

Biochemical, histopathological and ultra structural profile after pulsed water medication of enrofloxacin in broiler chickens

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Abstract

Aim: A pharmacological study was undertaken to evaluate the safety and adverse effects of enrofloxacin administration in broiler chickens by assessing the serum biochemical parameters, associated histopathological and ultra structural changes in liver and kidney.

Materials and Methods: Birds in the treatment group were administered with enrofloxacin at the recommended therapeutic dose 10mg/kg body weight via drinking water for five successive days, while the control group (untreated group) received non medicated water. Serum biochemical parameters viz., total protein, albumin, lactate dehydrogenase, alkaline phosphatase, creatine kinase, lipase, triglyceride, *gamma* glutamyl transferase, urea, uric acid and creatinine were estimated at 24hour and 48hour intervals during the dosing and withdrawal periods, respectively. Liver and kidney tissue samples collected from 1, 3, 5, 7, and 9 days post treatment groups were subjected to histopathological and ultrastructural examinations.

Results: There was no significant change ($p>0.05$) in total protein, albumin, lactate dehydrogenase, alkaline phosphatase, creatine kinase, lipase, triglyceride and urea levels in the enrofloxacin administered broiler chickens at all the time points evaluated. However, a significant increase ($p<0.05$) in *gamma* glutamyl transferase, uric acid and creatinine levels were observed after the 4th dose of the enrofloxacin and on day 1 post treatment. During the withdrawal period, the elevated levels declined gradually and showed the trend towards control values as evidenced by a statistically insignificant difference on 3, 5, 7 and 9 days post treatment when compared to that of control group. These biochemical changes were substantiated by histopathological and ultrastructural changes elicited in liver and kidney.

Conclusion: The reversible trend observed in serum biochemical parameters, histopathological and ultra structural alterations in liver and kidney during the withdrawal period suggests that enrofloxacin is safe if administered to broiler chickens at the recommended therapeutic dose and if the stipulated withdrawal period is strictly adhered to.

Keywords: biochemical parameters, broiler chickens, enrofloxacin, histopathology, ultrastructural, veterinary pharmacovigilance

Introduction

In the modern intensive poultry production, antimicrobial agents are being increasingly used to enhance feed efficiency, promote health, improve productivity, and are also used for disease prophylaxis and treatment [1, 2]. In this context, it is important to note that the fluoroquinolones are synthetic antimicrobial agents used extensively in the poultry industry for the control of premature mortality and for the prophylaxis and treatment of respiratory, renal, and digestive infections [3,4]. Enrofloxacin is a fluoroquinolone that was developed exclusively for veterinary use and is currently used in large-scale in poultry, especially for the treatment of chronic respiratory disease, colibacillosis, salmonellosis and fowl cholera [5,6]. *In vivo*, enrofloxacin is metabolised into pharmacologically active metabolite ciprofloxacin by liver microsomal enzymes that belong to the cytochrome P450 family [4,7].

The fluoroquinolones are considered relatively safe and well tolerated as indicated by earlier reports which demonstrated that enrofloxacin is well tolerated at the recommended dosage in cats [8, 9, 10]. However, it has been reported that fluoroquinolones are also associated with a low incidence of adverse effects related to gastrointestinal, skin, hepatic, central nervous system functions, and phototoxicity [11]. Further, there is evidence to show that enrofloxacin administration in broiler chickens resulted in a significant decline in the lymphocyte count [12] and reduction in haemagglutination inhibition (HI) titre and associated histopathological changes in the lymphoid organs [13].

Data on the safety regarding repeated oral administration of fluoroquinolones in poultry are very scarce [14]. Information on the residual effects of enrofloxacin and post market surveillance on serum biochemical parameters, histopathological and ultra structural changes in broiler chickens during the post treatment periods is lacking.

Hence, we conducted a pharmacological study in broiler chickens to assess the safety and adverse effects of enrofloxacin administration on serum biochemical

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Table-1. Effect of enrofloxacin administration (10mg/kg body weight, in drinking water for 5 successive days) on total protein, albumin, lactate dehydrogenase, alkaline phosphatase, creatine kinase, lipase and triglyceride levels (data is shown as Mean±SE, n=6)

	TP (g/dL)	Albumin (g/dL)	LD (U/L)	AP (U/L)	CK (U/L)	Lipase (U/L)	TG (mg/dL)
Control	2.88±0.13	1.59±0.06	1826.18±143.19	1351.30±86.91	1014.50±71.27	21.34±1.31	87.42±5.90
After 1st dose	3.01±0.04	1.50±0.01	1852.50±131.90	1249.35±44.61	1026.50±32.72	20.36±1.13	91.25±5.53
2nd dose	3.14±0.07	1.59±0.05	1933.18±159.53	1414.07±71.22	1027.83±35.24	23.67±1.79	95.60±4.98
3rd dose	3.03±0.06	1.66±0.04	1869.08±101.64	1510.78±57.48	1041.50±35.17	25.27±2.51	92.22±6.75
4th dose	2.94±0.14	1.51±0.08	2014.80±95.23	1358.47±86.64	1107.00±54.64	22.99±2.33	98.35±6.36
Post treatment							
Day 1	3.20±0.23	1.70±0.10	1918.37±192.28	1379.10±64.96	1118.33±27.09	26.67±1.36	106.63±8.38
Day 3	2.99±0.07	1.65±0.06	1985.72±103.05	1424.35±84.83	1060.50±33.56	24.79±1.60	106.10±4.75
Day 5	3.13±0.07	1.64±0.04	1966.23±166.54	1503.07±53.04	1000.33±55.94	25.62±1.84	101.23±3.13
Day 7	2.95±0.21	1.51±0.07	1871.40±160.28	1439.40±86.04	1021.00±51.46	25.66±2.06	97.97±8.39
Day 9	2.82±0.04	1.55±0.04	1830.00±131.12	1456.43±70.82	1010.17±24.39	20.34±1.30	95.60±1.42

TP : Total protein, LD: Lacto dehydrogenase, AP: Alkaline phosphatase, CK: Creatinine kinase, TG: Triglyceride

Table-2. Effect of enrofloxacin administration (10mg/kg body weight, in drinking water for 5 successive days) on gamma glutamyl transferase, urea, uric acid and creatinine levels (data is shown as Mean±SE, n=6)

	Gamma glutamyl transferase(U/L)	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)
Control	21.53 ^c ±2.28	3.38±0.31	4.86 ^c ±0.57	0.42 ^c ±0.01
After 1st dose	20.90 ^c ±0.49	3.25±0.18	5.26 ^c ±0.38	0.41 ^c ±0.01
2nd dose	25.20 ^{abc} ±0.89	3.40±0.36	5.66 ^{bc} ±0.00	0.43 ^{bc} ±0.02
3rd dose	26.35 ^{abc} ±5.67	4.02±0.36	6.10 ^{abc} ±0.21	0.42 ^c ±0.02
4th dose	33.31 ^a ±4.82	3.97±0.19	6.78 ^{ab} ±0.16	0.48 ^{ab} ±0.03
Post treatment				
Day 1	32.47 ^{ab} ±0.71	4.13±0.29	7.27 ^a ±1.06	0.52 ^a ±0.03
Day 3	28.85 ^{abc} ±1.69	3.90±0.34	5.89 ^{abc} ±0.74	0.46 ^{bc} ±0.01
Day 5	25.83 ^{abc} ±0.99	4.03±0.19	5.11 ^c ±0.36	0.45 ^{bc} ±0.02
Day 7	23.40 ^c ±1.12	3.30±0.09	5.53 ^{bc} ±0.21	0.44 ^{bc} ±0.03
Day 9	23.93 ^c ±4.08	3.43±0.12	5.50 ^{bc} ±0.36	0.45 ^{bc} ±0.03

Means bearing different superscripts (a,b,c) within the column differ significantly (p<0.05)

parameters, and to thoroughly evaluate the associated histopathological and ultrastructural alterations in liver and kidney.

Materials and Methods

Ethical approval: Institutional Animal Ethics Committee (IAEC), Madras Veterinary College, TANUVAS has accorded permission for conducting this biological trial.

Medication with enrofloxacin containing water: 36 day old broiler chicks (Broiler strain B,) acquired from Institute of Poultry Production and Management (IPPM), TANUVAS, Chennai-51 were randomly divided into control and treatment groups and were maintained under standard management conditions. Control group (I) received non medicated water while the treatment groups (II, III, IV, V and VI) were administered with enrofloxacin at recommended therapeutic dose of 10mg/kg body weight, via drinking water for five successive days from 43rd to 47th day of age [13, 15]. The pulsed water medication was given as per the methods described by Charleston et al. [16]. Birds received their freshly-prepared daily medication during a 4 hour period in the morning, and their water was antibiotic free for the remaining 20 hours in each day. The concentration of enrofloxacin in the water, to administer the required dose per kg of body weight, was calculated by determining the water consumption and body weight of each bird on the day of medication.

Serum biochemical parameters: Blood samples were

collected from the wing vein at 24 hour interval during the dosing period and at 48 hour interval during the withdrawal period up to 9 days post treatment. Serum samples thus obtained were subjected to total protein, albumin, lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatine kinase (CK), lipase, triglyceride, *gamma* glutamyl transferase (GGT), urea, uric acid and creatinine estimation by auto analyzer (A-15, Bio-Systems) using standard kits. Statistical analysis was carried out by following One way analysis of variance as described by Snedecor and Cochran [17].

Histopathology: Treatment groups were sacrificed ethically on 1, 3, 5, 7 and 9 days post treatment and control birds were sacrificed on day 9 post treatment. Liver and kidney tissue samples were collected in 10% formalin and were subjected to histopathological examination [18].

Ultrastructural study: Liver and kidney tissue samples from control and treatment groups (day 1 and day 9 post treatments) were collected immediately after sacrificing the birds and processed. Tissue sections were examined under a transmission electron microscope (Philips 2010, Netherlands) operated at 60KVA and photographed [18].

Results

Serum biochemical parameters: It is discernible from our results that there was no significant change (p>0.05) in total protein, albumin, LDH, ALP, CK, lipase, triglyceride and urea levels in enrofloxacin

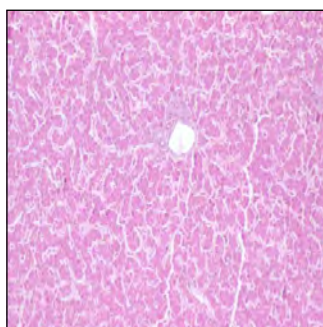


Figure-1. Liver of control group showing normal architecture. H&E, x10.

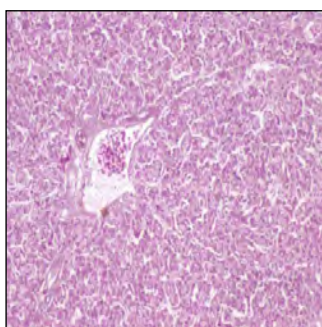


Figure-2. Liver of 1 day post treatment group showing degeneration of hepatocytes and areas of congestion. H&E, x10.

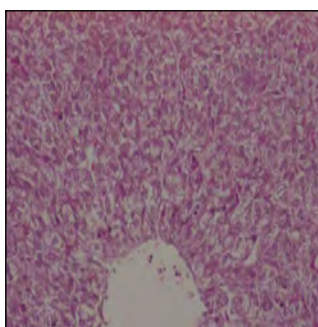


Figure-3. Liver of 3rd day post treatment group showing no sinusoidal spaces indicating swollen hepatocytes and few areas of vacuolar degeneration. H&E, x20.

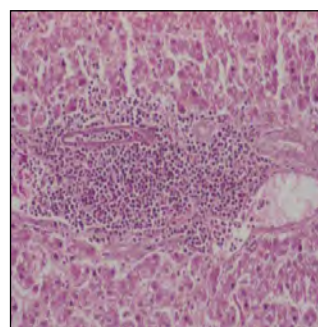


Figure-4. Liver of 5th day post treatment chicken showing focal areas of infiltrated inflammatory cells. H&E, x20.

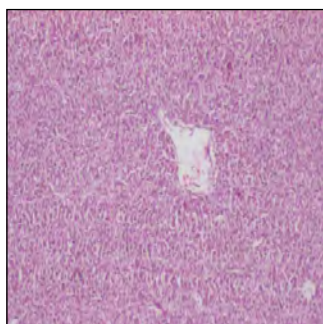


Figure-5. Liver of 9th day post treatment group showing mild vacuolation of hepatocytes. H&E, x10.

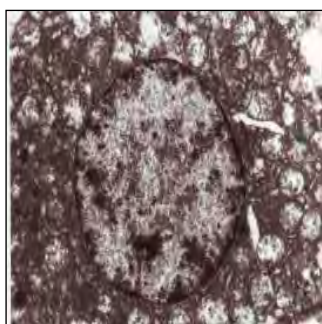


Figure-6. Liver of control group, normal hepatocytes with nucleus and mitochondria. Uranyl acetate & Reynold's solution, x10000.

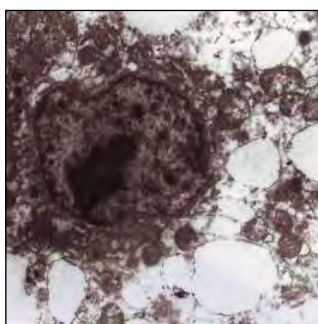


Figure-7. Liver of day 1 post treatment group showing hepatocytes with atrophied nucleus, cytoplasm with vacuolated cytoplasm and swollen mitochondria. Uranyl acetate & Reynold's solution, x10000.

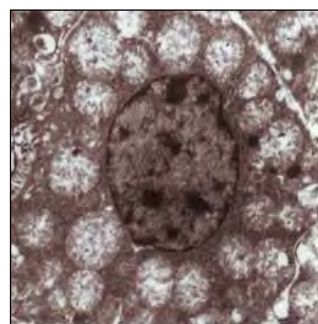


Figure-8. Liver of 9th day post treatment group showing hepatocytes with normal nucleus but few cells had shrunken nucleus. Mitochondrial swelling was the only appreciable lesion observed in few cases. Uranyl acetate & Reynold's solution, x10000.

administered broiler chickens at all the time points evaluated (Table-1). However, a significant increase ($p < 0.05$) in GGT, uric acid and creatinine levels were observed after the 4th dose of the enrofloxacin and on day 1 post treatment. The elevated levels declined gradually during the withdrawal period as evidenced by a statistically insignificant change starting from day 3 post treatment (Table-2).

Histopathology and ultra structural study

Histopathology of the liver: Liver of control group revealed no histological aberrations (Fig-1). On the other hand, on day 1 post treatment, hepatocytes were swollen and showed vacuolated cytoplasm. Degeneration of hepatocytes and areas of congestion were also observed (Fig-2). Whereas, on day 3 post treatment no sinusoidal space could be observed indicating that hepatocytes were highly swollen and also exhibited few areas of vacuolar degeneration with vacuolated cytoplasm (Fig-3). However, on day 5 post treatment sinusoidal spaces reemerged and samples from only a few birds showed focal areas of infiltrated inflammatory cells (Fig-4). Mild vacuolation of hepatocytes was the only appreciable lesion noticed on day 9 post treatment (Fig-5).

Ultrastructural changes in the liver: Control group showed normal hepatocyte morphology pertaining to organelles such as nucleus, mitochondria and endoplasmic reticulum (Fig-6). In the treatment group,

on day 1 post treatment, hepatocytes showed atrophied nucleus, cytoplasm was vacuolated with scattered cytoplasmic organelles and swollen mitochondria (Fig-7). While, in 9 day post treatment group hepatocytes showed normal nucleus but few cells had shrunken nucleus. Mitochondrial swelling was the only appreciable lesion observed in few cases (Fig-8).

Histopathology of the kidney: Control group showed normal features of kidney (Fig-9). Kidney from day 1 post treatment group revealed degeneration and necrosis of tubular epithelial cells with granular eosinophilic cytoplasm (Fig-10). On day 3 post treatment, degeneration of tubular epithelial cells with haemorrhagic areas were noticed. However, in few cases, cytoplasm of swollen tubular epithelial cells was filled with eosinophilic material (Fig-11). Degeneration of tubular epithelium persisted in day 5 post treatment group; however it was only mild and was also observed in only few cases (Fig-12). Indeed, mild swollen tubular epithelial cells were the only appreciable lesions observed on day 7 and 9 post treatment (Fig-13).

Ultrastructural changes in the kidney: Control group revealed normal glomeruli and tubular epithelial cells with unaltered brush border (Fig-14). In 1 day post treatment group, normal architecture of glomeruli and tubular epithelial cells vanished. Prominent features of the tubular epithelium include shrunken nucleus,

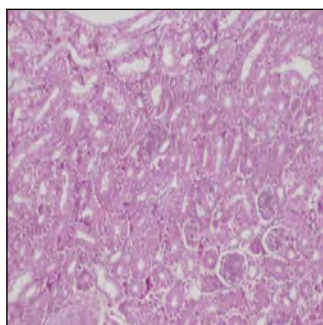


Figure-9. Kidney of control group showing normal glomeruli and tubular epithelial cells. H&E, x10.

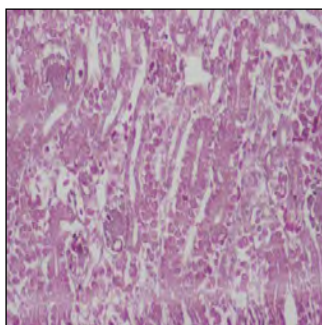


Figure-10. Kidney of 1 day post treatment group showing degeneration and necrosis of tubular epithelial cells with granular eosinophilic cytoplasm. H&E, x20.

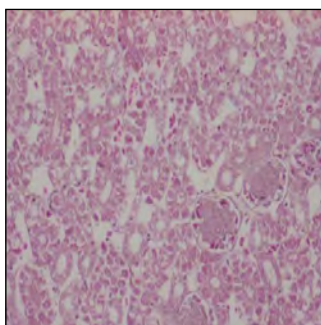


Figure-11. Kidney of 3rd day post treatment group showing degeneration of tubular epithelial cells with haemorrhagic areas. H&E, x20.

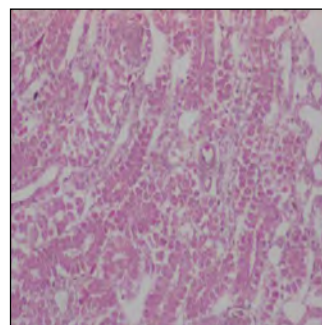


Figure-12. Kidney of 5th day post treatment chicken showing mild tubular degeneration. H&E, x20.

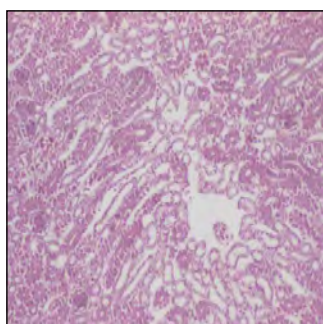


Figure-13. Kidney of 9th day post treatment group showing mild tubular degenerative changes. H&E, x10.

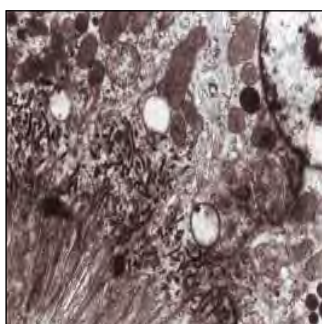


Figure-14. Kidney of control group showing normal tubular epithelial cells with unaltered brush border. Uranyl acetate & Reynold's solution, x10000.

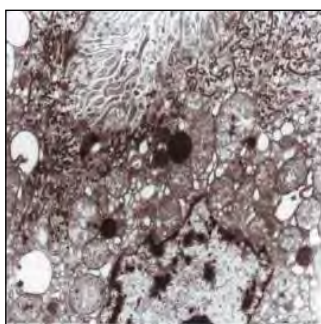


Figure-15. Kidney of day 1 post treatment group showing tubular epithelium with shrunken nucleus, vacuolated cytoplasm and attrition of brush border. Uranyl acetate & Reynold's solution, x10000.

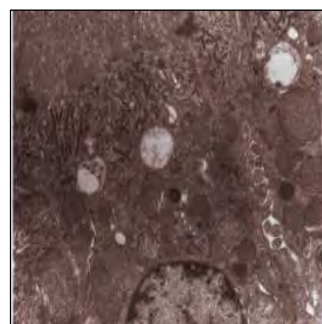


Figure-16. Kidney of 9th day post treatment group showing tubular epithelial cells with normal nucleus, brush border, but with mild mitochondrial swelling. Uranyl acetate & Reynold's solution, x10000.

swollen mitochondria, vacuolated cytoplasm and attrition of brush border (Fig-15). However, mild vacuolation in cytoplasm and swollen mitochondria were the only considerable lesions found in the 9 day post treatment group (Fig-16).

Discussion

In the present study, the serum biochemical parameters revealed insignificant changes in total protein, albumin, LDH, ALP, CK, lipase, triglyceride and urea levels in enrofloxacin administered broiler chickens at all the time points evaluated. This finding is in accordance with Elkholy et al. [19], who showed that administration of Enrotryl® and Baytril® at a dose equivalent to 10 mg enrofloxacin base/kg body weight daily for five successive days, to laying hens induced insignificant changes in serum glucose, cholesterol, creatinine levels, serum alanine amino transferase (ALT), aspartate amino transferase (AST) and ALP activities.

However, Moustafa et al. [20] reported that administration of enrofloxacin in high doses (100, 200 and 400 ppm through drinking water) for long periods (6 weeks) was accompanied with adverse effects on different organs along with elevated levels of AST, ALT, ALP, urea, uric acid and creatinine, hypoproteinaemia and hypoalbuminaemia. In addition, Tras et al. [21] showed that enrofloxacin injection at the

recommended dose of 5 mg/kg IM, once daily to healthy dogs for 14 days, caused transient increases in AST and mean corpuscular volume levels. Similar effects are reported in humans receiving ciprofloxacin and norfloxacin [22, 23].

Serum GGT is a highly specific and indicative enzyme for liver function and more sensitive than transaminases (AST and ALT) and ALP. A significant elevation in serum GGT level was observed after the 4th dose of enrofloxacin administration and on day 1 post treatment GGT levels showed a tendency to decline gradually during the withdrawal period. These findings were supported by Ellakany et al. [24] who also reported a similar trend that enrofloxacin at 100mg/kg body weight in broiler chicken for 5 consecutive days caused elevation of serum ALT, but 6 days after the withdrawal the values returned to the control levels. Similarly, the authors further reported that serum AST level was found to be higher than the control group, albeit not significant, in those receiving 100mg/kg body weight or 10mg/kg body weight enrofloxacin, then decreased after withdrawal of the drug.

In the present study, the elevated levels of GGT can be correlated with the histopathological changes in the liver observed during the withdrawal period. For example, on day 1 post treatment hepatocytes were swollen, degenerated with vacuolated cytoplasm and

showed areas of congestion. Vacuolar degeneration was persistent till 3rd day post treatment, however, during the subsequent withdrawal period the lesions were mild as indicated by clear sinusoidal spaces and focal areas of infiltrated inflammatory cells with only a mild vacuolation of hepatocytes. These observations are in conformity with Ellakany et al. [24]. Further, Shawky et al. [25] also showed that enrofloxacin administered at 400 ppm for 6 weeks produced histopathological changes in liver. Vaccaro et al. [26] opined that the major cause of enrofloxacin-induced hepato toxicity can be attributed to the inhibition of hepatic cytochrome P450 enzymes which are responsible for drug metabolism.

Hepatocellular injury represented by significant elevations in the serum GGT activities was further ascertained by the electron microscopic examination of liver samples of broiler chickens administered with enrofloxacin. Shrunken nucleus, swollen mitochondria and vacuolated cytoplasm with scattered cytoplasmic organelles are clearly indicative of hepatocyte degeneration and these changes were observed on day 1 post treatment group. However, in day 9 post treatment group mitochondrial swelling is the only appreciable lesion found in few cases. Similar observations using electron microscopy were made by El Daly [27] in the livers of rats that were administered with 10% enrofloxacin with a daily dose of 75mg/kg body weight for 10 days intraperitoneally. The authors demonstrated that enrofloxacin induces the production of free radicals that cause oxidative stress to hepatocytes and its organelles, particularly to mitochondria and cell membranes.

A significant but transient elevation in serum uric acid and creatinine was noticed after the 4th dose and on day 1 post treatment of enrofloxacin and evinced similar trend as that of GST during the withdrawal period. These biochemical changes were in harmony with histopathological alterations in kidneys which showed degeneration and necrosis of tubular epithelial cells with granular eosinophilic cytoplasm in day 1 post treatment group. However, a reversible tendency was noticed as evidenced by mild swollen tubular epithelial cells which were the only appreciable lesions observed on day 7 and 9 post treatment. Ellakany et al. [24] also found congestion, tubular degeneration, and areas of hemorrhage in kidney of broiler chicken administered with enrofloxacin at 100mg/kg body weight through drinking water for 5 consecutive days.

Kidney injury as evidenced by significant elevation in the serum uric acid and creatinine levels was further bolstered by results from the electron microscopic examination of kidneys isolated from enrofloxacin administered broiler chickens. Indeed, mild vacuolation and mitochondrial swelling were the only considerable lesions found in the day 9 post treatment group suggestive of a reversible trend in the lesions during the withdrawal period.

Conclusion

Enrofloxacin administration at recommended therapeutic dose caused insignificant changes in total protein, albumin, LDH, ALP, CK, lipase, triglycerides and urea levels. A significant but transient elevation in GGT, uric acid and creatinine was noticed and these biochemical alterations were suggestive of histopathological and ultrastructural changes observed in liver and kidney. The restoration towards the control values of biochemical parameters accompanied by reversal of histopathological and ultra structural lesions towards normal contour during the withdrawal period clearly suggests that enrofloxacin is extremely safe if administered at recommended therapeutic dose and if the stipulated withdrawal period is strictly followed. Adhering to these precautions can prevent any adverse drug reactions and untoward residual effects.

Authors' contributions

This study is a significant component of the work towards the Ph.D. thesis of the first author VS, submitted to Tamil Nadu Veterinary and Animal Sciences University, Chennai-51. GS was the Chairman and designed the trial. VS conducted the experimental trial. JR performed the sampling. GS and VS analysed and interpreted the data, organised and thoroughly revised the manuscript. All authors read and approved the final version of the manuscript.

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Competing interests

The authors declare that they have no competing interests.

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