



Immunological and haematobiochemical studies of enrofloxacin with special reference to residue level following oral administration in goats

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ABSTRACT

Effect of enrofloxacin on haematobiochemical and immunological parameters alongwith the residual level of enrofloxacin and its metabolite ciprofloxacin in black Bengal goat was carried out following daily oral administration at 5 mg kg⁻¹ body weight for 54 days. A significant decrease in haemoglobin %, total erythrocyte and lymphocyte count were noticed in experimental group in different days. Blood glucose and serum protein were not altered in both the groups. A significant decrease in IgG level on different days along with lymphocytopenia and decreased lymphocyte number in lymphoid organs of experimental animals indicate immunosuppressive effect. Increased serum AST and ALT activity concomitant with fatty changes and necrosis of liver suggest hepatitis. Degeneration of seminiferous tubule and depletion of spermatogenic cells were observed in testis of goats of experimental group. Vital organs contain residues of enrofloxacin and ciprofloxacin.

Key words: Enrofloxacin, Goat, Immunosuppressive, Lymphocytopenia, Residues

Enrofloxacin, a synthetic fluoroquinolone antimicrobial has a strong, broad spectrum bactericidal effect. It is the most extensively used fluoroquinolone in veterinary medicine with MIC values ranging from 0.008 to 0.06 µg/ml. In recent years, concerns about the use of antimicrobial products in food-producing animals have focused on human food safety because foods of animal origin are identified as vehicles of food borne disease in humans. The goat is considered to be a good choice of experimental animal because its meat and milk are widely accepted by human beings throughout the globe. Effect of enrofloxacin on haematobiochemical parameters were studied in cattle (Davidson *et al.* 1998), buffalo (Javid *et al.* 2004) and yak (Khargharia *et al.* 2007). Residue of enrofloxacin in poultry meat was reported by Reyes-Hererra *et al.* (2005) and Salehzadeh *et al.* (2007). But necessary data with respect to haematobiochemical, immunological and residue level of enrofloxacin are scarcely available in black Bengal goats and therefore it is to be generated in this animal before it is widely used and declared safe to avoid possible public health hazards. With this concept, the present experiment studied the effect of

enrofloxacin on haematobiochemical, immunological and residue level following oral administration in black Bengal goats at therapeutic dose level.

MATERIALS AND METHODS

Test drug: Enrofloxacin was purchased as 50 mg tablet for this study. Enrofloxacin and ciprofloxacin analytical grade (>97% purity) were used as standard to run HPLC.

Experimental animals: Clinically healthy black Bengal male and female adult goats (1–1.5 years age) weighing between 9–12 kg were used in the present experiment. They were caged individually in custom made stainless steel metabolic cage. The animals were stall-fed and water was provided *ad lib*. The temperature of the animal room was maintained at 22°C (±3° C). Animals were also dewormed twice with a mixture of albendazole and rafoxanide at 21 days interval. After one month of last dosing of anthelmintic, the animals were acclimatized for 7 days.

Design of experiment: Black Bengal goats (12) were divided into 2 groups, each consisting 6 animals with 3 male and 3 female animals. The first group was kept as control whilst the second group was considered as experimental. Animals of experimental group were given enrofloxacin tablet orally at the dose rate of 5 mg kg⁻¹ daily for 54 days. All the 12 goats including control animals were sacrificed on day 60. The technical programme was approved by the Institution Animal Ethics Committee, Department of

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Collection of samples: Blood samples (8 ml) were collected from jugular vein of each goat of experimental group before administration of enrofloxacin (0 day), and thereafter at every 7 days interval for 54 days. Blood samples were also collected from animals of control group as stated time interval. Out of 8 ml, 2 ml of blood was kept in glass vial containing ethylene di-amine tetraacetic acid (EDTA) as anticoagulant for haemogram study (Schalm *et al.* 1986). A drop of blood was taken on a clean grease free glass slide and a tongue shaped smear was prepared for differential count of leucocytes. The remaining 6 ml of blood was allowed to clot for separation of serum for estimation of biochemical parameters and IgG (immunoglobulin G).

All the goats were sacrificed on day 60 and small pieces of spleen, prescapular lymphnode, liver, heart, kidney, testes, ovary and uterus of each goat were collected and preserved in 10% (v/v) formal saline for histopathology study. After slaughter, vital organs were collected from each goat for analysis of enrofloxacin and ciprofloxacin.

Estimation of serum immunoglobulin G (IgG) level: A sandwich ELISA (enzyme linked immunosorbent assay) was conducted to estimate total serum immunoglobulin G (Heyman *et al.* 1984) using microplate ELISA reader.

Estimation of enrofloxacin: Analysis of enrofloxacin and its metabolite ciprofloxacin in plasma and tissues was done by the HPLC method of Teja-Isavadharm *et al.* (1991) with some modifications. High performance liquid chromatography (column: 250 × 4.6 mm 5 μ Luna 100A C₁₈) was used for analysis of enrofloxacin and its metabolite ciprofloxacin. Mixture of 0.1 M phosphoric acid (pH 2.5, adjusted with a solution of 45% potassium hydroxide) and

acetonitrile (70:30) was used as mobile phase. The wave length and flow rate were 290 nm and 1.2 ml/min respectively. Standard and samples (20 μ l) were injected into HPLC with the first and last being the standard.

Biochemical estimation: Tissue protein (Wooton 1974), blood glucose (Frankel *et al.* 1970) and serum AST and ALT (Yatazidis 1960) were estimated.

Statistical analysis: The results were expressed as mean $\pm$ standard error (SE). The data were analyzed by 1-way analysis of variance (ANOVA) using Duncan's method.

RESULTS AND DISCUSSION

Haematological and immunological parameters: Haemoglobin level in experimental group reduced significantly ($P < 0.05$) which were 5.58 ± 0.08 , 5.95 ± 0.08 , 5.52 ± 0.10 , 5.53 ± 0.13 , 5.53 ± 0.13 , 5.57 ± 0.09 , 5.67 ± 0.07 gm dl⁻¹ on day 7, 14, 21, 28, 35, 42 and 49 respectively in comparison to 6.62 ± 0.09 gm dl⁻¹ on day 0. A significant decrease ($P < 0.05$) in TEC values in experimental group was also observed from 7.05 ± 0.04 ($\times 10^{12}/L$) on day 0 to 6.02 ± 0.10 , 6.10 ± 0.10 , 6.02 ± 0.06 , 6.00 ± 0.06 , 6.17 ± 0.07 , 6.02 ± 0.10 ($\times 10^{12}/L$) on day 7, 14, 21, 28, 35, 42 respectively. TLC values were not significantly altered in experimental group in different days compared to day 0. The values of lymphocyte in experimental group was significantly decreased ($P < 0.05$) from day 28 ($4599.67 \pm 41.78 \times 10^6/L$) onwards to day 49 ($4360.83 \pm 97.35 \times 10^6/L$) compared to day 0 ($4849.50 \pm 66.01 \times 10^6/L$). There were no significant changes occurred in monocytes and granulocytes in experimental group. Control group did not show any significant alteration of haematological values in different days.

Serum IgG level in experimental group was significantly decreased ($P < 0.01$) from day 0 (11.92 ± 0.16 mg ml⁻¹) to day

Table 1. Effect of enrofloxacin on AST, ALT, blood glucose, serum protein and IgG level in goat following consecutive oral administration at 5mg kg⁻¹ b.w for 54 days

Groups	Days							
	0	7	14	21	28	35	42	49
	Serum AST (μ g pyruvic acid/h/ml)							
Control	75.00 $\pm$ 1.24	75.00 $\pm$ 1.24	74.17 $\pm$ 1.30	71.33 $\pm$ 2.11	76.33 $\pm$ 1.15	75.21 $\pm$ 1.35	74.50 $\pm$ 1.12	76.33 $\pm$ 1.15
Experiment	77.00 ^a $\pm$ 0.97	77.00 ^{ad} $\pm$ 0.97	79.67 ^d $\pm$ 0.88	80.83 ^d $\pm$ 1.35	77.83 ^{ad} $\pm$ 1.89	77.83 ^{ad} $\pm$ 1.89	77.00 ^{ad} $\pm$ 0.97	77.83 ^{ad} $\pm$ 1.89
	ALT (μ g pyruvic acid/h/ml)							
Control	27.50 $\pm$ 0.56	24.33 $\pm$ 1.45	29.00 $\pm$ 1.06	28.00 $\pm$ 0.73	27.50 $\pm$ 0.76	26.50 $\pm$ 1.26	27.50 $\pm$ 0.76	26.50 $\pm$ 1.26
Experiment	25.67 ^a $\pm$ 1.15	29.67 ^c $\pm$ 0.88	30.67 ^c $\pm$ 1.28	34.83 ^d $\pm$ 0.95	25.67 ^a $\pm$ 1.15	25.83 ^{ac} $\pm$ 1.08	25.67 ^a $\pm$ 0.88	24.33 ^a $\pm$ 1.45
	Blood glucose (m mol L ⁻¹)							
Control	2.44 $\pm$ 0.05	2.43 $\pm$ 0.04	2.42 $\pm$ 0.03	2.41 $\pm$ 0.04	2.47 $\pm$ 0.04	2.43 $\pm$ 0.07	2.46 $\pm$ 0.06	2.48 $\pm$ 0.06
Experiment	2.44 $\pm$ 0.08 ^a	2.44 $\pm$ 0.05 ^a	2.56 $\pm$ 0.05 ^a	2.43 $\pm$ 0.07 ^a	2.41 $\pm$ 0.06 ^a	2.43 $\pm$ 0.04 ^a	2.44 $\pm$ 0.05 ^a	2.48 $\pm$ 0.05 ^a
	Serum protein (g L ⁻¹)							
Control	67.50 $\pm$ 0.76	67.00 $\pm$ 1.15	67.50 $\pm$ 0.76	67.50 $\pm$ 0.76	67.00 $\pm$ 1.46	67.00 $\pm$ 1.46	67.83 $\pm$ 1.35	68.83 $\pm$ 0.95
Experiment	67.17 $\pm$ 0.95 ^a	67.33 $\pm$ 1.33 ^a	67.50 $\pm$ 0.76 ^a	68.83 $\pm$ 0.95 ^a	68.00 $\pm$ 1.29 ^a	66.33 $\pm$ 1.12 ^a	64.67 $\pm$ 0.88 ^a	63.83 $\pm$ 1.01 ^a
	Serum IgG (mg ml ⁻¹)							
Control	11.21 $\pm$ 0.07	11.40 $\pm$ 0.13	11.40 $\pm$ 0.13	11.38 $\pm$ 0.14	11.23 $\pm$ 0.07	11.38 $\pm$ 0.14	11.27 $\pm$ 0.08	11.40 $\pm$ 0.13
Experiment	11.92 $\pm$ 0.16	6.97 ^c $\pm$ 0.12	7.53 ^b $\pm$ 0.16	6.98 ^c $\pm$ 0.30	7.10 ^{bc} $\pm$ 0.12	7.18 ^{bc} $\pm$ 0.09	7.03 ^{bc} $\pm$ 0.12	7.08 ^{bc} $\pm$ 0.14

Control group received no drug. Experimental group received enrofloxacin daily orally at 5mg kg⁻¹ b.w for 54 days; mean values of 6 replicates with S E; *mean values in rows bearing different superscripts differ significantly at $P < 0.05$.

49 ($7.08 \pm 0.14 \text{ mg ml}^{-1}$) with respect to day 0 (Table 1). The values of IgG were 7.03 ± 0.12 and $11.25 \pm 0.13 \text{ mg ml}^{-1}$ respectively in experimental and control group on day 60 (5th day post immunization). Serum level of IgG depends on correct function of the immunological system. Many factors have direct or indirect influence on antibody level in the body. One of the factors is antibacterial agent. In the present study enrofloxacin was administered orally in the experimental group and it is evident that enrofloxacin is responsible for the decreased level of serum IgG. It is logical to propose that antimicrobials should not impair the host's defense mechanisms against pathogens, particularly in immune-compromised patients. Therefore significantly decreased level of IgG (Table 1) concomitant with lymphocytopenia and depletion of lymphocytes in lymphoid organs may suggest immunodepression effect of enrofloxacin at therapeutic dose following 54 days administration. Stainslaw *et al.* (2002) also reported that enrofloxacin have a suppressive effect on the immunological system and observed the decrease level of IgG in laying hens following administration of enrofloxacin in drinking water for 10 days. So, the present study is corroborated with the result of Stainslaw *et al.* (2002).

Biochemical parameters in blood: No significant differences in the mean value of blood glucose among goats of different groups were found on different days. This result is supported by non significant changes in glucose level after repeated administration of levofloxacin in birds (Patel *et al.* 2009) and moxifloxacin in rat (Sadariya *et al.* 2010). The level of serum protein in experimental group did not show any significant changes in different days compared to day 0. A significant increase ($P < 0.05$) in serum AST activity (Table 1) was observed on day 14 and 21 compared to day 0. Tras *et al.* (2001) also found an increase in AST level following administration of enrofloxacin intramuscularly @ 5 mg/kg daily for 14 days. The goats of experimental group showed a significant increase ($P < 0.05$) in serum ALT activity

(Table 1) on day 7 to 21 compared to day 0. Kim *et al.* (2003) found an increase in ALT activity following 26 week oral administration of a fluoroquinolone in rat.

Histopathology: Section of liver in goats of experimental group treated with enrofloxacin orally showed fatty changes in the hepatocytes (Fig 1) and the fatty vacuoles pressed the nucleus of the hepatocytes. But section of liver of control animals received no drug revealed the absence of any fatty changes.

The result of increased serum AST and ALT activity indicates that enrofloxacin has caused damage to the liver. This is corroborative with the histopathological finding of fatty changes and necrosis in liver. The observation may suggest enrofloxacin as a hepatotoxic drug. Tras *et al.* (2001) also showed a temporary increase in AST and ALT activity in dogs following intramuscular administration of enrofloxacin at 5 mg kg^{-1} once in a day.

There were marked thickening of renal capsule and degeneration of tubules in kidney tissues and marked thickening of the capsule with depletion of lymphocytes in splenic tissues. Marked thickening of the trabeculae and depletion of lymphocytes in prescapular lymphnodes were also noticed. Section of testes of goats of experimental group showed degeneration of seminiferous tubules and depletion of spermatogenic cells (Fig 2). But no histopathological changes in ovary and uterus of goats of control and experimental group were found in corresponding slides. Degeneration of seminiferous tubules and depletion of spermatogenic cells suggest that enrofloxacin may have some effect on fertility of male goats. This result corroborates with the finding of Aral *et al.* (2008).

Residue level of enrofloxacin and its metabolite ciprofloxacin: Higher concentration of enrofloxacin and ciprofloxacin were recorded (Table 2 and 3) in liver, kidney and heart compared to the other organs. Concentration of enrofloxacin and ciprofloxacin in skeletal muscle were 160.67 ± 8.58 and $12.34 \pm 1.89 \text{ } \mu\text{g/gm}$ respectively. Residues

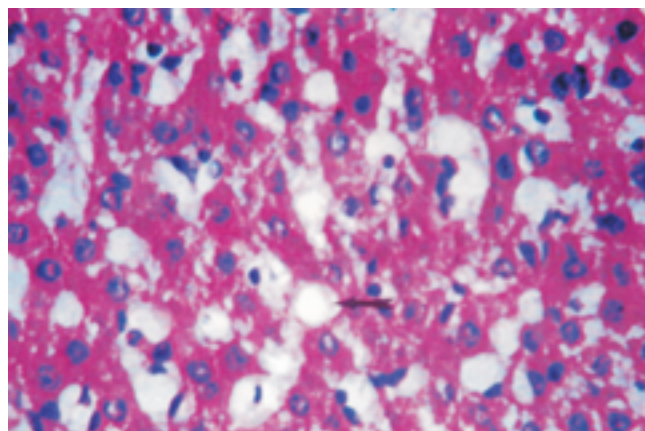


Fig 1. Section of liver showing fatty changes in the hepatocyte (H&E; 450 $\times$).

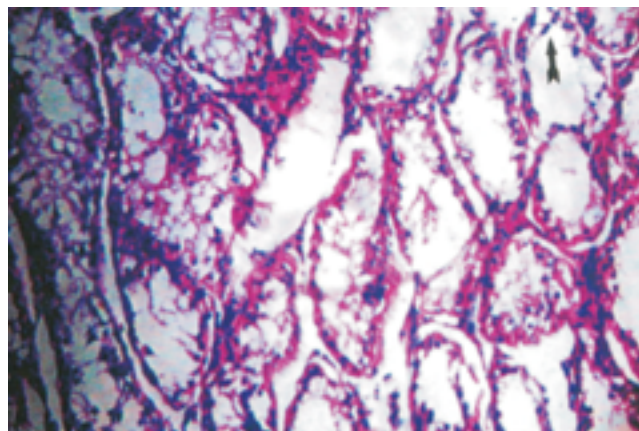


Fig 2. Section of testes showing degeneration of seminiferous tubules and depletion of spermatogenic cells (H&E; 450 $\times$).

Table 2. Plasma concentration of enrofloxacin ($\mu\text{g/ml}$) and its metabolite ciprofloxacin ($\mu\text{g/ml}$) in goats following daily oral administration at 5 mg kg^{-1} for 54 days
Mean values of 6 replicates with S E

Days	Plasma concentration($\mu\text{g/ml}$)	
	Enrofloxacin	Ciprofloxacin
7	20.84 $\pm$ 1.40	4.34 $\pm$ 1.15
14	21.50 $\pm$ 1.57	7.84 $\pm$ 1.17
21	34.67 $\pm$ 2.62	22.50 $\pm$ 2.65
28	45.67 $\pm$ 2.34	16.34 $\pm$ 1.09
35	61.17 $\pm$ 2.19	21.84 $\pm$ 2.00
42	129.84 $\pm$ 5.25	15.67 $\pm$ 1.48
49	139.67 $\pm$ 5.34	21.50 $\pm$ 1.79

Mean values of 6 replicates with SE.

Table 3. Tissue concentration of enrofloxacin($\mu\text{g/g}$) and its metabolite ciprofloxacin($\mu\text{g/g}$) in goats on day 60 following daily oral administration at 5 mg kg^{-1} for 54 days

Days	Tissue concentration ($\mu\text{g/g}$)	
	Enrofloxacin	Ciprofloxacin
Liver	165.00 $\pm$ 13.32	204.00 $\pm$ 9.12
Kidney	174.67 $\pm$ 6.49	107.00 $\pm$ 6.84
Heart	164.84 $\pm$ 13.28	68.00 $\pm$ 3.52
Skeletal muscle	160.67 $\pm$ 8.58	12.34 $\pm$ 1.89
Fat	32.67 $\pm$ 1.81	8.67 $\pm$ 1.88
Testes	64.00 $\pm$ 4.09	8.00 $\pm$ 1.99
Ovary	43.67 $\pm$ 3.00	7.34 $\pm$ 1.95
Uterus	33.67 $\pm$ 3.28	5.67 $\pm$ 1.40

Mean values of 6 replicates with SE.

of enrofloxacin and ciprofloxacin at high concentration in most of vital organs including skeletal muscle indicate that enrofloxacin has the higher affinity to accumulate in tissues which subsequently may cause public health hazards during consumption. Intorre *et al.* (1997) reported a 68% bioavailability of enrofloxacin after administration directly into crop while the metabolic conversion of enrofloxacin to ciprofloxacin was quite low (<10%) with both routes of administration of enrofloxacin in muscovy duck at a single dose of 10 mg kg^{-1} . The result therefore supports the findings of Intorre *et al.* (1997).

With the above findings it may be concluded that enrofloxacin may cause immunosuppression, hepatotoxicity and infertility in black Bengal goats following prolong exposure. Long term use of enrofloxacin should be done very cautiously as it may cause public health hazards because of its accumulating tendency in tissues.

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