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Renal handling of ciprofloxacin in domestic ruminant species

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Abstract: Investigations regarding renal clearance and urinary excretion of ciprofloxacin were carried out in buffaloes, cows, sheep, and goats. The drug was administered intramuscularly at a dose of 5 mg/kg body weight. The plasma and urine concentrations of the drug were determined with HPLC. Renal clearance of endogenous creatinine was used as an index of glomerular filtration rate (GFR). In buffaloes, it was 0.72 ± 0.15 (mean ± SE), in cows 0.36 ± 0.09, in sheep 1.33 ± 0.35, and in goats 1.87 ± 0.38 mL/min/kg. The renal clearance of ciprofloxacin in buffaloes was 0.47 ± 0.08, in cows 0.29 ± 0.08, in sheep 0.39 ± 0.06, and in goats 0.98 ± 0.17 mL/min/kg. Besides glomerular filtration, ciprofloxacin was reabsorbed from the renal tubules of all ruminants and actively secreted in buffaloes. The cumulative percentage of intramuscular dose of ciprofloxacin excreted into the urine over 10 h in buffaloes was 10.04 ± 1.17, in cows 7.22 ± 1.12, in sheep 11.26 ± 1.74, and in goats 13.03 ± 2.07. Species difference in the urinary excretion of ciprofloxacin revealed that the excretion of the drug was highest in goats, followed by sheep, buffaloes, and cows. This species variation in the percentage of urinary excretion in these ruminants coincides with their respective GFR being the highest in goats and lowest in cows.

Key words: Ciprofloxacin, domestic ruminants, renal clearance, urinary excretion

Introduction

It has been observed that the pharmacokinetic behaviour, optimal dosage, renal clearance, and urinary excretion of many investigated drugs were different under local conditions when compared with the values given in the literature by Nawaz et al. (1) and Javed et al. (2,3). The fluoroquinolones are synthetic antibacterial agents, used for the treatment of a variety of bacterial infections. All fluoroquinolones exhibit such distributional and antimicrobial properties that make them potentially useful in veterinary medicine. Vancutsem et al. (4) reported that these have extensive application in clinical practice because of their good bioavailability and pharmacokinetic profile, arousing great interest in the field of chemotherapy. Ciprofloxacin is an important member of the fluoroquinolone group of antibiotics. It is a broad spectrum antibiotic used to combat various infectious diseases in humans and animals (5). However, the biodisposition of ciprofloxacin has not been studied in local ruminant species. Keeping in view the vast clinical use of ciprofloxacin in local animals, the present study was designed to determine the renal handling of this drug in domestic ruminant species.

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Materials and methods

Experimental animals

Renal clearance and urinary excretion of ciprofloxacin were investigated in domestic ruminants, 8 each in Nili Ravi buffaloes (368 ± 44 kg), Sahiwal cows (280 ± 13 kg), Lohi sheep (40 ± 2 kg), and Teddy goats (35 ± 2 kg). In this way a total of 32 experiments were conducted. All the ruminant animals were clinically healthy adult females maintained under similar environmental and management conditions at the Livestock Experimental Farm, Institute of Nutrition and Feed Technology, University of Agriculture, Faisalabad, Pakistan. All the animals were stall fed with dry wheat straw and green fodder of the season. The animals had free access to drinking water. The experiments were performed during November and December 2006.

Methodology

In each animal, one of the jugular veins was cannulated in the standing position under strict aseptic conditions with plastic cannula No. 90 (Protex Ltd., England) for the collection of blood samples. A sterilised disposable balloon catheter (Rush No. 14, 30 mL) was inserted into the urinary bladder through the urethra of each animal. The external opening of the catheter was connected through rubber tubing to a urine collecting reservoir in which all the voided urine was quantitatively collected. A commercial injectable preparation of ciprofloxacin, CIPROCIN-100®, batch No. 539009 (Han Dong Corporation Ltd., Korea) was used in these studies. Each millilitre of the injection contained 100 mg of ciprofloxacin. A single dose of the above-mentioned preparation of ciprofloxacin was injected intramuscularly into the neck region at a dose rate of 5 mg/kg body weight. In all experiments, prior to drug administration, a blank blood sample and blank urine sample were collected. For renal clearance studies, the urinary bladder was emptied completely and washed with distilled water through the catheter 45 min after the drug administration. After washing, the first urine sample was collected at 75 min and then more samples were collected at 105, 135, and 165 min, post-drug administration. The volume of each urine sample was measured. Following drug administration, blood samples were drawn at 30, 60, 90, and 120 min. Blood samples were collected in heparinized plastic centrifuge tubes. The pH of blood and urine samples was recorded by a pH meter (Beckman HS, Germany) with a glass electrode at 37 °C. Blood samples were centrifuged and plasma was separated and stored at -20 °C until analysis. For the study of urinary excretion, the urine voided until 4, 6, 8, and 10 h after drug administration was collected quantitatively. The pH of all urine samples was recorded as mentioned earlier.

Analytical procedures

Ciprofloxacin

Ciprofloxacin concentration in plasma and urine was determined using HPLC according to the method described by Soback et al. (6). Chromatography was performed with a high performance liquid chromatograph (Sykam, S-1122) and analytes were determined using UV/Vis detector (Sykam, S-3210). The output of the detector was monitored with computer software (Peak Simple Chromatography Data System, Buck Scientific Inc., East Norwalk, CT, USA). A stainless steel column packed with YMC pack A-312 (Thermo Hypersil-Keystone, BDS-C_{18} with 250 × 4.6 mm dimensions and 5 µm particle size) was used. The column was protected with a pre-column (Guard-Pak™) filled with a µBondapak™ C_{18} cartridge (Thermo Hypersil, England). Separation of ciprofloxacin was achieved at 37 °C, using an isocratic mode. The mobile phase consisted of a mixture of 800 mL of 14 mL/L phosphoric acid and 200 mL of acetonitrile per litre. The UV detector was set at 275 nm and the flow rate was 1 mL/min. The lower limit of detection was 0.075 µg/mL while the limit of quantitation was 0.25 µg/mL.

For the determination of the ciprofloxacin concentration in the plasma samples, in a centrifuge tube 2 mL of acetonitrile was added to 1 mL of plasma or plasma calibrator. The mixture was vortexed for 1 min and centrifuged for 30 min at 4000 rpm. The supernatant was transferred into a glass tube and evaporated to dryness in a water bath. The residue was then reconstituted in 10 µL of IS and 1 mL of 14 mL/L phosphoric acid. The final solution was again vortexed for 30 s and filtered through cellulose acetate membrane filter (Sartorius AG, Germany, 13 mm diameter and 0.2 µm pore size), and 20 µL was injected into the HPLC system.

For determination of ciprofloxacin concentration the urine samples were diluted 1:20 (by volume) with the mobile phase. In a centrifuge tube 10 μL of the working solution of paracetamol was added to 1 mL of the diluted urine. The mixture was vortexed and filtered, and 20 μL was injected directly into the HPLC system.

Creatinine

The concentration of creatinine in plasma and urine samples was determined as described by Thomas (7) with the help of BTS-330 (Bio-Systems S.A., Spain) according to the Jaffe reaction. Creatinine forms an orange-red complex in alkaline picrate solution. The difference in absorbance at fixed times during the conversion is proportional to the concentration of creatinine in the sample. For this purpose 50 μL plasma sample was mixed with 1000 μL a mono reagent having 4 parts of 0.16 mol/L sodium hydroxide and 1 part of 4.0 mmol/L picric acid and the first absorbance was read after 60 s and the second absorbance after a further 120 s. Concentration of creatinine was determined from the difference between these 2 absorbance values. The creatinine concentration in urine samples was determined by the same method but samples were diluted 50 times with the distilled water before mixing them with mono reagent as the creatinine concentration is several-fold higher in urine as compared to plasma.

Calculations

Ciprofloxacin

The concentration of ciprofloxacin in plasma and urine samples was determined by the following regression equation: $y = bx + a$, where y = peak area for unknown concentration of ciprofloxacin determined through HPLC, a = intercept, b = slope of the regression line, and x = concentration of ciprofloxacin.

Renal clearance

The renal clearance of endogenous creatinine was used for the estimation of glomerular filtration rate (GFR). Renal clearance of ciprofloxacin and endogenous creatinine was calculated by the formula $Cl_{ren} = Uc \times Uv/Pc$, where Cl_{ren} is renal clearance, and Uc and Pc are concentrations of a substance in urine and plasma, respectively. Uv is urine rate flow (diuresis, mL/min/kg). Influence of urine pH, rate of urine flow, and plasma drug concentration on the renal clearance of drug was examined by regression/correlation analysis.

Urinary excretion

The mean ± SE (standard error of mean) values for the ciprofloxacin in the urine samples of buffaloes, cows, sheep, and goats at different time intervals after the intramuscular injection were calculated. The urine concentrations versus time data were used to determine the cumulative percentage of the dose excreted until 10 h after the intramuscular drug administration.

Statistical analysis

The mean values and standard error of mean (±SE) for each concentration and parameter were calculated. The relationship between the urine flow, blood pH, and plasma concentration of the drug was calculated with regression correlation analysis. Statistical analysis was carried out with PC-program MStat-C by Freed, R.D. and Eisensmith. S.P., Michigan State University, USA, and the figures were prepared using Microsoft Excel version 2003.

Results

Renal clearance

Mean ± SE values of diuresis, plasma, and urine concentration and renal clearance of endogenous creatinine and ciprofloxacin for 32 sampling periods, 4 each in 8 buffaloes, cows, sheep, and goats are presented in the Table.

The values of rate of urine flow were 0.012 ± 0.003 mL/min/kg in buffaloes, 0.01 ± 0.003 mL/min/kg in cows, 0.032 ± 0.007 mL/min/kg in sheep, and 0.073 ± 0.014 mL/min/kg in goats. The pH in blood and urine was 8.41 ± 0.03 and 9.08 ± 0.05, 8.41 ± 0.03 and 9.08 ± 0.05, 7.29 ± 0.02 and 8.18 ± 0.01, and 7.29 ± 0.02 and 8.18 ± 0.01, respectively, in buffaloes, cows, sheep, and goats. The mean ± SE values for the concentration of endogenous creatinine in plasma and urine were 18.94 ± 0.60 and 1132.7 ± 44.85 μg/mL, 20.37 ± 0.36 and 778.12 ± 51.86 μg/mL, 15.69 ± 0.59 and 615.6 ± 37.6 μg/mL, and 14.47 ± 0.50 and 364.1 ± 13.2 μg/ml in buffaloes, cows, sheep, and goats, respectively,

Table. Mean ± SE values for body weight, diuresis, plasma and urine concentrations and renal clearance of endogenous creatinine and ciprofloxacin in 8 female buffaloes, cows, sheep and goats following a single intramuscular administration of ciprofloxacin at dose rate of 5 mg/kg body weight.

Ruminant species	Body weight (kg)	Diuresis mL/min/kg	pH		Creatinine conc. µg/mL		Ciprofloxacin conc. µg/mL		Renal clearance mL/min/kg		Ratio Cip/Creat.
			Blood	Urine	Plasma	Urine	Plasma	Urine	Creat.	Cipro.	
Buffaloes	367.6 ± 47.39	0.012 ± 0.003	8.41 ± 0.03	9.08 ± 0.05	18.94 ± 0.60	1132.7 ± 44.85	3.84 ± 0.19	156.80 ± 9.25	0.72 ± 0.15	0.47 ± 0.08	0.69 ± 0.06
Cows	279.5 ± 13.55	0.010 ± 0.003	8.41 ± 0.03	9.08 ± 0.05	20.37 ± 0.36	778.12 ± 51.86	3.42 ± 0.21	97.75 ± 5.31	0.36 ± 0.09	0.29 ± 0.08	0.78 ± 0.05
Sheep	39.87 ± 2.30	0.032 ± 0.007	7.29 ± 0.02	8.18 ± 0.01	15.69 ± 0.59	615.6 ± 37.6	1.48 ± 0.09	18.60 ± 2.90	1.33 ± 0.35	0.39 ± 0.06	0.37 ± 0.07
Goats	35.38 ± 1.97	0.073 ± 0.014	7.29 ± 0.02	8.18 ± 0.01	14.47 ± 0.50	364.1 ± 13.2	1.38 ± 0.15	18.60 ± 2.90	1.87 ± 0.38	0.98 ± 0.17	0.57 ± 0.08

Each data point shows mean of 32 observations in 8 experiments, each consisting of 4 experimental periods.

while the respective values of ciprofloxacin concentration in buffaloes, cows, sheep, and goats were 3.84 ± 0.19 and 156.80 ± 9.25 µg/mL, 3.42 ± 0.21 and 97.75 ± 5.31 µg/mL, 1.48 ± 0.09 and 18.60 ± 2.90 µg/mL, and 1.38 ± 0.15 and 18.6 ± 2.90 µg/mL. The renal clearance of endogenous creatinine and ciprofloxacin was in buffaloes 0.72 ± 0.15 and 0.47 ± 0.08 mL/min/kg, in cows 0.36 ± 0.09 and 0.29 ± 0.08 mL/min/kg, in sheep 1.33 ± 0.35 and 0.39 ± 0.06 mL/min/kg, and in goats 1.87 ± 0.39 and 0.98 ± 0.17 mL/min/kg. The ratio between the clearance of ciprofloxacin and the clearance of endogenous creatinine was 0.69 ± 0.06, 0.78 ± 0.05, 0.37 ± 0.07, and 0.57 ± 0.08 in buffaloes, cows, sheep, and goats, respectively. In buffaloes the regression correlation analysis revealed a significant ($P < 0.05$) negative correlation ($r = -0.436$) between plasma concentration of ciprofloxacin and its renal clearance (Figure 1) while diuresis also revealed a significant ($P < 0.05$) positive correlation ($r = 0.369$) with renal clearance (Figure 2). However, urine pH ($r = 0.141$) did not show any significant correlation with the renal clearance of the drug. Both in cows and sheep the regression/correlation analysis did not show any influence of diuresis ($r = 0.143, 0.132$), pH of urine ($r = 0.050, 0.065$), or plasma concentration of ciprofloxacin ($r = -0.247, -0.213$) on the renal clearance of the drug. In goats a significant ($P < 0.05$) positive correlation ($r = 0.420$) between diuresis and the renal clearance of ciprofloxacin was observed (Figure 3). However, plasma concentration ($r = -0.087$) and urine pH ($r = 0.131$) did not show any influence on the renal clearance of the drug.

Urinary excretion

The mean ± SE values of the cumulative percentage of dose excreted for each species are presented in Figure 4. This figure shows that the cumulative percentage of dose of ciprofloxacin excreted at 10 h in the urine of goats was 13.03 ± 2.07, followed by 11.26 ± 1.74 in sheep, 10.04 ± 1.17 in buffaloes, and 7.22 ± 1.12 in cows.

Discussion

The primary mechanism of renal excretion for ciprofloxacin and all other fluoroquinolones is by glomerular filtration (8). However, Blum (9) reported that ciprofloxacin was eliminated by both the renal (glomerular filtration and tubular secretion) and non-renal (hepatic and trans-intestinal) routes.

In the present study the renal clearance of endogenous creatinine was 0.723 ± 0.148 mL/min/kg. The clearance value was lower than 1.74 mL/min/kg (10) but higher than 0.44 and 0.34 mL/min/kg in summer and winter, respectively (1), which appears to be attributed to the normal biological variations.

The mean value for renal clearance of ciprofloxacin in buffaloes was 0.469 ± 0.082 mL/min/kg. A significant ($P < 0.05$, Figure 1) negative correlation between plasma concentration of ciprofloxacin and ratio of renal clearance of ciprofloxacin and renal clearance of endogenous creatinine was attributed to the saturation of excretory mechanism at higher drug plasma levels, which is indicative of the involvement of active tubular secretion. Diuresis revealed a significant ($P < 0.05$, Figure 2) positive correlation with the renal clearance

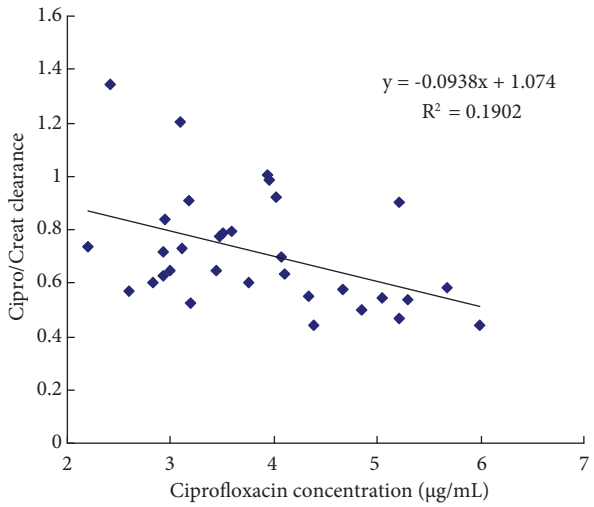

Figure 1. Effect of plasma concentration of ciprofloxacin on its renal clearance in buffaloes. Each data point shows 1 of the 32 observations in 8 experiments, each consisting of 4 experimental periods.

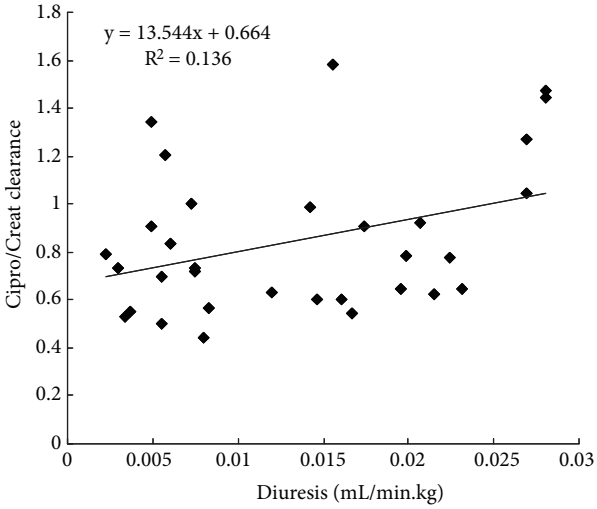

Figure 2. Effect of diuresis of ciprofloxacin on its renal clearance in buffaloes. Each data point shows 1 of the 32 observations in 8 experiments, each consisting of 4 experimental periods.

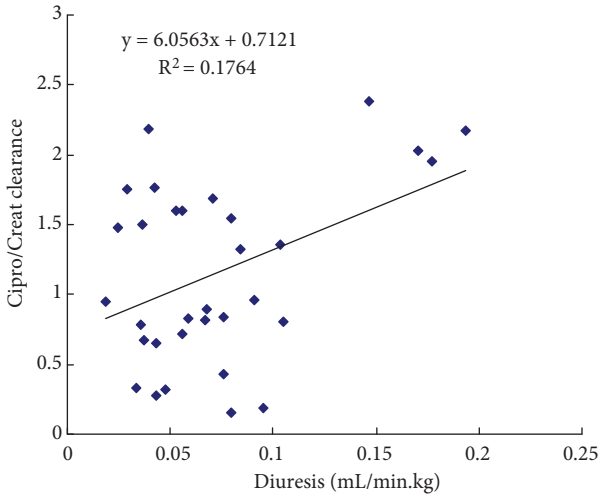

Figure 3. Effect of diuresis of ciprofloxacin on its renal clearance in goats. Each data point shows 1 of the 32 observations in 8 experiments, each consisting of 4 experimental periods.

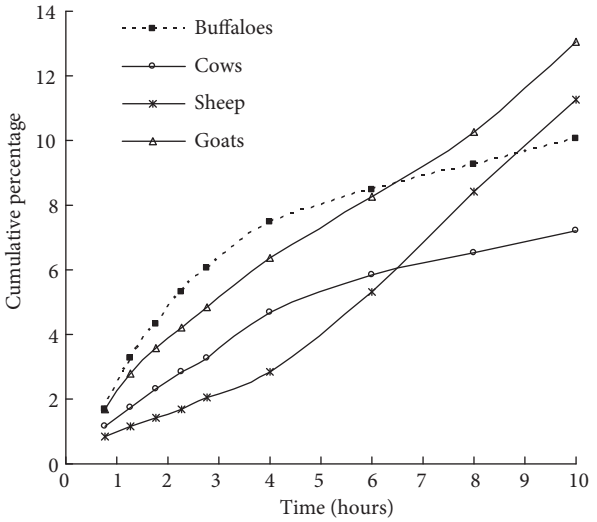

Figure 4. Mean cumulative percent of ciprofloxacin intramuscular dose (5 mg/kg) excreted in urine of ruminant species.

ratio. This indicates that with increasing diuresis renal clearance of ciprofloxacin also increased. This observation also indicates that at lower diuresis the drug had more time to stay in the tubules from where it would be reabsorbed. Thus the renal handling of ciprofloxacin in buffaloes besides glomerular filtration involved active tubular secretion and back diffusion also.

The renal clearance of endogenous creatinine was 0.362 ± 0.09 mL/min/kg, which was comparable to 0.49 mL/min/kg (3). However, this value was lower than 0.60 ± 0.08 mL/min/kg (11).

Analysis of the data revealed that the rate of urine flow, urine pH, and the plasma concentration of ciprofloxacin did not influence the renal clearance of

drug in cows. This indicates that the renal handling of ciprofloxacin in cows mainly involves the glomerular filtration.

The renal clearance of endogenous creatinine showed a mean value of 1.325 ± 0.35 mL/min/kg, which is comparable to previously reported values, e.g. 1.0 mL/min/kg (12), and also with those in ewes, 1.05 ± 0.06 mL/min/kg, and sheep, 1.13 ± 0.24 mL/min/kg (13). However, the clearance value in the present study is much higher than 0.042 ± 0.005 mL/min/kg (2) and lower than 17 mL/min/10 kg in sheep (14).

In sheep regression/correlation analysis revealed that there was no significant correlation between diuresis, plasma concentration of ciprofloxacin, and urine pH with the renal clearance of drug. Thus in sheep the renal excretion of ciprofloxacin mainly involved glomerular filtration.

The goats used in the present study showed a mean value for the renal clearance of endogenous creatinine of 1.87 ± 0.385 mL/min/kg. However, the value was lower than the clearance in Danish goats of 26.2 mL/min/10 kg (15) but comparable with the local goats, 1.43 and 1.86 mL/min/kg, during summer and winter, respectively (1).

The analysis of data on diuresis, urine pH, plasma concentration of ciprofloxacin, and renal clearance of endogenous creatinine and ciprofloxacin showed a significant ($P < 0.05$, Figure 3) positive correlation between diuresis and renal clearance of the drug, which is indicative of back diffusion. However, plasma concentration of ciprofloxacin and urine pH did not influence the renal excretion of the drug. Hence, renal handling of ciprofloxacin in goats involves glomerular filtration and back diffusion.

In indigenous buffaloes, cows, sheep, and goats GFR is lower in the subtropical environment when compared with the dwellers of temperate environments as has been explained by an original term "geonetics" reported by Nawaz et al. (1).

In all ruminant species the mean ± SE ratio of renal clearance of ciprofloxacin and renal clearance of endogenous creatinine was less than unity. This reflects the lower renal clearance of drug than the respective GFR in individual species (almost 2 times in buffaloes, 1 time in cows, 3 times in sheep, and 2 times in goats), which is indicative of back diffusion of the drug during its renal handling. It shows that during renal clearance of ciprofloxacin almost 31%, 22%, 63%, and 43% of the drug is reabsorbed at the kidney tubular level in buffaloes, cows, sheep, and goats, respectively. Hence, on the basis of these observations it is evident that besides glomerular filtration back diffusion is involved in all ruminant species during their renal excretion of the drug. However, regression/correlation analysis also evidenced the involvement of active tubular secretion and back diffusion in buffaloes while only back diffusion was manifested in goats.

Urinary excretion of endogenous and exogenous substances is manipulated by multiple mechanisms of glomerular filtration, tubular secretion, and resorption in a passive or active way. Drugs are regarded as interfering with homeostasis and thus must be excreted by means of existing mechanisms.

Cumulative percentage of dose of ciprofloxacin excreted at 10 h in the urine is maximum in goats (13.03%) and minimum in cows (7.22%), followed by intermediate values: 11.26% in sheep and 10.04% in buffaloes (Figure 4). The species variation in the percentage of urinary excretion in these domestic ruminants coincides with their respective GFR values being highest in goats, lowest in cows, and intermediate in sheep and buffaloes.

Higher urinary excretion, 28.3% of intravenous dose of ciprofloxacin after 24 hours, in buffalo calves has been reported by Saini and Srivastava (16). Besides ciprofloxacin, higher urinary excretion has also been reported for other fluoroquinolones. Urinary excretion of danofloxacin of 22.9% was observed after 48 h of the drug administration in buffalo calves reported by Sappal et al. (17). Montay et al. (18) documented 29% urinary recovery of the pefloxacin dose in mice, 37.8% in rats, 36.3% in dogs, 26.5% in monkeys, and 58.9% in humans. However, contrary to the above findings, Singh and Srivastava (19) reported a lower urinary excretion of ciprofloxacin of 10% of intramuscular dose in cross-bred cow calves.

Lower urinary excretion of ciprofloxacin in domestic ruminants in the current investigation may

be evidenced by the results regarding its renal handling. These results show that regardless the involvement of active tubular secretion in buffaloes 22%-63% of the administered dose of the drug has been absorbed at kidney tubular level. For ciprofloxacin being the zwitterions at physiological pH, the pKa values have been reported as 6.1 and 7.8 at its carboxylic and amino groups, respectively. Takács-Novák et al. (20) reported that ciprofloxacin is most lipophilic drug. Moreover, if we consider ciprofloxacin as a weakly basic drug with a pKa value of 7.8, it means that 50% of the drug will be un-ionized at pH 7.8. When the pH of urine in domestic ruminants increases, the drug will be more un-ionized. A high degree of un-ionization provides more of the drug for reabsorption and therefore high clearance values may not be observed when the pH of urine is high.

Renal clearance of endogenous creatinine in indigenous domestic ruminant species has been found lower than that of their foreign counterparts. Renal handling of ciprofloxacin besides glomerular filtration involved back diffusion. Active tubular secretion has been observed in buffaloes only. Urinary excretion of ciprofloxacin in domestic ruminant species was found to be lower than most literature values and coincides with their respective GFR values being the highest in goats, lowest in cows, and intermediate in sheep and buffaloes.

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