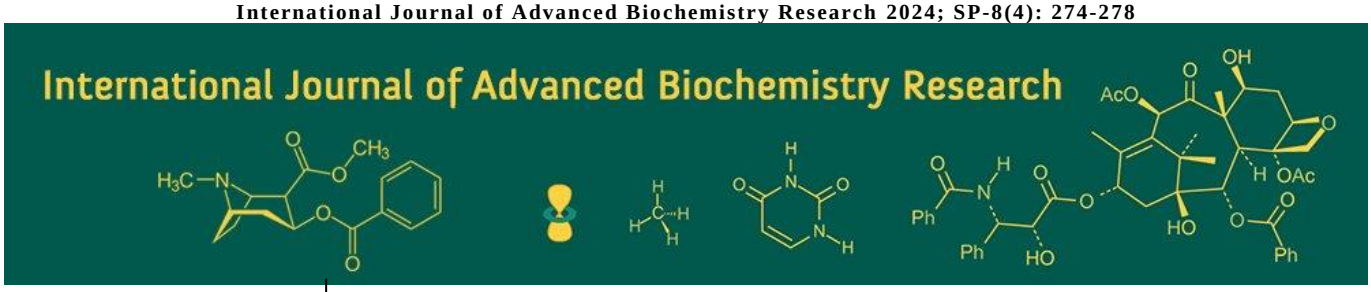

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Disposition profile of Danofloxacin in broiler chickens

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Abstract
Danofloxacin, a fluoroquinolones antimicrobial was exclusively used in veterinary clinical practice for treating a variety of bacterial infections in avian species. Accompanying other fluoroquinolones, Danofloxacin has broad-spectrum bacterial action against Enterobacteriaceae (*Escherichia coli*, *Klebsiella*, *Salmonella*) and some gram-positive *cocci*, such *Staphylococcus*. The pharmacokinetics studies in avian species are very scare and the there is huge gap of information on pharmacokinetic behaviour of Danofloxacin in broilers. The present study was done to explores the disposition kinetics of Danofloxacin after single dose intravenous administration at the dose rate of 5.0 mg/kg body weight. The study was performed on eight healthy broiler chicken, weighing between 0.9-1.2 kg. Danofloxacin concentrations were measured using the reversed-phase ultra-high-performance liquid chromatography (UHPLC) with UV detector. A non-compartmental technique was used to analyse the plasma concentration-time data. Following single dose intravenous administration of Danofloxacin at the dose rate of 5.0 mg/kg, the immediate plasma concentration of Danofloxacin at 0.083 h was observed as 2.97 ± 0.05 µg/mL, which rapidly declined to 0.17 ± 0.01 µg/mL at 12 h. The mean value of elimination rate constant (β) was found as 0.15 ± 0.002 h^{-1}. The drug was eliminated from the body of bird with an average half-life ($t_{1/2\beta}$) of 4.64 ± 0.07 h. The mean values of area under curve (AUC) and mean residence time (MRT) were observed to be 7.63 ± 0.11 µg.h/mL and 5.87 ± 0.09 h, respectively. The average values of volume of distribution ($V_{d(area)}$) and volume of distribution at steady state ($V_{d(ss)}$) were calculated as 4.40 ± 0.06 and 3.85 ± 0.03 L/kg, respectively. The body of bird was able to eliminate the drug from the body at a rate was found to be 0.66 ± 0.01 L/h/kg, which was indicated by total body clearance (CL_B). Danofloxacin has clinically superior pharmacokinetic properties, including the longer elimination half-life, wide volume of distribution and slower clearances in broiler chicken. The pharmacokinetics data obtained from this study may serve an important tool for PK/PD relationship to optimize the dosage regimen of Danofloxacin targeted for the treatment of diseases caused by susceptible bacteria with known minimum inhibitory concentration values in broiler chicken.

Keywords: Disposition, Danofloxacin, broiler, chickens

Introduction
The second-generation animal-specific fluoroquinolone, developed by Pfizer Inc. danofloxacin, is a fluorinated quinolone that is used to treat and prevent bacterial infections in domestic animals and birds. It is a promising antimicrobial for poultry due to its bactericidal effect, wide range of activity, and concentration-dependent killing mechanism. (Brown, 1996; Gosal *et al.*, 2009; Sarasola *et al.*, 2002) [3, 10, 13]. Being a fluoroquinolone, It acts on bacterial DNA synthesis via inhibition of bacterial DNA gyrase and topoisomerase IV, compromising bacterial DNA replication (Drlica and Zhao, 1997) [7]. The clinical prospect of the danofloxacin is promising because of greater cell permeability, larger plasma and tissue drug concentrations, and more potent antibacterial activity (Wang *et al.*, 2022) [14]. The European Medicines Agency has authorized the use of danofloxacin in all food-producing species (EMEA, 2002) [8].
The use of antibiotics to prevent and treat infectious diseases is critical to the profitability of broiler production. However, emergence of antimicrobial resistance has caused the loss of effectiveness of the conventional antimicrobials. In this case, danofloxacin may be a potential antimicrobial for broiler production. However, before introducing it into clinical practice, pharmacokinetics data must be obtained to determine the ideal dosing schedules suitable to local climatic and management practice. Hence, to fulfil the objectives of generating pharmacokinetics data applicable for calculating optimum dosage regimens, the

present investigation on disposition kinetics of danofloxacin at a dosing rate of 5.0 mg/kg body weight after single dose intravenous administration in broilers was carried out.

Materials and Methods

Animal treatment

A total of 8 healthy broiler chickens at the age of 3 to 3.5 weeks, weighing between 0.9 to 1.2 kg were obtained from a registered commercial poultry farm. Each chicken was individually placed in a wire cage measuring 55 × 55 × 45 cm, which was equipped with an automatic water dispenser. All the chickens were provided with an antimicrobial-free diet and had unrestricted access to water. The feeding environment was maintained at a temperature of 21 ± 0.6 °C, with proper ventilation, and a daily light exposure of 16 h. Before beginning of the study procedures, the chickens were given a two-week acclimation period, during which the health of birds was monitored clinically through daily examination. The study protocol received approval from the Institutional Animal Care and Use Committee (IACUC) at College of Veterinary Science and Animal Husbandry, Sardarkrushinagar with the approval number VETCOLL/IAEC/2022/20/PROTOCOL-31.

Chemicals and reagents

The analytical standard powder (Certified reference Material) of danofloxacin was purchased from Sigma-Aldrich (USA) having batch number BCCD0344 and lot number CAS# 112398-08-0. The purity of assay was more than 99.6%. Water, methanol, perchloric acid (70-72%), formic acid, triethylamine and acetonitrile of HPLC grade were purchased from S.D. Fine chemical Ltd., Mumbai.

Experimental protocol

All broiler chicken were given danofloxacin by intravenous route at the dose rate of 5 mg/kg body weight via brachial vein using 25 G needle. Periodical blood samples were collected from the poultry in sterilized and heparinized test tube at 0 minutes (before administration), 5 minutes (0.083 hours), 10 minutes (0.167 hours), 30 minutes (0.5 hours), 1 hours, 2 hours, 4 hours, 8 hours, 12 hours, and 24 hours. Approximately 0.2 mL of blood was collected at every interval. The plasma was separated after centrifugation of blood samples at 4000 revolution per minute for 10 minutes. The plasma samples were transferred in cryo-vials (0.5 mL capacity) and stored at -20 ºC until assayed for danofloxacin concentration using UHPLC procedure.

Danofloxacin quantification

Danofloxacin was extracted from plasma sample by method suggested in Abo-El-Sooud *et al.* (2017) [1] with few modifications. To 100 µl aliquot of chicken plasma, 100 µl of 20% perchloric acid was added. The mixture was vortexed for 1 minute, and then centrifuged at 10,000 rpm for 10 min at 4 ºC. An aliquot of the supernatant solution was transferred to another Eppendorf tube, aliquot of 20 µl was injected into UHPLC system.

UHPLC instrumentation

The concentration of danofloxacin was determined using the Thermofischer's Dionex Series UHPLC system, which was connected to pump (Dionex ultimate 3000) and UV detector (model 2020 Plus), and controlled by Chromleon Software (Version 6.8). The separation process utilized a (Chromolith RP – 18e, 100 × 4.6 mm, Merck KGaA, Germany). Each sample (20 µL) was injected, and the column was maintained at room temperature for elution over a duration of 12 min. UV detection was carried out at 282 nm wave length. The isocratic mobile phase consisted of 0.01 M formic acid buffer (prepared in HPLC water): acetonitrile (82:18 v/v %) and pumped into system at a flow rate of 0.5 mL/min. To prepare buffer containing 0.01 M formic acid, exactly 460 µl of formic acid was dissolved in 1.0 litter of HPLC water and pH was adjusted to 3.2 by adding 400 µl of triethylamine

Validation of analytical method

The method was validated prior to it's deployment in quantification of danofloxacin from broilers' plasma samples for study. The validation criteria calculated were the linearity, accuracy, and precision (intraday and inter-day) as per CDER guidelines (CDER, 1994) [4]. The quantification of danofloxacin was observed to be linear over the concentration range of 0.10 to 16.00 µg/mL in chicken plasma with regression co efficient value of 0.9991. The intra-day and inter-day coefficients of variation for three samples were satisfactory, with relative standard deviations (RSD) less than 5.6%. Mean values of recovery of danofloxacin from chicken's plasma was observed to as 95%.

Pharmacokinetics

With the aid of the software "PK solver 2.0," a menu-driven add-in programme for Microsoft Excel created in visual basic for application (VBA), the plasma concentration time curve of individual chicken was examined for derived pharmacokinetic parameters. Non-compartmental analysis performed by this software is based on the basic theory of statistical moment concepts. The mean values along with the standard error (Mean ± SE) were calculated for plasma concentration analysed and data generated after pharmacokinetics using the MS-Excel programme.

The concentration–time data of danofloxacin in each broiler chicken plasma were analysed using the non-compartment model analysis (NCA) method in "PK solver 2.0," (a menu-driven add-in programme for Microsoft Excel created in visual basic for application VBA) to obtain pharmacokinetic parameters. For intravenous routes of danofloxacin administration, the first-order rate constant associated with the terminal phase (λz) was calculated using linear regression, from which the terminal half-life ($t_{1/2\lambda z}$) was determined as $\ln 2/\lambda z$. The area under the concentration–time curve (AUC) and the first moment curve (AUMC) were calculated using the linear trapezoidal rule with extrapolation to infinity. AUC% represents the ratio of $AUC_{last-\infty}$ to $AUC_{0-\infty}$. The mean residence time (MRT) was calculated as $MRT = AUMC_{0-\infty}/AUC_{0}$. Total body clearance (Cl) was determined as the ratio of the intravenous dose to AUC, while the volume of distribution (V_Z) was calculated as V_Z = Dose/AUC/λz, where Dose represents the intravenous dose. The volume of distribution at steady state (V_{SS}) was determined as $V_{SS} = MRT_{IV} \times Cl$. (Jo *et al.* 2023) [20].

Results and Discussion

The extraction and quantification methods employed for danofloxacin in present study showcased robust specificity, high accuracy and precision, as well as excellent selectivity,

in detecting danofloxacin in plasma. The retention time of danofloxacin was determined to be approximately 6.8 min, effectively avoiding interference from impurity peaks. The linearity of employed quantification method was observed over the concentration range of 0.1 to 16 µg/mL. With an R^2 of 0.9991, the regression equation for the calibration curve was $C = 7 \times 10^{-9} S + 0.0021$, (where C is the predicted danofloxacin concentration and S is the peak area of danofloxacin in the chromatogram). Under these conditions, the recovery rate of danofloxacin was above 95% (at concentration of 0.1, 1 and 10 µg/mL). the intra-day variation coefficient ranged from 1.35% to 4.15%, and the inter-day variation coefficient ranged from 0.33% to 5.67%.

All the broilers birds were free from any adverse effects associated with intravenous administration of danofloxacin (5 mg/kg body weight) and remained apparently healthy throughout experiment and even after experiment.

The assayed plasma concentrations of danofloxacin at different time points after its single dose intravenous (IV) administration at the dose rate of 5 mg/kg in birds are tabulated as the mean and standard error (SE) values for all eight birds in Table 1 and graphically represented by semi-logarithmic plot of mean danofloxacin concentration in plasma versus time following intravenous administration (Figure 1).

Mean danofloxacin concentration in plasma at the first collection time point i.e., 0.083 h (5 minutes) after drug administration was found to be 2.97 µg/mL and declined by about 57% to 1.30 µg/mL at 0.5 h. The average plasma danofloxacin concentration at 1, 2, 4, 8 and 12 h were found to be 1.12, 0.80, 0.53, 0.29 and 0.17 µg/mL, respectively. The plasma concentrations were not detectable in the post-treatment samples collected at 24 and 36 h in all eight birds.

In the present study, the mean plasma concentration of danofloxacin after IV administration at the dose rate of 5.0 mg/kg body weight of chicken was maintained above the therapeutic concentration up to 12 h (0.17 µg/mL), which covers MIC_{90} values of 0.13 µg/mL against most susceptible bacterial infections in broiler chicken (Watts *et al.*,1997) [5].

Table 1: Plasma concentrations of danofloxacin following single dose intravenous administration at the dose rate of 5 mg/kg body weight in birds (n=8)

Time point (h)	Plasma danofloxacin concentration (µg/mL)	
	Range	Mean ± SE
0.083	2.36 – 3.36	2.97 ± 0.05
0.167	1.84 – 2.75	2.45 ± 0.04
0.5	1.07 – 1.44	1.30 ± 0.02
1	0.91 – 1.26	1.12 ± 0.02
2	0.74 – 0.88	0.80 ± 0.01
4	0.43 – 0.71	0.53 ± 0.01
8	0.23 – 0.38	0.29 ± 0.01
12	0.13 – 0.26	0.17 ± 0.01
24	ND	ND

ND - Not detected

Table 2: Pharmacokinetics (PK) parameters of danofloxacin following single dose intravenous administration (5 mg/kg b. wt.) in birds (n=8)

Pk parameter	Unit	P1	P2	P3	P4	P5	P6	P7	P8	Mean ± SE
B	h^{-1}	0.16	0.13	0.15	0.19	0.16	0.16	0.14	0.13	0.15 ± 0.002
$t_{1/2\beta}$	h	4.33	5.49	4.59	3.71	4.28	4.44	5.01	5.28	4.64 ± 0.07
Cp^0	µg/mL	3.49	3.53	3.33	5.09	5.07	3.03	3.17	4.09	3.85 ± 0.10
$AUC_{0\rightarrow\infty}$	µg.h/mL	7.78	9.62	6.99	7.23	6.76	7.59	7.43	7.64	7.63 ± 0.11
MRT	h	5.89	7.39	5.52	4.82	5.38	6.05	5.87	6.07	5.87 ± 0.09
$V_{d(area)}$	L/kg	4.01	4.12	4.73	3.70	4.56	4.22	4.86	4.99	4.40 ± 0.06
V_{dss}	L/kg	3.78	3.84	3.94	3.33	3.98	3.98	3.95	3.97	3.85 ± 0.03
Cl_B	L/h/kg	0.64	0.52	0.71	0.69	0.74	0.66	0.67	0.65	0.66 ± 0.01

(Notations used:- β: Elimination rate constant; $t_{1/2\beta}$: Elimination half-life; Cp^0: Theoretical concentration of drug in plasma at zero-time; $AUC_{0\rightarrow\infty}$: Area under the plasma concentration–time curve from time 0 to ∞; MRT: Mean Resident Time; $V_{d(area)}$: Apparent volume of distribution; V_{dss}: Volume of distribution at steady state; Cl_B: Total body clearance)

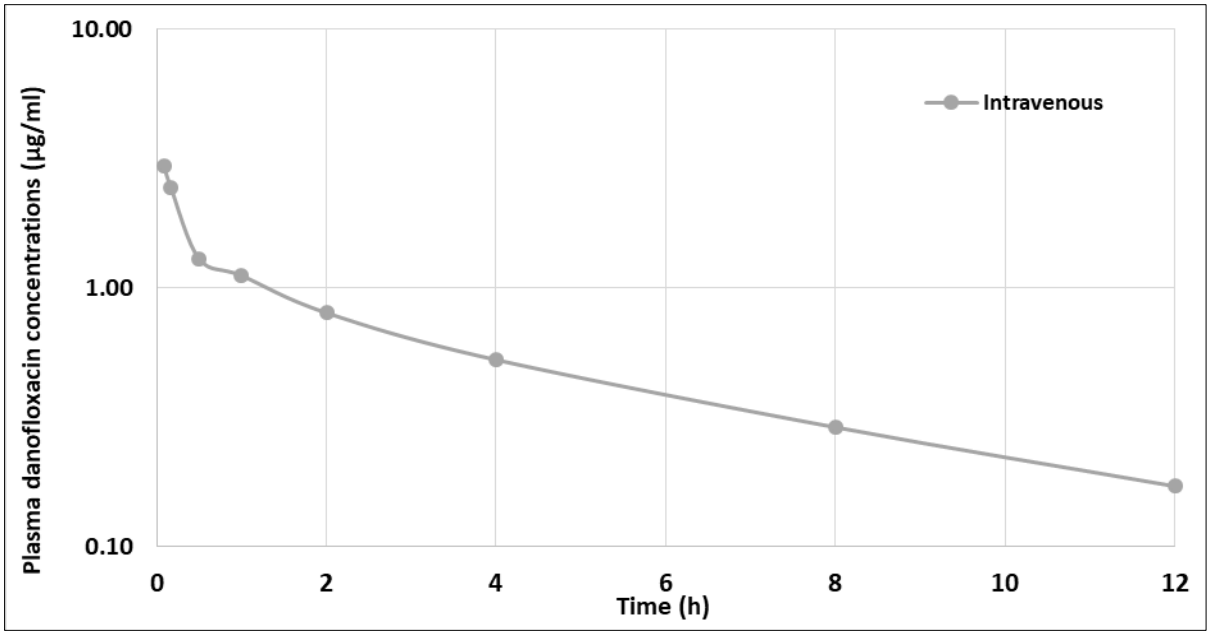

Fig 1: Semi logarithmic plot of mean ± SE concentration of danofloxacin in plasma following IV administration (5.0 mg/kg body weight) in broiler chicken

The pharmacokinetic (PK) parameters calculated from plasma concentrations of danofloxacin after its single dose IV administration (5 mg/kg body weight) in birds are shown in Table 2. The calculation of pharmacokinetic parameters was based on a non-compartmental approach. The results were expressed as a mean ± SE value of eight birds. The pharmacokinetics is not reported in any species in India. There are few references in other species of animals abroad. The value of the elimination rate constant (β) was observed to be 0.15 ± 0.002 h^{-1} which was in close approximation to values reported in chicken as 0.12 h^{-1} (Lych *et al.*, 1994) [12]. The elimination half-life ($t_{1/2\beta}$) was shown to be 4.64 ± 0.07 h comparatively shorter half-lives were observed in guinea fowl (3.31 h) and quils (3.84 h) by Dimitrova *et al.* (2014) [6]. Higher value of elimination half-life was reported in pheasant (6.82 h) by Dimitrova *et al.*, 2014 [6] and non-laying hen (7.69 ± 3.40 h) by Chen *et al*., 2023 [19]. Variation in half-lives because of properties of the body fluids of birds, including volume status, protein content, body fluid pH and presence of drugs which compete for binding sites, pathophysiological states, including age, gender, obesity and blood delivery to organs of clearance (Yartsev, 2023a) [16]. The values of volume of distribution at steady state (V_{dss}) and apparent volume of distribution ($V_{d(area)}$) were observed to be 4.40 and 3.85 L/kg, respectively. Because it is sufficiently lipid-soluble to penetrate tissues and is supported by large distribution volumes of danofloxacin. Higher concentrations of danofloxacin in tissues than in plasma of broiler chickens (Knoll *et al*., 1999) [11]. The value of volume of distribution at steady state obtained in present study was close to the values observed to be 4.20 L/kg in guinea fowl and 5.81 L/kg in quil (Dimitrova *et al.*, 2014) [6]. Higher values also reported as 8.57 L/kg in pheasant (Dimitrova *et al.*, 2014) [6], 10.02 L/kg in chicken (Knoll *et al.*, 1999) [11]. Drug characteristics that alter tissue and protein binding are the main factors of volume of distribution. These include the lipid/water partition coefficient, pKa, charge, and molecule size. Other physiological factors like age, gender, body muscle/fat proportion, level of hydration and water distribution also affect the volume of distribution in birds (Gibaldi and McNamara, 1978; Yartsev, 2023b) [9, 17]. The value of the area under time concentration curve from 0 to ∞ ($AUC_{0\rightarrow\infty}$) was observed to be 7.63 µg.h/mL. It suggested that danofloxacin covers a vast body area upon intravenous administration. Very close value of area under time concentration curve from 0 to ∞ ($AUC_{0\rightarrow\infty}$) was reported to be 8.29 µg.h/mL in guinea fowl (Dimitrova *et al.*, 2014) [6]. The lower values of area under concentration time curve from 0 to ∞ ($AUC_{0\rightarrow\infty}$) reported in chicken (3.55 and 4.07 µg.h/mL) by Knoll *et al.*, 1999 [11] and Lynch *et al.*, 1994, respectively, pheasant (4.90 µg.h/mL) and quils (6.49 µg.h/mL) by Dimitrova *et al.*, 2014 [6]. The difference in AUC values might be due to variation in the physiology of fluid compartment and total physiological body space available to the drug. The mean residence time (MRT) was calculated to be 5.87 ± 0.09 h. The value obtained in present study was consistent to value reported in quils (5.54 h) by Dimitrova *et al.*, 2014 [6]. Lower value of MRT was reported in guinea fowl (4.91 h) by Dimitrova *et al.*, 2014 [6] and higher value of MRT was reported in pheasant (9.84 h) by Dimitrova *et al.*, 2014 [6]. In present study low MRT value indicates that danofloxacin remains for shorter span of time in birds due to relatively faster elimination of drug compared to other species. The total body clearance (Cl_B) was observed to be 0.66 L/h/kg. Relatively faster values of total body clearance were reported in chicken (1.41 and 1.23 L/h/kg) by Knoll *et al.*, 1999 [11] and Lynch *et al.*, 1994 [12], respectively, guinea fowl (1.23 L/h/kg) and quills (1.61 L/h/kg) by Dimitrova *et al.*, 2014 [6]. Relatively slower value of total body clearance was studied in pheasant (0.45 L/h/kg) by Dimitrova *et al.*, 2014 [6]. The variation in values of clearance in the present study is due to individual factors that can influence clearance, such as the intrinsic functions of the liver or kidneys. Therefore, variations in clearance can be anticipated when there is a major impairment of these organs. Blood flow to the organs of elimination can also affect clearance.

Conclusions

It is concluded that, since general adverse reactions were not observed in any broiler chicken of the study, and in light of the favourable pharmacokinetic properties such as long half-life, slower clearance and volumes of distribution, danofloxacin administered at 5.0 mg/kg via intravenous routes could be effective in broiler chicken. However, further pharmacokinetics/pharmacodynamics studies are necessary for the treatment of diseases caused by susceptible bacteria with known minimum inhibitory concentration values in broiler chicken.

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