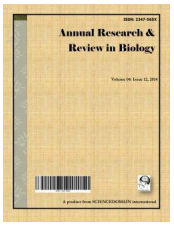

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Effect of Enrofloxacin on the Joint Fluid/Blood Oxidative Status and Organ Damage Markers

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DC, KP, HEF and EB treated drug and obtained blood and joint fluid samples. Authors BD and AE analysed all samples. Authors EY and AE made statistics and wrote the article. All authors read and approved the final manuscript.

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ABSTRACT

Aim: It has been hypothesized that chondrotoxicity, the main side effect of enrofloxacin use, may be derived from oxidative stress, and this side effect can be confirmed by measuring malondialdehyde and endogen antioxidants following drug application. The primary aim of this research is to determine the effect of enrofloxacin on the joint fluid and blood oxidative status parameters, and it is also to determine the effect on the organ damage parameters.
Materials and Methods: In the study, 10 rams received enrofloxacin (10 mg/kg/day, SC) for 14 days. Blood and joint fluids were taken on day 0 (Control) before drug application and 1.5 hours

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after the last drug application. Plasma and joint fluid malondialdehyde, total antioxidant status, superoxide dismutase, glutathione peroxidase and catalase levels were determined by an ELISA reader. Cardiac (CK-MB mass, troponin I), liver (AST, ALT, ALP, GGT, total protein, albumin) and kidney (Creatinine, BUN) damage markers and hemogram (WBC, RBC, platelet, hematocrit, haemoglobin) values were measured.

Results: Enrofloxacin decreased the joint fluid catalase level ($P<0.05$), while there was no effect observed in the other oxidative status parameters of joint fluid or blood samples. Statistically significant changes ($P<0.05$) were found in some hemogram and biochemical parameters within the reference range. However, enrofloxacin increased ($P<0.05$) the levels of cardiac damage markers (CK-MB mass, troponin I).

Conclusion: It may be stated that enrofloxacin does not cause oxidative stress in the joint fluid and blood in rams, and it is generally accepted to be safe when the effect on the organ/system is considered, but the long-term use and high doses require caution in terms of possible heart related damage.

Keywords: Enrofloxacin; joint; organ damage; oxidative status.

1. INTRODUCTION

Fluoroquinolone antibiotics are widely used in both human and veterinary medicine because of their broad antimicrobial spectrum. Enrofloxacin belongs to the family of fluoroquinolone antibiotics which is used in the therapy of septic shock, gastrointestinal, urogenital, respiratory, dermal, mycoplasmal and staphylococcal infections in many animal species such as ruminants, equidae, poultry, pet animals and exotic animals. Although fluoroquinolone antibiotics are widely preferred in veterinary or human medicine, they may cause adverse effects such as chondrotoxicity, renal damage, retinal damage, dysglycemia, cardiac arrhythmia and even tendon rupture. These side effects of fluoroquinolones are associated with reducing collagen synthesis and inducing oxidative stress [1,2,3,4,5]. Although enrofloxacin may cause degeneration in the joint cartilages of calves, piglets and puppies, similar effects of enrofloxacin and its active metabolite ciprofloxacin have not been reported in lambs [6,7].

Metabolic functions, especially mitochondrial electron transport, continuously produces reactive oxygen radicals (ROS; singlet oxygen, superoxide radical, hydroxyl radical, hydrogen peroxide etc) in the body. However, ROS are detoxified by endogen antioxidants such as total antioxidant status (TAS), superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT). Hence, ROS induced cellular damage may be inhibited in the cells. SOD enzyme dismutase superoxide radicals to hydrogen peroxidase. Hydrogen peroxidase is converted to molecular oxygen and water by CAT or GPX enzymes. If ROS are not detoxified due to inadequate antioxidants and/or excessive producing ROS, pathological reactions are observed in the cells. This reaction may term cellular death. This situation is termed oxidative stress; hence, lipid peroxidation develops in the cells [8,9]. After developing lipid peroxidation, malondialdehyde (MDA) is produced, which is the main and much-measured lipid peroxidation marker in the oxidative stress studies [10,11]. The total antioxidant status (TAS) parameter may be generally accepted as the total antioxidant activity of the body [12,13].

Although drugs have beneficial effects, adverse or side effects may be observed even when used at the recommended dose and duration for the target species [14]. Some parameters measured from blood and/or other biological samples reflect abnormalities in the normal physiological functions of organ and systems in the body. Although hemogram values are primarily related to bone marrow function, these values are also affected by infection and fluid-electrolyte balance disorder [15]. Blood creatine kinase-MB (CK-MB)mass activity and troponin I level are specific markers of cardiac damage [16,17,18]. Cardiac troponins are heart-specific proteins. Firstly, cardiac troponin concentrations increase within minutes after cardiac damage and reach to pick the level at 2-6 hours following cardiac damage. Heart-specific creatine kinase-MB (CK-MB) enzyme is a subunit of creatine kinase. After cardiac damage, CK-MB activity increase first 24 hours, and a higher level of CK-MB may be observed till 72 hours [19,20]. Levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), total protein

and albumin are measured to define damage of the bile duct and liver, whereas blood urea 2qwedx yhbn7nitrogen (BUN) and creatinine levels are measured to determined kidney damage [15].

In this research, considering that the side effects of ciprofloxacin, active metabolite of a fluoroquinolone antibiotic enrofloxacin, are caused by oxidative stress [5], it was hypothesized that the chondrotoxic effects of fluoroquinolones may be shown in sheep without regard to animal species and age difference, and this chondrotoxic effect can be determined by measuring MDA, main indicator of oxidative stress [10,11] and other antioxidant parameters in joint fluid.

The primary aim of this research was to determine the effect of enrofloxacin (10 mg/kg, SC, SID, 14 days) on the oxidative status parameters (MDA, TAS, SOD, GPX, CAT) of joint fluid and plasma. Determination of the effect of enrofloxacin on the hemogram (WBC, RBC, platelet, hematocrit, hemoglobin), heart (CK-MBmass, troponin I), liver (ALP, AST, ALT, GGT, total protein, albumin) and renal (BUN, creatinine) damage parameters were also targeted.

2. MATERIALS AND METHODS

In the current research, 10 Akkaraman rams were used and the research procedure was approved by the Ethics Committee (SUVDAMEK, 2016-110). In the study, blood and joint fluid samples were initially taken from 10 rams to provide 'Control values' where no drugs were applied. The joint used to generate the control values was not used for the final sample collection. After obtaining the Control values, a 10 mg/kg (SC, SID) dose of enrofloxacin was administered to rams for 14 days [21]. At 1.5 hours after the last enrofloxacin treatment, joint fluids and blood samples were obtained. MDA (Bioxytech MDA-586 Kit, OxisResearch, OR, USA), TAS (Total Antioxidant Status Kit, Rel Assay Diagnostics, Gaziantep, Turkey), SOD (Cayman Superoxidase Dismutase Assay Kit, MI, USA), GPX (Cayman Glutathione Peroxidase Assay Kit, MI, USA) and CAT (Cayman Catalase Assay Kit, MI, USA) levels of plasma and joint fluid were measured by an ELISA reader (MWGt Lambda Scan 200, Bio-Tec Instruments, Winooski, VT, ABD). Hemogram values (WBC, RBC, platelet, hemoglobin, hematocrit) were determined with a hemacell counter (BC-2800 Auto Hematology Analyser, Mindray Bio-Medical Electronics, Shenzen, China), while hepatic (AST, ALT, ALP, GGT, albumin, total protein) and renal (BUN, creatinine) damage markers were measured by auto-analyser (ILab-300 plus, Instrumentation Laboratory, Milano, Italy). Cardiac damage markers (CK-MBmass and troponin I) were measured with the chemiluminescence immunoassay technique (Siemens AdviaCentaur XP, Erlangen, Germany).

Research data was presented as mean±SEM. Data was evaluated by Paired t test (SPSS 22.0). $P<0.05$ level was accepted as statistically significant.

3. RESULTS

No clinical abnormalities were observed during the research period. Oxidative status values (MDA, TAS, SOD, GPX, CAT) are shown in the Table 1, whereas data for hemogram (WBC, RBC, platelet, hemoglobin, hematocrit), liver (ALP, AST, ALT, GGT, total protein, albumin) and kidney (Creatinine, BUN) damage markers are shown in Table 2. Enrofloxacin decreased joint fluid CAT activity ($P<0.05$), whereas there were no statistically significance differences determined ($P>0.05$) in the other oxidative status values of joint fluid and blood (Table 1). Enrofloxacin decreased ($P<0.05$) RBC and hematocrit levels, whereas it increased ($P<0.05$) platelet counts (Table 2). In addition, statistically significant ($P<0.05$) changes were determined in ALP, AST, ALT, total protein, BUN and creatinine levels (Table 2).

CK-MBmass activity and troponin I levels are shown in Graphics 1 and 2, respectively, and CK-MBmass activity and troponin I levels increased ($P<0.05$).

4. DISCUSSION

Oxidative stress induced by fluoroquinolone antibiotics has been reported previously [4]. In this research, enrofloxacin was found to slightly increase the joint fluid and plasma MDA levels, but this increase was not statistically significant ($P>0.05$, Table 1). In the experimental studies, fluoroquinolone-induced chondrotoxicity has been histopathologically defined [22,23]. Although fluoroquinolones may exhibit chondrotoxicity by destroying collagen formation

Table 1. Effect of enrofloxacin (10 mg/kg, SID, SC, 14 days) on the joint fluid and plasma oxidative status parameters (mean±SE)

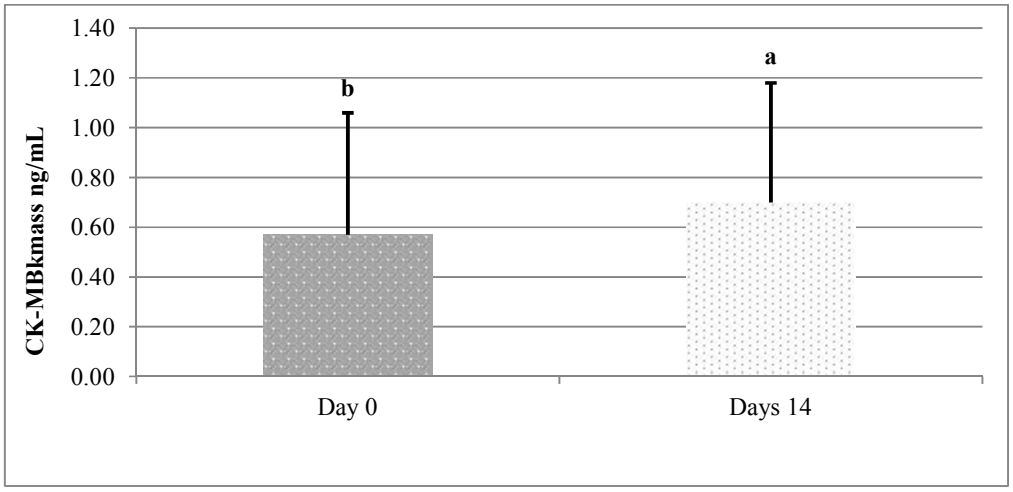
Parameters	Control (0.) day	Days 14	P value
$MDA_{joint\ fluid}$ µM	0.35±0.01	0.38±0.03	>0.05
MDA_{plasma} µM	1.05±0.26	1.29±0.67	>0.05
$TAS_{joint\ fluid}$ mmol/L	1.07±0.02	1.11±0.09	>0.05
TAS_{plasma} mmol/L	0.80±0.07	0.75±0.10	>0.05
$SOD_{joint\ fluid}$ U/mL	0.036±0.001	0.038±0.001	>0.05
SOD_{plasma} U/mL	0.043±0.001	0.047±0.001	>0.05
$GPX_{joint\ fluid}$ nmol/min/mL	85.32±28.29	375.67±135.00	>0.05
GPX_{plasma} nmol/min/mL	151.79±51.14	179.30±41.66	>0.05
$CAT_{joint\ fluid}$ nmol/min/mL	20.80±2.42	11.90±1.54	<0.05
CAT_{plasma} nmol/min/mL	161.60±21.31	136.30±18.14	>0.05

MDA; Malondialdehyde, TAS; Total antioxidant status, SOD; Superoxide dismutase, GPX; Glutathione peroxidase, CAT; Catalase.

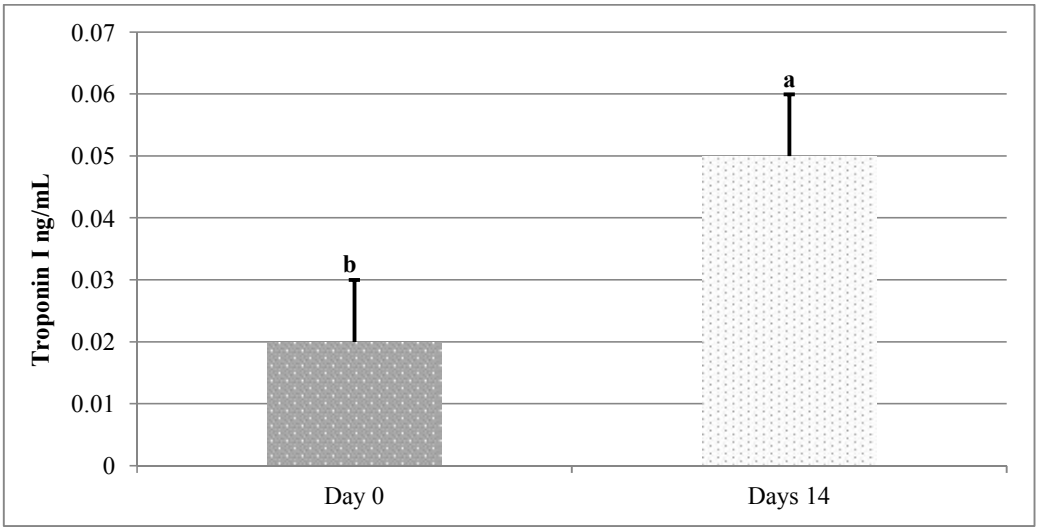
Table 2. Effect of enrofloxacin (10 mg/kg, SID, SC, 14 days) on the hemogram values, liver and kidney damage markers (mean±SE)

Parameters	Control (0.) day	Days 14	P value	Reference range*
WBC $x10^9$/L	9.75±0.66	9.31±1.16	>0.05	8.0-18
RBC $x10^{12}$/L	11.04±0.70	10.40±0.67	<0.05	2.5-12
Platelet $x10^9$/L	202.40±21.41	259±14.93	<0.05	250-1100
Hemoglobin g/dL	9.48±0.48	9.09±0.41	>0.05	9.0-16
Hematocrit %	35.66±1.32	33.24±1.13	<0.05	24-49
ALP U/L	102.90±16.78	78.10±12.26	<0.05	68-387
AST U/L	91.30±4.57	116.50±6.25	<0.05	55-280
ALT U/L	25.60±1.68	32.90±1.96	<0.05	12-34
GGT U/L	70.50±3.13	70.30±4.14	>0.05	20-130
Total protein g/dL	7.92±0.19	7.56±0.17	<0.05	6.0-8.0
Albumin g/dL	2.32±0.04	2.26±0.04	>0.05	2.4-4.0
BUN mg/dL	20.60±1.12	14.01±0.58	<0.05	8.0-20
Creatinine mg/dL	0.75±0.03	0.81±0.04	<0.05	0.5-1.9

*WBC; White blood cell, RBC; Red blood cell, ALP; Alkaline phosphatase, AST; Aspartate aminotransferase, ALT; Alanine aminotransferase, GGT; Gamma glutamyltransferase, BUN; Blood urea nitrogen [*16,36,37]*



Graphic 1. Effect of enrofloxacin (10 mg/kg, SID, SC, 14 days) on the serum CK-MBmass activity (mean±SE)



Graphic 2. Effect of enrofloxacin (10 mg/kg, SID, SC, 14 days) on the serum troponin I levels (mean±SE)

via the production of ROS or interacting with magnesium ions [2,24,25], the mechanism of chondrotoxicity is not clearly defined [25]. To the best of our knowledge, there has been no information published about the MDA level of joint fluid, which is the most analyzed value as oxidative stress indicator, after enrofloxacin application in the literatures. However, increased MDA levels in the brain, kidney, cell culture, serum and erythrocyte were reported [5,26,27, 28,29] after treatment with fluoroquinolone antibiotics (Ciprofloxacin, danofloxacin, moxifloxacin, pazufloxacin). It has been declared that fluoroquinolone antibiotics may produce ROS [30,31] and/or affect the antioxidants [5, 32]. Hence, some side effects including retinal [4, 32], renal [5] and tendon [30] damage may be observed. In the current research, high-dose and long-term administered enrofloxacin did not increase MDA levels in the joint fluid and plasma, and this result might arise from animal species diversity. Ewes may have a higher antioxidant capacity than other animals, thus causing them to be more resistant to oxidative stress.

In the present research, enrofloxacin decreased ($P<0.05$) joint fluid CAT levels, while it did no change any other oxidative status parameters both joint fluid and plasma (Table 1). Decreased CAT level in the joint fluid may be derived from excess hydrogen peroxide because of main function of CAT is detoxifying of hydrogen peroxidase [8]. Increased or decreased GPX levels, unchanged, decreased or increased SOD levels, decreased or increased CAT levels, decreased GSH levels in the different biological samples have been reported [5,26,29,33,34,35] after treatment with fluoroquinolone antibiotics (Enrofloxacin, danofloxacin, ciprofloxacin, pazufloxacin, norfloxacin). The results of these studies show that the effects of fluoroquinolones on the antioxidants are not similar, and different results obtained may be derived from differences of antioxidant capacity, tissue, animal species and dose and duration of antibiotics treatment.

Enrofloxacin caused statistically significantly ($P<0.05$) changes in some hemogram (RBC, platelet, hematocrit), liver (ALP, ALT, AST, total protein) and kidney (BUN, creatinine) function parameters (Table 2) within reference range [16, 36,37]. Enrofloxacin caused similar results have been reported in dogs [38]. Considering the high doses of the drug and long application period, it can be said that it is very safe for the rams at least in terms of lack of damage to bone marrow, liver and kidneys. On the contrary to these, enrofloxacin increased ($P<0.05$) CK-MBmass and troponin I levels, specific cardiac damage markers, in the rams (Graphics 1 and 2). Although fluoroquinolone-induced cardiotoxicity [39] derived from nitric oxide [40] have been rarely reported, seriously cardiotoxicity has been also declared after some fluoroquinolone antibiotics (Sparfloxacin, grepafloxacin) treatments [39,41]. It should be considered that cardiotoxic effects of enrofloxacin may be partially observed in high dose and long-term administration in rams.

5. CONCLUSION

In conclusion, it may be stated that enrofloxacin does not cause oxidative stress in rams, and it has no negative effect on the bone marrow, liver and kidney, but it may cause cardiotoxicity in rams. However, the cardiotoxic effect of enrofloxacin should be supported by further histopathological examination, especially when it is used higher and long-term used in rams.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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