was found $0.75 \pm 0.04 \mu\text{g/g}$ after fourth dose and $0.22 \pm 0.07 \mu\text{g/g}$ after tenth dose respectively in liver, whereas in skeletal muscles the concentration of gatifloxacin was below the limit of quantification after fourth dose and after tenth dose gatifloxacin was not detected.

Keywords: Chicken; biomarkers; chicken

Introduction

Fluoroquinolones are gaining widespread acceptance in veterinary medicine as they have broad spectrum activity against Gram-negative and Gram-positive bacteria, mycoplasma, rickettsia as well as against bacteria resistant to other antibiotics (Brown, 1996). At present only few fluoroquinolones are used in veterinary medicine. Resistance of bacteria against fluoroquinolone is great threat for future survival of the fluoroquinolone drugs as an antibiotic class in veterinary medicine (Sharma *et al.*, 1994). Gatifloxacin is a newer fourth generation fluoroquinolones. It is active L-isomer of the racemate ofloxacin having twice antimicrobial activity than parent compound (Saravolatz and Leggett, 2003). The numbers of pharmacokinetic studies are being undertaken in domestic animals with a view to adopt this drug in veterinary medicine as well. Its spectrum of activity and pharmacokinetic properties favour its use in veterinary practice. Pharmacokinetics of gatifloxacin have been studied in buffalo calves (Raipuria *et al.*, 2006, Raipuria *et al.*, 2007a, Raipuria *et al.*, 2007b) and goats (Verma and Roy,

2006, Verma *et al.*, 2007). However, the data on safety of repeated oral administration of gatifloxacin in broiler chickens are lacking. Therefore, the present study was planned to evaluate safety of gatifloxacin following multiple oral dose administration in broiler chickens.

Materials and methods

Experimental Animals

The present study was conducted on 14 broiler chickens reared at Central Poultry Research Station, Anand Agricultural University, Anand, Gujarat, India. The birds were weighing between 1.50-2.00 kg on 8-10 weeks of age. The birds were examined clinically to evaluate health status and to rule out the possibility of any diseases. They were kept individually as a single bird per cage and were maintained on standard antibiotics free starter 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).

Drugs and chemicals

Gatifloxacin sesquihydrate technical grade powder

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was received as a gift sample from Sun Pharmaceutical Ltd, Vadodara, Gujarat. Gatifloxacin oral tablet (200 mg; Gatispan®, Lupin Ltd., Mumbai) was purchased from local market. 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 Design for Safety Assessment

Eight broiler chickens were employed to assess safety of the drug. Birds were administered 10 mg/kg body weight of gatifloxacin at 12 hours interval for 14 days. Blood samples were withdrawn from wing vein into sterile heparinized (2 ml) and non-heparinized (3 ml) test tubes at 0 day (before drug administration) and on 3rd, 5th, 7th, 9th, 11th, 13th and 15th day for haematological [Hemoglobin (Hb), Packed cell volume (PCV) and Total leukocytes count (TLC)] and serum biochemical analysis [Aspartate aminotransferase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), Serum uric acid, Serum creatinine, Blood glucose and Total bilirubin], respectively. Haemoglobin was determined by Sahli's acid hematin method, PCV and TLC as described by Schalm, (1967). Biochemical parameters were estimated using standard assay kits (Crest Biosystems, A Division of Coral Clinical Systems, Goa, India) with the help of clinical chemistry analyser (Junior Selectra, Vital Scientific NV) at Teaching Veterinary Clinical Service Complex, Veterinary College, AAU, Anand.. Birds were scarified to collect tissue samples (liver, kidney and femoro-tibial and tibio-tarsal joint cartilages) for histopathological examination at 15th day. Tissues were processed for histopathological examination by standard procedure and were stained with hematoxylin and eosin (H & E). Statistical analysis of data was done by software SPSS (Version 12.0.1).

Experimental Design for Tissue Residue Assessment

Six broiler chickens were employed to determine the accumulation of the drug in tissues following multiple oral administrations at dose rate of 10 mg/kg body weight at 12 hours interval for 5 days. Birds were sacrificed at 12 hours after the last dose of the drug to collect the samples of liver and skeletal

muscle. The drug concentration in tissue samples was determined by HPLC assay (Santoro et al., 2006 and Najma Sultana et al., 2006).

Extraction of gatifloxacin from Tissues

The extraction of gatifloxacin from tissues was done by using a modified method previously reported by Goudah, (2009). A 0.5 g sample of tissue was homogenized at ambient temperature with 2ml of acetonitrile, centrifuged at 5000 revolutions per minute (rpm) for 10 minutes; the supernatant was diluted 4-fold with mobile phase and mixed on vortex mixer for 1 minute. Five hundred microlitre of supernatant was transferred to a 2 ml micro-centrifuge tube. 2.5 µg of ciprofloxacin was added as an internal standard in each sample. Thereafter, the perchloric acid (50 µl) was added in supernatant to precipitate proteins. The mixture was shaken on a vortex mixer for 1 minute and centrifuged for 2 minute at 5000 revolutions per minute (rpm). The supernatant was decanted in glass vials and 20 µl supernatant was injected into the loop injector using 25 µl glass syringe.

High Performance Liquid Chromatography (HPLC) Assay:

The HPLC system (Laballiance, USA) comprised of quaternary gradient solvent delivery pump (model AIS 2000) and UV detector (model 500). Chromatographic separation was done on reverse phase C18 column (Thermo, 5 µ ODS; 250 X 4.6 mm ID) at room temperature. 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 methanol (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 at ambient temperature. The effluent was monitored at 290 nm.

Standard curves were also prepared using homogenates of tissues like liver and skeletal muscle in mobile phase. Known concentrations of gatifloxacin were prepared by diluting the stock standard with drug-free poultry tissue homogenate. The sensitivity of the assay method was 0.1 µg gatifloxacin per ml. The mean correlation coefficient R² was 0.999 in both liver and skeletal muscle tissue homogenate.

Results

After oral administration of the drug at the dose rate of 10 mg/kg body weight repeated at twelve hours interval, the values of hematological and biochemical parameters were evaluated and are presented in Table 1 and 2, respectively. No alterations in hematological and serum biochemical parameters

have been found in the present study.

All organs including liver, heart and kidney were found with normal colour, texture and consistency on gross necropsy examination of the birds. On histopathological examination, no alterations at cellular level have been found in liver (Fig. 1), heart (Fig. 2), kidney (Fig. 3) and joint cartilage (Fig. 4).

Table 1. Hematological parameters (mean \pm S.E.) after oral administration of gatifloxacin (10mg/ kg of body weight) in broiler chicken (n=8).

Day	Haematological Parameters		
	Haemoglobin (g/dl)	PCV (%)	TLC ($\times 10^3/\text{mm}^3$)
0	9.5 \pm 0.42	26.5 \pm 0.71	3.3 \pm 0.08
3	9.2 \pm 0.28	26.5 \pm 0.71	3.3 \pm 0.07
5	9.5 \pm 0.14	26.0 \pm 1.41	3.2 \pm 0.16
7	9.7 \pm 0.71	27.5 \pm 2.12	3.6 \pm 0.07
9	9.4 \pm 0.57	28.0 \pm 2.83	3.6 \pm 0.11
11	9.8 \pm 0.85	27.5 \pm 0.71	3.2 \pm 0.03
13	9.9 \pm 0.42	27.5 \pm 0.71	3.5 \pm 0.23
15	9.6 \pm 0.28	28.0 \pm 0.00	3.4 \pm 0.01

Non significant at $P < 0.05$ level

Table 2. Biochemical parameters (m \pm S.E.) after oral administration of gatifloxacin (10mg/kg of body weight) in broiler chicken (n=8).

Day	Biochemical Parameters							
	AST (IU)	ALT (IU)	ALP (IU)	LDH (IU)	Creatinine (mg/dl)	Glucose (mg/dl)	Total Bilirubin (mg/dl)	Uric acid (mg/dl)
0	222.45 \pm 6.39	42.73 \pm 2.29	123.08 \pm 3.40	643.17 \pm 5.43	0.28 \pm 0.01	242.66 \pm 3.61	0.16 \pm 0.01	8.02 \pm 0.25
3	251.08 \pm 4.42	45.22 \pm 2.42	124.91 \pm 3.13	646.45 \pm 3.56	0.29 \pm 0.01	247.25 \pm 3.23	0.17 \pm 0.01	7.80 \pm 0.26
5	252.74 \pm 6.24	44.38 \pm 1.62	124.69 \pm 2.50	640.01 \pm 4.24	0.27 \pm 0.01	234.87 \pm 3.33	0.19 \pm 0.01	8.09 \pm 0.20
7	260.93 \pm 8.48	44.99 \pm 2.26	126.72 \pm 2.96	640.36 \pm 3.46	0.28 \pm 0.01	241.98 \pm 3.73	0.18 \pm 0.01	8.14 \pm 0.22
9	255.69 \pm 5.20	45.19 \pm 2.32	128.16 \pm 1.54	647.82 \pm 2.69	0.28 \pm 0.01	250.85 \pm 3.89	0.19 \pm 0.01	7.95 \pm 0.13
11	249.62 \pm 7.27	47.66 \pm 1.93	127.94 \pm 1.35	642.33 \pm 5.71	0.29 \pm 0.01	245.29 \pm 3.12	0.19 \pm 0.005	7.98 \pm 0.22
13	260.05 \pm 5.29	43.41 \pm 2.18	129.27 \pm 2.26	639.10 \pm 3.48	0.27 \pm 0.01	248.89 \pm 3.10	0.18 \pm 0.02	8.01 \pm 0.11
15	266.02 \pm 6.56	48.45 \pm 2.65	128.17 \pm 2.08	643.54 \pm 3.20	0.30 \pm 0.00	242.92 \pm 3.73	0.17 \pm 0.02	8.07 \pm 0.15

Non significant at $P < 0.05$ level

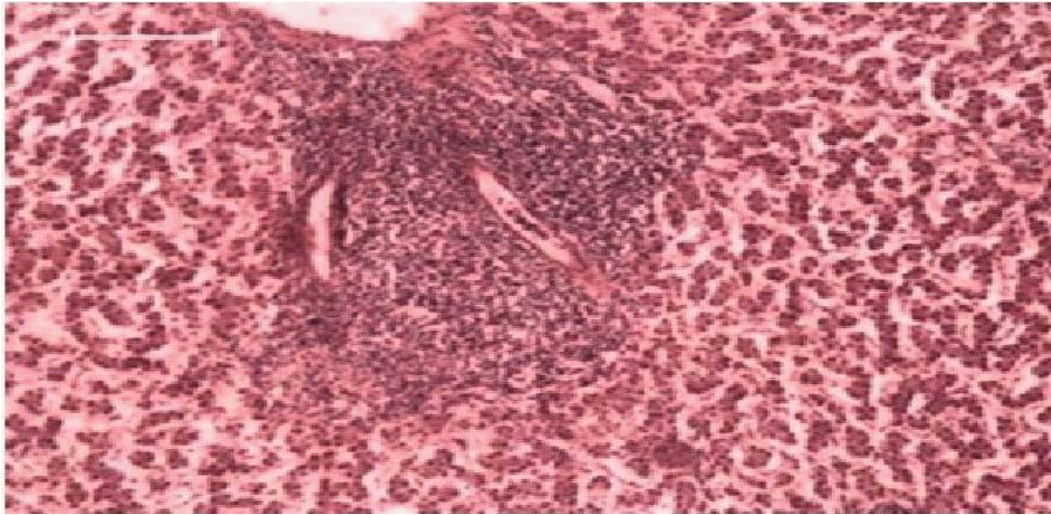


Fig. 1. Histopathological section of Liver



Fig. 2. Histopathological section of Heart.

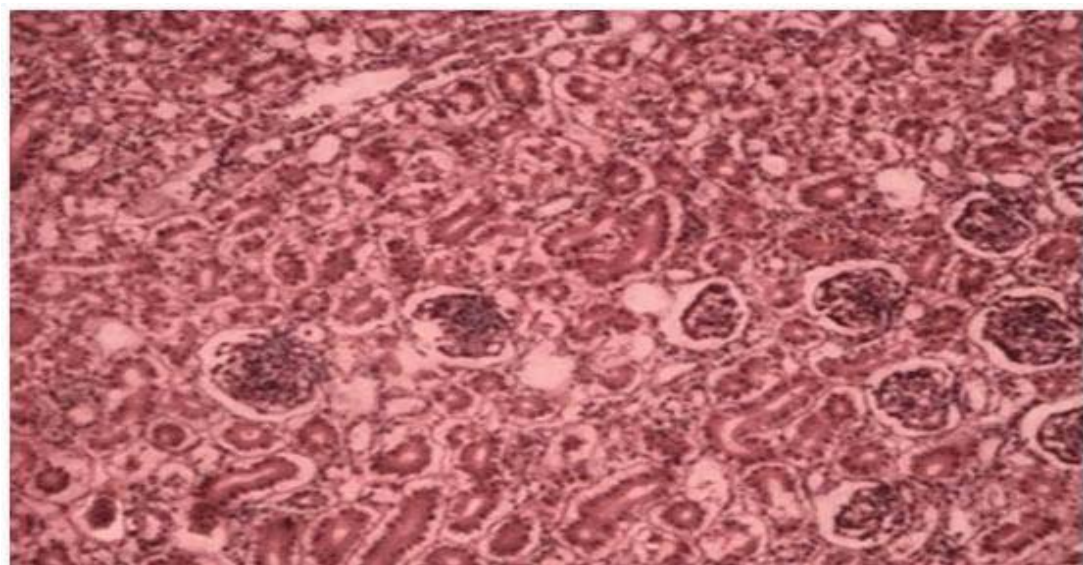


Fig. 3. Histopathological section of Kidney.

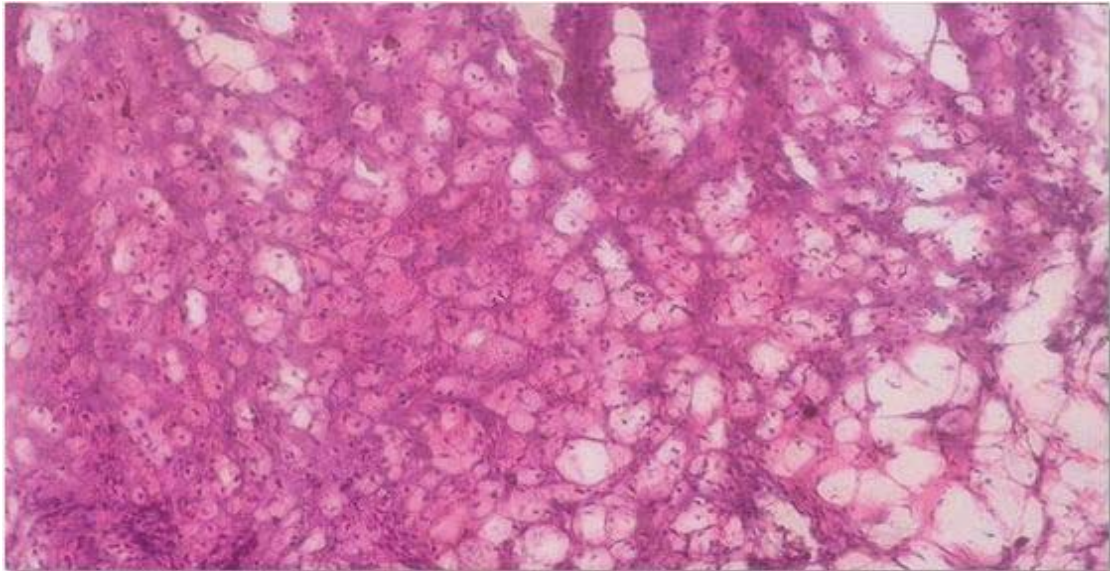


Fig. 4. Histopathological section of Joint cartilage.

Discussion

After oral administration of the drug at the dose rate of 10 mg/kg body weight repeated at twelve hours interval, the values of hematological and biochemical parameters were evaluated and are presented in Table 1 and 2, respectively. No alterations in hematological and serum biochemical parameters have been found in the present study. It is in agreement with the no significant alterations in haematological and biochemical parameters reported in layer birds (Patel *et al.*, 2009) and broiler birds (Varia, 2008) at 10 mg/kg of body weight dose of levofloxacin respectively. However mild transient decrease in serum glucose and mild transient intravenous site reaction was associated with the end of the 1-hour infusion of gatifloxacin daily via intravenous route for 14 days in healthy adult men by Gajjar *et al.* (2000).

In the present study, all organs including liver, heart and kidney were found with normal colour, texture and consistency on gross necropsy examination of the birds. On histopathological examination, no alterations at cellular level have been found in liver (Fig. 1), heart (Fig. 2), kidney (Fig. 3) and joint cartilage (Fig. 4). Potential of the drug to induce arthropathic changes was evaluated by observation of the gait, behaviour and reaction to palpation of joint (pain) during the experiment and by histopathological examination of joint cartilage in broiler chickens. In the present study, no abnormality in gait and pain at joints were observed during the treatment period. Moreover,

histopathological examination of the joint cartilage did not reveal any microscopic changes in the articular cartilages of treated birds. However excessive dosages of various quinolones have caused lesions in articular cartilages of skeletally immature dogs, rats and other skeletally immature laboratory animals (Gough *et al.* 1979; Howard *et al.* 1979; Kato and Onodera, 1988, Tatsumi *et al.* 1978). Gross and microscopic observations of the present study are in agreement with the result observed in broiler and layer birds treated with repeated oral dose of levofloxacin at the rate of 10 mg/kg of body weight (Patel *et al.*, (2009), Varia *et al.*, (2009)). Ciprofloxacin was also found safe in cow calves following repeated administration at dose rate 5 mg/kg body weight as no alterations were found in joint cartilage (Bhavsar *et al.*, 2004).

Following oral administration of gatifloxacin at the dose rate of 10 mg/kg at 12 hours interval for 5 days revealed gatifloxacin concentration in liver was 0.75 ± 0.04 $\mu\text{g/g}$ after fourth dose and 0.22 ± 0.07 $\mu\text{g/g}$ after tenth dose respectively, whereas in skeletal muscles the concentration was below the limit of quantification (0.012 ± 0.002 $\mu\text{g/g}$) after fourth dose and after tenth dose gatifloxacin was not detected. This persistence of drug in tissue indicates the high volume of distribution and low protein binding of gatifloxacin in chickens. The equivalent high concentration of pefloxacin (10 mg/kg of body weight) after 1 day of last dose was 3.20 ± 0.40 in liver and 1.42 ± 0.18 in muscle, whereas tissue concentration of moxifloxacin (5 mg/kg of body weight) after 72 hour was 0.27 ± 0.06 in liver and 0.13 ± 0.06 in skeletal muscle obtained

by Pant *et al.* (2005) and Goudah (2009) in broiler chickens respectively. The results indicate that, for daily oral administration of gatifloxacin at 10 mg/kg of body weight repeated at twelve hours interval following four and ten oral doses, a pre-slaughter withdrawal time of more than 7 days is needed to ensure that the drug is eliminated from the tissues.

Conclusion

Following multiple oral administration, gatifloxacin shown good accumulation in the tissues at third day. Results obtained in the present study indicate that gatifloxacin is well tolerated following multiple oral administrations at 10 mg/kg body weight in broiler bird.

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