TÜBİTAK

Turkish Journal of Zoology

http://journals.tubitak.gov.tr/zoology/

Research Article

Turk J Zool
(2016) 40: 513-521
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doi:10.3906/zoo-1510-51

# Cardiotoxic effects of enrofloxacin on electrophysiological activity, cardiac markers, oxidative stress, and haematological findings in rabbits

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**Received:** 21.10.2015 • **Accepted/Published Online:** 22.02.2016 • **Final Version:** 09.06.2016

**Abstract:** The aim of this research was to investigate the effect of normal and high doses of enrofloxacin on basal electrocardiographic (ECG) parameters including corrected QT (QTc) values along with biochemical and haematological findings in healthy rabbits. In total 21 New Zealand rabbits were used. The animals were randomly divided into three groups including 7 rabbits in each. The normal dose (5 mg/kg) of enrofloxacin and a toxic dose (50 mg/kg) were given intravenously in group 1, and the normal dose was given for 6 weeks in group 2. The last group was designated as a negative control (group 3). ECG, serum biochemistry parameters, and total antioxidant capacity (TAC) and total oxidant status (TOS) were determined every week. At the end of the experiment, necropsy was performed and histology was evaluated. Some oxidative parameters were measured in the heart tissue. No cardiotoxic effect was observed on ECG, in biochemical or haematological findings, or macroscopic or histological findings in groups 2 and 3. The results indicated that enrofloxacin is not harmful for the heart in clinically healthy rabbits at normal and toxic doses compared with the negative control group in terms of ECG, biochemical or haematological findings, or macroscopic or histological findings.

**Key words:** Rabbit, enrofloxacin, ECG and QT prolongation, haematology, biochemistry

## 1. Introduction

Prolongation of the QT interval is commonly used as a surrogate marker of torsades de pointes (TdP), which is a known clinical risk factor for the development of severe, life-threatening, ventricular arrhythmias (Abi-Gerges et al., 2004). Noncardiovascular drug-induced prolongation of the QT interval is often associated with the onset of TdP (Haverkamp et al., 2000; De Ponti et al., 2001). Fluoroquinolones are among the drugs of choice for the treatment of common bacterial infections due to their wide spectrum against respiratory, gastrointestinal, and genitourinary pathogens (Elmas et al., 2006). QT interval prolongation is also a class effect of fluoroquinolones but there are great differences between the various members of this group (Camm, 2005). Thus, there are significant differences in the potency to prolong QT interval among the fluoroquinolones and the risk of arrhythmia varies between drugs and with co-risk factors (Frothingham, 2001; Owens and Ambrose, 2002). Because a QT prolongation and potentially fatal ventricular arrhythmias associated with quinolones such as grepafloxacin due to several cases of sudden death and TdP have been encountered retrospectively, enrofloxacin needs to be further investigated in detail. Therefore, the purpose of this study was to investigate the effect of normal and high doses of enrofloxacin on basal electrocardiographic parameters including heart rate (HR), P, QRS, QT, and RR intervals and corrected QT (QTc) values as well as biochemical and haematological findings in healthy conscious rabbits.

## 2. Materials and methods

### 2.1. Approvals

This study was approved by the Institutional Laboratory Animal Care and Use Committee of Mustafa Kemal University (2011 - 01 - 09).

### 2.2. Chemicals

All chemicals were obtained from Sigma Chemical Co, and solutions were prepared fresh daily from concentrated stock solutions.

### 2.3. Animal preparation

After 1 week of quarantine and acclimatisation, a total of 21 healthy New Zealand rabbits of both sexes, 12–18 months old, and of 2–4 kg body weight were used. The rabbits were housed individually in stainless-steel wire mesh cages and provided with food and water ad libitum. All rabbits were

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treated with sulfaquinoxaline to decrease the incidence of coccidial infections. The animals were housed in a room maintained at a temperature of 23 ± 3 °C and a relative humidity of 50 ± 10% with artificial lighting from 0800 to 2000 and with 13–18 air changes per hour.

The rabbits were randomly divided into three equal groups including 7 rabbits in each. In the first group, normal (5 mg/kg) and toxic doses (50 mg/kg) of enrofloxacin (10 times the normal dose) were given (group 1). In the second group, the normal dose (5 mg/kg) of enrofloxacin was given for 6 weeks (group 2). The last group received no drug and was designated as a negative group (group 3).

In the first group, enrofloxacin was injected intravenously via the auricular vein at the dose of 5.0 mg/kg bw (Enrofloxacin HCl, Baytril, 10%, Bayer, Turkey) over 10 min. The animals were assessed by ECG, haematology, and serum biochemical analysis at 5, 10, 15, 20, and 30 min after the end of the first infusion. Then infusion continued to peracute toxic effect, enrofloxacin at a 10-fold dose of 50 mg/kg was injected over 10 min, and each parameter was re-evaluated at 5, 10, 15, 20, 30, 45, and 60 min after the end of the infusion to the same animals. Serum enrofloxacin concentration and its metabolite ciprofloxacin were measured by using a standard high-performance liquid chromatographic method. In the second group, for chronic toxic effect, the normal dose (5 mg/kg) of enrofloxacin was given for 6 weeks. ECG of all the conscious animals was recorded every week. Serum LDH, AST, CK, and CK-MB isoenzyme activities, and cTnI levels and TAC and TOS were determined in all animals at the sampling time. A clinical examination was performed, body weights were measured weekly, and food and water consumption recorded in the second group. For all groups, at the end of experiment, necropsy was performed and gross findings and histology were evaluated.

**2.4. Assessment of ECG recordings in the conscious rabbit**

ECG records were taken by direct writing electrocardiograph (Nihon Kohden, Cardiofax Vet, Japan). ECG was standardised at 1 mV = 20 mm, with a chart speed of 50 mm/s with the filter off. Lead II was used for the measurements of intervals/durations and amplitudes. Leads I, III, aVR, aVL, and aVF were also checked for any abnormalities. Corrected QT values (QTc) were calculated by using Carlsson's formula (Carlsson et al., 1993) [QTc = QT– 0.175(RR – 300)].

**2.5. Biochemical and haematological measurement**

The activities of LDH, AST, CK, and CK-MB isoenzyme, and cTnI levels were assayed colorimetrically, according to the standard procedures using commercially available diagnostic kits (DiaSys Diagnostic Systems, Holzheim, Germany). Furthermore, haematological parameters were determined using an automatic analyser (Abacus Junior Vet Systems, Budapest, Hungary).

**2.6. Serum total antioxidant capacities, total oxidant status, and oxidative stress index**

Total antioxidant capacities and total oxidant status capacities were determined colorimetrically (PowerWave XS, BioTek Instrument, Bedfordshire, UK) using a commercial kit (Rel Assay Diagnostic, Gaziantep, Turkey) as previously described by Durgut et al. (2013). Serum TAC was determined using a novel automated measurement method developed elsewhere (Erel, 2004). The results are expressed as μmol of Trolox equivalent/litre.

Total oxidant status levels were measured using commercially available kits (Rel Assay Diagnostic, Gaziantep, Turkey). The assay was calibrated with hydrogen peroxide ($H_2O_2$), and the results were expressed in terms of micromolar $H_2O_2$ equivalent per litre (μmol $H_2O_2$ equivalent/L) (Erel, 2005). The OSI was calculated as the ratio of TOS to TAC to determine the extent of oxidative stress (Erel, 2004, 2005). OSI values were calculated using the following formula: OSI = [(TOS, μmol/L)/(TAC, mmol Trolox equivalent/L) × 100] (Erel, 2005).

**2.7. Measurement of serum drug concentration**

A volume of 3 mL of blood was drawn from the auricular vein to measure the serum drug concentration. The blood samples were centrifuged at 1500 × *g* for 30 min at 4 °C. The serum was stored at –80 °C until the drug concentration was measured. Determinations of the enrofloxacin concentration were performed using a standard high-performance liquid chromatographic method (Model LC-6A with UV spectrophotometric detector model SPD-6A, data processor model chromatopac CR-6A; Shimadzu Corp, Analytical Instrument Plant, Kyoto, Japan), as described by Devreese et al. (2014). Enrofloxacin was extracted from plasma using dichloromethane and analysed by reverse-phase chromatography. The mobile phase was a mixture of buffer (pH 2.2) and acetonitrile (80:20, v/v). Heptane sulfonic acid-Na (1.1 g/L) added as the ion-pairing reagent. Ultraviolet absorbance measured at 278 nm was used for detection, with the flow rate maintained at 2 mL/min. The linearity of the method was examined by linear regression analysis of calibration curves in plasma from goats. The recovery of enrofloxacin was >90%. The limit of quantification of enrofloxacin was 0.01 pg/mL.

**2.8. Necropsy and histopathology**

At scheduled termination, all rabbits were sacrificed by exsanguination from the neck. Complete gross postmortem examinations were performed on all terminated animals. The heart was obtained from all rabbits. The tissues were fixed with 10% neutral buffered formalin solution. The tissues were routinely processed, embedded in paraffin, and sectioned at 3–5 μm. The sections were stained with haematoxylin–eosin stain for microscopic examination. All organs and tissues taken from all animals in the control

and highest dose groups were examined microscopically. All gross lesions as defined by the study histologist were also included in the examination.

### 2.9. Statistical analysis

The groups and different time changes were evaluated by one-way analysis of variance (ANOVA) with repeated measurement design. Two-way ANOVA with repeated measurement between groups was done with Duncan's multiple comparison test, and Bonferroni's multiple comparison test was used for differences between weeks. The results were presented as means ± SEM. A P value <0.05 was regarded as statistically significant.

## 3. Results

### 3.1. Peracute enrofloxacin administration

Two discrete results emerged from the study. First, no evidence of weakness and no adverse effects on general health were observed in rabbits treated at the recommended dosage regimes or in a 10 times higher dose of 50 mg/kg of enrofloxacin in all groups. Second, there was no significant difference in the QT interval and QTc, and other ECG parameters with the normal dose of 5 mg/kg, and the highest dose of 50 mg/kg in rabbits during the experiment. The electrophysiological effects of enrofloxacin are summarised in Tables 1A and 1B.

A number of issues were identified. No statistically significant changes in heart rate were observed at any of the dose levels in group 1 during the experimental procedure, but there was a dose-related increase in plasma concentrations of enrofloxacin. The results of this investigation show that enrofloxacin at clinically relevant dose of 5 mg/kg and in a 10 times higher dose of 50 mg/kg should have no torsadogenic potential. One of the more significant findings to emerge from this study is that enrofloxacin in the conscious rabbits had no effect on QTc and causes QT prolongation only when administered at normal and higher doses injected intravenously (Tables 1A and 1B).

As shown in Table 2, MCHC count significantly increased in group 1 when compared with that at 0, 30, and 60 min, and MCV count was significantly decreased at 30 min and increased at 60 min. As shown in Table 3, CK significantly was increased at 60 min compared with that at 0 and 30 min.

No statistically significant changes in serum biochemical findings (as can be seen in Table 3), or TAC, TOS, or OSI were observed in group 1 (Table 4). As Table 5 shows, there was a significant difference ($P < 0.05$) in the serum concentration of enrofloxacin and its metabolite ciprofloxacin between the sampling times.

### 3.2. Subacute enrofloxacin administration

As shown in Table 6, the body weight gain (g) of rabbits did not significantly change in group 2 during the experimental period. Food consumption by the rabbits in group 2 did not change, and water consumption decreased during the experimental period (data not shown).

The experimental design showed that enrofloxacin or its metabolite ciprofloxacin, when administered at the recommended dose of 5 mg/kg once daily for 6 weeks in group 2, did not cause a significant prolongation in the QT interval (Tables 7 and 8). It appears that enrofloxacin, at clinically relevant doses and in a 10 times higher dose of 50 mg/kg, had no torsadogenic potential either. No statistically significant changes were observed in haematological findings or serum biochemistry, TAC, TOS, or OSI during the experimental period in group 2 (as can be seen in Tables 9–11). No statistically significant changes were observed in mean serum concentration of enrofloxacin or its metabolite ciprofloxacin between the sampling times either (Table 12).

**Table 1A.** Interval amplitudes and other electrocardiographic parameters measured over lead II after the normal-dose injection of enrofloxacin in group 1.

| | Time | | | | | |
|---|---|---|---|---|---|---|
| | 0 min | 5 min | 10 min | 15 min | 20 min | 30 min |
| Heart rate (bpm) | 251.4 ± 11.9 | 263.4 ± 15.6 | 259.71 ± 15.07 | 253.29 ± 14.13 | 237 ± 17.13 | 245.57 ± 13.45 |
| QT interval (ms) | 153.43 ± 11.57 | 170.29 ± 4.12 | 172.7 ± 9.12 | 174.29 ± 8.66 | 168 ± 6.32 | 164.43 ± 6.32 |
| QTc interval (ms) | 204.14 ± 6.47 | 208.43 ± 4.04 | 205 ± 9.64 | 214.71 ± 9.55 | 198.57 ± 4.63 | 198.71 ± 5.82 |
| PR interval (ms) | 79.57 ± 9.44 | 50.57 ± 14.29 | 71.43 ± 2.72 | 62.86 ± 3.90 | 55 ± 9.37 | 57.43 ± 10.56 |
| P interval (ms) | 68.57 ± 8.75 | 50.14 ± 10.16 | 59.43 ± 3.64 | 51.14 ± 3.97 | 41.86 ± 7.10 | 46.43 ± 8.81 |
| P amp (mV) | 0.04 ± 0.01 | 0.02 ± 0.01 | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 |
| QRS complex (ms) | 46.57 ± 2.39 | 42.43 ± 2.05 | 49.86 ± 9.13 | 48.14 ± 5.56 | 42 ± 8 | 46.86 ± 3.33 |

There was no statistical significance in the same column ($P > 0.05$). Values are presented as mean ± SEM.

**Table 1B.** Interval amplitudes and other electrocardiographic parameters after in a 10 times higher dose of 50 mg/kg over 10 min in group 1.

| | Time | | | | | |
|---|---|---|---|---|---|---|
| | 5 min | 10 min | 15 min | 30 min | 45 min | 60 min |
| Heart rate (bpm) | 254.14 ± 13.40 | 254.43 ± 20.43 | 216.86 ± 34.87 | 272.14 ± 9.50 | 246.86 ± 14.61 | 249.71 ± 16.86 |
| QT interval (ms) | 175.86 ± 17.43 | 175.86 ± 4.13 | 169.86 ± 4.01 | 172.86 ± 5.94 | 178.57 ± 10.74 | 169.43 ± 5.91 |
| QTc interval (ms) | 199.29 ± 4.98 | 214.71 ± 3.30 | 207 ± 4.03 | 211.14 ± 5.07 | 214.57 ± 8.81 | 205.57 ± 6.24 |
| PR interval (ms) | 55.14 ± 9.57 | 44.14 ± 16.07 | 60.43 ± 11.20 | 75.14 ± 8.46 | 66.86 ± 4.60 | 68.86 ± 2.80 |
| P interval (ms) | 44.71 ± 7.74 | 46.71 ± 12.80 | 59.29 ± 12.83 | 55.00 ± 3.57 | 56.43 ± 5.11 | 57.14 ± 3.78 |
| P amp (mV) | 0.03 ± 0.01 | 0.01 ± 0.01 | 0.03 ± 0.11 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.01 |
| QRS complex (ms) | 43.57 ± 2.28 | 50.14 ± 2.23 | 49.14 ± 4.59 | 41.43 ± 1.51 | 50.86 ± 7.12 | 50 ± 7.34 |

There was no statistical significance in the same column ($P > 0.05$). Values are presented as means ± SEM.

**Table 2.** Haematological parameters in rabbits treated enrofloxacin in group 1.

| | 0 min | 30 min | 60 min |
|---|---|---|---|
| Leukocytes ($\times 10^9$/L) | 8.78 ± 1.13 | 8.69 ± 0.98 | 8.25 ± 0.53 |
| Erythrocytes ($\times 10^{12}$/L) | 5.49 ± 0.23 | 5.43 ± 0.27 | 5.13 ± 0.30 |
| Haematocrit (%) | 33.39 ± 3.06 | 31.91 ± 2.96 | 31.23 ± 3.43 |
| MCV (fL) | 58.14 ± 0.91 | 58 ± 0.54 | 62.29 ± 0.81* |
| RDW (%) | 15.96 ± 0.46 | 16.60 ± 0.75 | 16.94 ± 0.88 |
| MCH (pg) | 20.46 ± 1.49 | 21.77 ± 1.67 | 22.16 ± 0.82* |
| MCHC (g/dL) | 35.36 ± 0.34 | 35.44 ± 0.25 | 35.39 ± 0.23 |
| Platelets ($\times 10^9$/L) | 359.86 ± 79.78 | 368.57 ± 67.24 | 360.14 ± 26.25 |
| Lymphocytes ($\times 10^9$/L) | 2.86 ± 0.57 | 2.51 ± 0.55 | 3.40 ± 0.66 |
| Monocytes ($\times 10^9$/L) | 0.54 ± 0.07 | 0.47 ± 0.07 | 0.86 ± 0.25 |
| Granulocytes ($\times 10^9$/L) | 5.98 ± 0.99 | 4.64 ± 0.78 | 5.45 ± 0.76 |
| Haemoglobin (g/dL) | 10.69 ± 1.40 | 11.51 ± 0.97 | 11.17 ± 0.91 |

MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RDW, red blood cell distribution width. Values are presented as means ± SEM. *There was statistical significance in the same line ($P < 0.05$).

**Table 3.** Serum biochemical findings in rabbits treated with enrofloxacin in group 1.

| | Aspartate aminotransferase (IU/L) | Creatine phosphokinase (IU/L) | Creatine phosphokinase-MB (IU/L) | Lactate dehydrogenase (IU/L) | Cardiac troponin I (ng/mL) |
|---|---|---|---|---|---|
| 0 min | 31.14 ± 8.65 | 487.14 ± 66.28 | 332.57 ± 94.94 | 64.43 ± 11.42 | 0 ± 0 |
| 30 min | 23.86 ± 6.99 | 478.71 ± 77.66 | 333.43 ± 95.78 | 68.57 ± 10.52 | 0.004 ± 0.003 |
| 60 min | 36.57 ± 5.34 | 2149.3 ± 363.43* | 489.56 ± 154.10 | 195.43 ± 60.18 | 0.041 ± 0.016 |

*There was statistical significance in the same column ($P < 0.05$). Values are presented as means ± SEM.

**Table 4.** Serum TAC, TOS, and OSI in group 1.

| | TAS (mmol Trolox equiv./L) | TOS ($\mu$mol $H_2O_2$ equiv./L) | OSI (arbitrary unit) |
|---|---|---|---|
| 0 min | 0.13 ± 0.03 | 5.49 ± 1.05 | 7298.29 ± 3182.72 |
| 30 min | 0.04 ± 0.02 | 6.25 ± 1.59 | 71,961.29 ± 24,024.4 |
| 60 min | 0.09 ± 0.02 | 20.73 ± 14.61 | 57,652.86 ± 37,453.99 |

There was no statistical significance in the same column ($P > 0.05$). Values are presented as means ± SEM. TAC: Total antioxidant capacity, TOS: Total oxidant status, OSI: Oxidative stress index.

**Table 5.** Mean serum concentration of enrofloxacin and its metabolite ciprofloxacin in group 1.

| | 0 min | 30 min | 60 min |
|---|---|---|---|
| Enrofloxacin (mg/L) | $0.000 \pm 0.000^a$ | $3.57 \pm 1.31^b$ | $85.71 \pm 9.53^c$ |
| Ciprofloxacin (mg/L) | $0.000 \pm 0.000^a$ | $0.16 \pm 0.03^b$ | $7.75 \pm 1.96^c$ |

Statistically significant among minutes marked with different letters ($P < 0.05$); the same letters show no significance. Values are presented as means ± SEM.

**Table 6.** Body weights comparison during the experimental period in group 2.

| | Week 0 | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|---|---|---|---|---|---|---|---|
| Body weights | 2275.7 ± 510.3 | 2462.8 ± 341.7 | 2602.7 ± 353.8 | 2652.1 ± 209.7 | 2632.8 ± 224.5 | 2638.8 ± 247.9 | 2605.7 ± 185.4 |

There was no statistical significance in the same line ($P > 0.05$). Values are presented as means ±SEM.

**Table 7.** Interval amplitudes and other electrocardiographic parameters after treatment with enrofloxacin during the experimental period in group 2.

| | Weeks | | | | | |
|---|---|---|---|---|---|---|
| | Week 0 | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 |
| Heart rate (bpm) | 227.72 ± 11.53 | 251.43 ± 8.48 | 238.00 ± 16.81 | 246.57 ± 13.80 | 233.43 ± 16.19 | 262.71 ± 13.49 |
| QT interval (ms) | 167.00 ± 3.57 | 162.00 ± 5.91 | 176.28 ± 7.42 | 158.57 ± 6.39 | 176.57 ± 7.45 | 162.00 ± 3.49 |
| QTc interval (ms) | 202.00 ± 4.55 | 199.14 ± 6.45 | 210.43 ± 6.64 | 194.57 ± 7.24 | 210.57 ± 8.45 | 200.43 ± 4.26 |
| PR interval (ms) | 78.571 ± 6.64 | 77.14 ± 5.97 | 70.14 ± 4.04 | 86.86 ± 8.41 | 73.86 ± 5.51 | 76.85 ± 5.86 |
| P interval (ms) | 61.714 ± 4.18 | 64.00 ± 6.07 | 55.14 ± 4.31 | 75.00 ± 10.60 | 63.85 ± 5.16 | 63.14 ± 6.55 |
| P amp (mV) | 0.016 ± 0.07 | 0.027 ± 0.01 | 0.025 ± 0.01 | 0.026 ± 0.01 | 0.031 ± 0.01 | 0.021 ± 0.01 |
| QRS complex (ms) | 43.28 ± 4.20 | 43.71 ± 2.53 | 45.14 ± 2.06 | 48.74 ± 6.654 | 55.57 ± 7.86 | 42.14 ± 3.47 |

There was no statistical significance in the same line ($P > 0.05$). Values are presented as means ± SEM.

No histopathological changes were observed in the treatment groups except for some minor abnormalities including hyperaemia in some heart areas when compared to the negative control group (Figure 1A–1C).

## 4. Discussion

Fluoroquinolones are widely used and well tolerated antibacterial agents for veterinary medicine. In our review of the literature, no data were found on the association between enrofloxacin and QT prolongation and TdP in

**Table 8.** Interval amplitudes and other electrocardiographic parameters at the end of week 6 after treatment with enrofloxacin during the experimental period in group 2.

| | Week 6 | | | | | |
|---|---|---|---|---|---|---|
| | 0 min | 5 min | 10 min | 15 min | 20 min | 30 min |
| Heart rate (bpm) | 240.86 ± 15.59[a] | 246.71 ± 11.87[b] | 245.57 ± 13.96[c] | 237.57 ± 15.68[d] | 225.00 ± 11.87[e] | 221.00 ± 16.58[f] |
| QT interval (ms) | 172.43 ± 5.17[a] | 184.57 ± 7.19[a] | 172.14 ± 4.64[a] | 176.57 ± 7.18[a] | 172.43 ± 4.34[a] | 170.29 ± 5.88[a] |
| QTc interval (ms) | 207.57 ± 4.18[a] | 220.71 ± 7.77[a] | 202.86 ± 3.99[a] | 210.71 ± 7.41[a] | 204.57 ± 2.17[a] | 201.00 ± 3.22[a] |
| PR interval (ms) | 96.29 ± 12.90[a] | 81.14 ± 5.79[a] | 71.29 ± 4.60[a] | 82.86 ± 5.46[a] | 75.29 ± 5.58[a] | 80.86 ± 6.26[a] |
| P interval (ms) | 85.43 ± 13.46[a] | 71.71 ± 6.76[a] | 59.14 ± 5.19[a] | 71.14 ± 6.01[a] | 64.86 ± 5.97[a] | 70.14 ± 6.89[a] |
| P amp (mV) | 0.018 ± 0.01[a] | 0.03 ± 0.01[a] | 0.02 ± 0.01[a] | 0.03 ± 0.01[a] | 0.02 ± 0.01[a] | 0.03 ± 0.01[a] |
| QRS complex (ms) | 49.14 ± 1.91[a] | 61.29 ± 10.52[a] | 49.00 ± 3.38[a] | 54.86 ± 8.39[a] | 51.00 ± 2.18[a] | 46.86 ± 1.41[a] |

Statistically significant among minutes marked with different letters ($P < 0.05$); the same letters show no significance. Values are presented as means ± SEM.

**Table 9.** Haematological findings in rabbits treated with enrofloxacin during the experimental period in group 2.

| | Week 0 | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|---|---|---|---|---|---|---|---|
| Leukocytes ($\times10^9$/L) | 8.79 ± 1.1[a] | 8.68 ± 0.9[a] | 8.15 ± 0.5[a] | 7.41 ± 0.5[a] | 8.43 ± 0.7[a] | 9.15 ± 0.6[a] | 8.91 ± 0.9[a] |
| Erythrocytes ($\times10^{12}$/L) | 5.66 ± 0.2[a] | 5.28 ± 0.2[a] | 5.13 ± 0.2[a] | 5.26 ± 0.3[a] | 5.52 ± 0.2[a] | 5.71 ± 0.2[a] | 5.64 ± 0.3[a] |
| Haematocrit (%) | 33.49 ± 1.3[a] | 32.32 ± 1.1[b] | 31.56 ± 1.3[c] | 32.56 ± 1.6[d] | 33.31 ± 1.4[e] | 34.20 ± 1.0[f] | 33.54 ± 1.4[g] |
| MCV (fL) | 59.00 ± 1.3[a] | 61.28 ± 1.2[a] | 61.71 ± 0.8[a] | 61.85 ± 1.0[a] | 60.43 ± 0.8[a] | 59.85 ± 0.7[a] | 59.71 ± 1.0[a] |
| RDW (%) | 16.16 ± 0.5[a] | 16.78 ± 0.7[a] | 16.70 ± 0.8[a] | 16.48 ± 0.6[a] | 15.44 ± 0.4[a] | 14.97 ± 0.3[a] | 15.08 ± 0.3[a] |
| MCH (pg) | 20.80 ± 0.5[a] | 22.03 ± 0.4[a] | 21.81 ± 0.3[a] | 21.75 ± 0.3[a] | 22.03 ± 0.2[a] | 21.27 ± 0.2[a] | 21.55 ± 0.2[a] |
| MCHC (g/dL) | 35.200 ± 0.3[a] | 35.94 ± 0.3[a] | 35.37 ± 0.2[a] | 35.15 ± 0.2[a] | 36.50 ± 0.3[a] | 35.50 ± 0.3[a] | 36.05 ± 0.5[a] |
| Platelets ($\times10^9$/L) | 371.57 ± 35[a] | 377.28 ± 37[a] | 403.00 ± 28[a] | 490.28 ± 46[a] | 399.43 ± 9.0[a] | 315.14 ± 44[a] | 305.28 ± 36.72[a] |
| Lymphocytes ($\times10^9$/L) | 30.61 ± 6.55[a] | 3.22 ± 0.82[a] | 3.26 ± 0.65[a] | 2.52 ± 0.60[a] | 2.94 ± 0.7[a] | 4.15 ± 0.91[a] | 2.29 ± 0.60[a] |
| Monocytes ($\times10^9$/L) | 7.23 ± 1.09[a] | 0.46 ± 0.09[a] | 0.49 ± 0.14[a] | 0.33 ± 0.03[a] | 0.43 ± 0.1[a] | 0.48 ± 0.08[a] | 0.35 ± 0.06[a] |
| Granulocytes ($\times10^9$/L) | 62.15 ± 6.28[a] | 4.99 ± 0.46[a] | 3.94 □0.35[a] | 4.56 ± 0.87[a] | 5.05 ± 0.6[a] | 4.50 ± 0.78[a] | 4.67 ± 1.05[a] |
| Haemoglobin (g/dL) | 11.81 ± 0.55[a] | 11.61 ± 0.38[a] | 11.13 ± 0.46[a] | 11.45 ± 0.62[a] | 12.16 ± 0.55[a] | 12.14 ± 0.35[a] | 12.18 ± 0.63[a] |

Statistically significant among minutes marked with different letters ($P < 0.05$); the same letters show no significance. Values are presented as means ± SEM.

clinically healthy rabbits. Therefore, the aim of this study was to assess the importance of enrofloxacin using the in vivo clinically healthy rabbit model with the normal dose and in a 10 times higher dose injection with various electrocardiographic parameters including RR, PQ, and QT intervals; QRS duration; QT/QTc prolongation; or TdP. Some authors speculated that increases in heart rate (HR), with accompanying decreases in QT intervals, could be masking an inherent increase in QT interval, identified with numerous drugs (Haverkamp et al., 2000; De Ponti et al., 2001; Redfern et al., 2003; Kijtawornrat et al., 2006; Ozkanlar et al., 2014). The reason for this was that a correction formula that adequately corrected QT intervals was used in the current study.

**Table 10.** Serum biochemical findings in in rabbits treated with enrofloxacin during the experimental period in group 2

| | Aspartate aminotransferase (IU/L) | Creatine phosphokinase (IU/L) | Creatine phosphokinase-MB (IU/L) | Lactate dehydrogenase (IU/L) | Cardiac troponin I (ng/mL) |
|---|---|---|---|---|---|
| Week 0 | 31.71 ± 4.71 | 652.71 ± 212 | 551.86 ± 224 | 167.85 ± 57 | 0.039 ± 0.034 |
| Week 1 | 32.85 ± 4.23 | 2867.85 ± 715 | 928.28 ± 457 | 194 ± 66 | 0.01 ± 0.006 |
| Week 2 | 61.14 ± 28.02 | 1990.57 ± 442 | 429.14 ± 122 | 128 ± 18 | 0.003 ± 0.002 |
| Week 3 | 41.86 ± 8.33 | 2865.28 ± 314 | 410.71 ± 116 | 157.71 ± 21 | 0.011 ± 0.007 |
| Week 4 | 85.72 ± 23.56 | 3316.57 ± 469 | 350.71 ± 65 | 123.43 ± 14 | 0.006 ± 0.002 |
| Week 5 | 196.57 ± 70.57 | 4700.28 ± 221 | 300 ± 27 | 254.28 ± 57 | 0.014 ± 0.005 |
| Week 6 | 200.14 ± 70.64 | 3410.57 ± 625 | 320.43 ± 31 | 177.86 ± 37.16 | 0.007 ± 0.003 |

There was no statistical significance in the same line (P > 0.05). Values are presented as means ± SEM.

**Table 11.** Serum TAC, TOS, and OSI during the experimental period in group 2.

| | TAS (mmol Trolox equiv./L) | TOS (μmol $H_2O_2$ equiv./L) | OSI (arbitrary unit) |
|---|---|---|---|
| Week 0 | 0.196 ± 0.04 | 20.363 ± 9.899 | 8732.86 ± 2089 |
| Week 1 | 0.036 ± 0.013 | 6.008 ± 0.758 | 91,978.57 ± 4864 |
| Week 2 | 0.005 ± 0.001 | 10.178 ± 5.281 | 266,430.4 ± 1165 |
| Week 3 | 0.085 ± 0.039 | 5.790 ± 0.572 | 75,142.57 ± 6106 |
| Week 4 | 0.080 ± 0.038 | 6.438 ± 0.526 | 103,966.1 ± 6377 |
| Week 5 | 0.065 ± 0.038 | 7.329 ± 1.049 | 58,177.43 ± 1966 |
| Week 6 | 0.083 ± 0.021 | 11.256 ± 1.550 | 42,212.14 ± 1874 |

There was no statistical significance in the same column (P > 0.05). Values are presented as means ± SEM. TAC: Total antioxidant capacity, TOS: Total antioxidant status, OSI: Oxidative stress index.

**Table 12.** Mean serum concentration of enrofloxacin and its metabolite ciprofloxacin during the experimental period in group 2.

| | Week 0 | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 |
|---|---|---|---|---|---|---|---|
| Enrofloxacin (mg/L) | 0 ± 0 | 0 ± 0 | 0 ± 0 | 1.52 ± 0.14 | 0 ± 0 | 0.98 ± 0.16 | 0 ± 0 |
| Ciprofloxacin (mg/L) | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0.55 ± 0.22 | 0 ± 0 | 0.11 ± 0.01 | 0 ± 0 |

There was no statistical significance in the same line (P > 0.05). Values are presented as means ± SEM.

Drug-induced QT prolongation is relatively common in clinical practice (De Ponti et al., 2001; Redfern et al., 2003; Ozkanlar et al., 2005b). It has been reported that sparfloxacin and grepafloxacin can prolong the QT interval to cause lethal ventricular arrhythmias (Bertino and Fish, 2000; Frothingham, 2001; Owens, 2001). Therefore, this finding has important implications for developing a therapeutic strategy. To date, there has been no scientific research on enrofloxacin associated effects on the rabbit's heart. Rabbits may be dosed with 2.5–5 mg/kg bw per day for 3–5 days by oral administration or by intramuscular injection (Elmas et al., 2006). In the present study, the normal dose of 5 mg/kg and the higher dose of 50 mg/kg were used in rabbits peracutely to investigate the relationship between serum concentration (the normal-dose and the higher-dose injection of enrofloxacin). In the current experimental period of peracute (group 1) and subacute application (Group 2), no QT prolongation, recurrent syncope, or sudden cardiac death secondary to atypical polymorphic ventricular tachycardia were

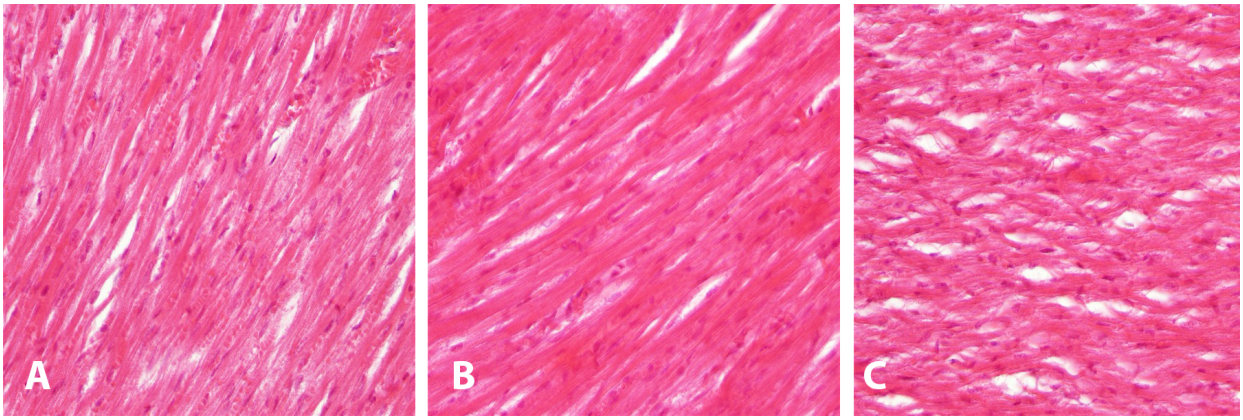


**Figure 1.** Histological examination of rabbit heart in the negative control (A) shows normal morphology. Histological examination of rabbit heart in group 1 (B) and group 2 (C) shows normal morphology except for some minor abnormalities including hyperaemia in some areas (H&E, 400×).

observed. In the current study, no evidence of abnormal ECG parameters was detected including RR, PQ, and QT intervals; QRS duration and QTc; and heart rate at normal and even a 10 times higher dose. A possible explanation for these results may be that heart tissue of the rabbit may be affected by even higher doses than those used in this study.

TdP is very important in terms of developing effective preclinical testing strategies and interpreting the data accurately (Frothingham, 2001; Redfern et al., 2003). Nevertheless, there was a significant difference ($P < 0.05$) in the serum concentration of enrofloxacin and its metabolite ciprofloxacin (a major metabolite of enrofloxacin) at sampling intervals (as can be seen in Tables 5 and 12). In contrast to earlier findings, however, no evidence of QT/QTc prolongation or potentially fatal ventricular arrhythmias associated with quinolones (Bertino and Fish, 2000; Frothingham, 2001; Owens, 2001) was detected in the current study. Hence, the data presented here demonstrate that enrofloxacin had no torsadogenic potential in rabbits at 5 mg/kg or higher dose of 50 mg/kg. It is possible, therefore, that this would create a more clinically relevant situation.

The plasma AST, LDH, and CK enzyme activities are important measures of both early and late phases of cardiac injury especially during clinical follow up (Saad et al., 2001). In the present study, the activities of all these three enzymes in plasma did not increase. They are not specific for myocardial injury individually; however, evaluation of these enzymes together may be an indicator of myocardial injury. Serum cTnI and CK are the preferred markers for the detection of minor ischemic cardiac injury (Apple et al., 1997). By treating the rabbits with enrofloxacin, AST, LDH, and CK enzyme activities and cTnI did not significantly change. This suggests that enrofloxacin may not have adverse effect on enzyme activities.

Oxidative stress is generally described as an imbalance between oxidant and antioxidant levels (Lykkesfeldt and Svendsen, 2007). Nitric oxide (NO), essential for the proper functioning of the cardiovascular system, is derived from L-arginine by NO synthase (NOS) in endothelial cells. NO synthase inhibition produces various cardiovascular abnormalities and ventricular contractile dysfunction (Loscalzo and Welch, 1995; Dickinson and Forman, 2002). Although no previous report has investigated TAS, TOS, and OSI as biomarkers of oxidative stress and antioxidant defence related to treating the rabbits with enrofloxacin, a possible explanation for this might be that free oxidant radicals are not produced.

The histopathology of the heart in the treatment groups did not show any remarkable changes, which was similar to the negative control when stained with haematoxylin and eosin and assessed using light microscopy. It can thus be suggested that peracute and subacute enrofloxacin application may have not adverse effects on myocardial tissue.

The first major finding is that enrofloxacin, at clinically relevant dose of 5 mg/kg and even excessive dosing (10 times higher dose of 50 mg/kg), has no electrophysiological abnormalities particularly QT prolongation or torsadogenic effect. It was also shown that administration of a daily single dose of enrofloxacin intravenously was a safe application protocol. This finding might be of use for safe administration of enrofloxacin to animals and especially to rabbits during infectious disease. However, more clinical studies are needed to identify possible combinations of medications that have a potential for a proarrhythmic effect.

**Acknowledgement**
This study was financially supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK, 111O048).

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