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# Evaluation of ameliorative potential of supranutritional selenium on enrofloxacin-induced testicular toxicity

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ABSTRACT

The study was designed to assess the ameliorative potential of selenium (Se) on enrofloxacin-induced testicular toxicity in rats. There was a significant decrease in body weight and non-significant decrease in mean testicular weight of enrofloxacin treated rats. In enrofloxacin treated rats, total sperm count and viability decreased where as sperm abnormalities increased. Testicular histopathology revealed dose dependent dysregulation of spermatogenesis and presence of necrotic debris in seminiferous tubules which was marginally improved with Se. Enrofloxacin also produced a dose dependent decrease in testosterone level. The activity of testicular antioxidant enzymes decreased where as lipid peroxidation increased in a dose-dependent manner. Se supplementation partially restored oxidative stress and sperm damage and did not affect the plasma concentrations of enrofloxacin or ciprofloxacain. The results indicate that enrofloxacin produces a dose-dependent testicular toxicity in rats that is moderately ameliorated with supranutritional Se.



## 1. Introduction

Quinolones have widely been prescribed to treat infections related to respiratory tract, urinary tract, skin and skin structure, as well as, intra-abdominal infections, sexually transmitted diseases, bone and joint infections [1]. Enrofloxacin, a 6-fluoroquinolone, is one of the most widely used antimicrobials in veterinary practice. It is a bactericidal drug with a broad spectrum antimicrobial activity against a range of bacteria, including the strains resistant to other antimicrobial agents [2]. Fluoroquinolones have been reported for detrimental effects on male reproductive system [3–5]. Induction of oxidative stress, reported with several fluoroquinolones, has been incriminated as a mechanism for several of their adverse effects [2,4,6,7]. Selenium (Se), an essential dietary element in human and animal nutrition, is an integral component of various antioxidant proteins and is required for normal testicular development and spermatogenesis [8]. Organic Se, a natural form of Se, is more antioxidantive than inorganic form [9]. Fodder is a source of organic Se for animals where it is found as amino acid conjugates; selenomethionine and selenocysteine [10]. Se improves testicular antioxidant status by enhancing the activities of antioxidant enzymes, in particular, glutathione peroxidase (GPx) and thioredoxin [11,12]. Although amelioration of xenobiotic induced testicular toxicity is documented with Se [13–15], there is paucity of literature on the effect of Se on fluoroquinolone-induced testicular toxicity. Taking the above facts into consideration, the current investigation was undertaken to evaluate ameliorative effect of Se on the dose-dependent enrofloxacin-induced testicular toxicity in rats.

## 2. Materials and methods

Male Wistar rats of 60–65 days age were maintained in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. The study is approved by Institutional Animal Ethics Committee vide order number; VMC/13/1786-1806. Prior to the start of experiment, rats were acclimatized to laboratory conditions for a period of seven days. *Ad libitum* water and handmade pellets were

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provided to the animals. The rats were randomly divided into 5 groups of 8 rats each. One group served as control. Two groups were administered enrofloxacin (Floxidin VetR 10%, Intervet India Pvt. Ltd.) at the dose rate of 20 and 80 mg/kg body weight per day, respectively, for 21 days. Two more groups were co-fed organic Se at supranutritional level and gavaged enrofloxacin at the same dose rates of above groups, respectively. The therapeutic dose of enrofloxacin in rats is 5–20 mg/kg body weight [16]. The selected dose (20 mg/kg body weight) corresponded to the highest limit of the above therapeutic dose range, whereas, 80 mg/kg body weight per day corresponded four times this limit. The dosing was carried out in morning. Rats were kept under constant observation during the entire period of study. After the end of 21 day study period, animals were anaesthetized with diethyl ether and blood was collected in heparinised vials by cardio-puncture. Blood was centrifuged at 3000 rpm for 15 min to separate the plasma which was then stored at −20 °C till further analysis.

Immediately after humanely sacrificing the rats, testes were removed, cleaned of the adhering tissue and weighed. For epididymal sperm analysis, epididymus was excised and placed in prewarmed petri dish containing 0.5 mL phosphate buffered saline (PBS, pH 7.4) at 37 °C. Testis were fixed in 10% neutral buffer formalin, processed by acetone benzene method. Paraffin embedded tissue sections (5 μm thick) were taken on clean glass slides and stained with haematoxylin and eosin for histopathological evaluation [17]. Sperm count of epididymal samples was determined with haemocytometer under high power magnification of microscope (400X) (Nikon, Japan). The total number of spermatozoa in five squares was counted and number of spermatozoa was calculated by using the following formula $= X \times 10 \times 10^6$ spermatozoa/mL, where X is number of spermatozoa in five squares [18]. A drop of seminal fluid containing spermatozoa was mixed with a drop of Eosin/Nigrosin stain, kept at 37 °C for 2 min; smeared on clean glass slides and examined at 1000X for live (unstained) and dead (pink) spermatozoa. About 100 live and dead spermatozoa were counted in different fields and percentage of live spermatozoa was calculated [18]. For the calculation of abnormal spermatozoa about 100 spermatozoa with normal and abnormal morphology were counted in different fields and the percentage of abnormal spermatozoa was estimated.

Plasma levels of testosterone hormone were assayed by Lumax™ Model 4101 Chemiluminiscence Immuno Assay (CLIA) Strip Reader (Monobind, Inc. USA), using Acculite CLIA microwells (Monobind, Inc. USA), as per the standard manufacturer protocol. After plasma sample processing [19], concentration of enrofloxacin and ciprofloxacin in plasma was determined simultaneously using reverse-phase HPLC with UV detection system [20]. Testicular homogenate (10%) prepared in chilled phosphate buffer saline (pH 7.4) was centrifuged at 4000 rpm for 15 min to harvest supernatant which was used for estimation of activities of enzymes; superoxide dismutase (SOD) [21], catalase (CAT) [22], and (GPx) [23] and levels of malondialdehyde (MDA) an indicator of lipid peroxidation (LPO) [24].

The results for body weight, testicular weight, sperm parameters, testosterone levels, enrofloxacin and ciprofloxacin concentration and oxidative stress parameters were analyzed by one-way ANOVA. The differences between means were compared with Duncan's test using SPSS®16 software. The significance was assessed at $P < 0.05$ and $P < 0.01$.

## 3. Results

### 3.1. Body weight and testicular weight

The results for the effect of repeated administration of enrofloxacin on body weight and testicular weight of rats are presented in Table 1. The repeated oral administration of enrofloxacin at low and high dose for 21 consecutive days did not produce any apparent clinical signs of toxicity. However, there was a significant decrease in body weight of all enrofloxacin treated groups when compared to the control group. The mean testicular weight of various groups differed non-significantly from each other.

### 3.2. Testicular histopathology

Testicular histopathology revealed that the group, administered lower dose of enrofloxacin, showed very little deviation from the control group (Figs. 1 and 2). However, higher dose enrofloxacin resulted in dysregulation of spermatogenesis with decreased spermatogenesis and sperm maturation, presence of necrotic debris and/or proteinaceous fluid in the seminiferous tubules (Fig. 3). Se supplementation in feed improved spermatogenesis as evident from the lesser degree of histoarchitectural damage in the groups co-treated with enrofloxacin and Se, when compared with the parallel enrofloxacin alone treated groups (Figs. 4 and 5).

### 3.3. Total sperm count, live sperm percentage and sperm abnormalities

The results for the effect of repeated administration of enrofloxacin and Se on total sperm count, live sperm percentage and sperm abnormalities are presented in Table 2. There was a significant decrease in total sperm count in all enrofloxacin-treated groups, with lowest count found in the group treated with enrofloxacin alone at 80mg/Kg body weight. The percentage of live spermatozoa and abnormal spermatozoa, in all enrofloxacin treated groups differed significantly from each other as well as control group with lowest viable percentage found in the group treated exclusively with enrofloxacin at 80mg/Kg body weight. When the percentage of live spermatozoa in enrofloxacin alone treated groups is compared to their Se co-treated counter groups, the later groups had significantly higher percentage. A reverse trend was noted with the percentage of abnormal spermatozoa. Bent or coiled tail, tailless head and decapitated head were the major sperm abnormalities observed.

### 3.4. Plasma concentration of testosterone, enrofloxacin and ciprofloxacin

Table 3 represents results for plasma concentration of testosterone, enrofloxacin and ciprofloxacin in various study groups. When compared to control rats and the groups receiving low dose enrofloxacin, there was a significant reduction in the levels of plasma testosterone in rats receiving high dose enrofloxacin both alone and in combination with Se. However, the plasma testosterone levels of the group receiving high dose enrofloxacin with Se were significantly higher than the group receiving high dose enrofloxacin alone. Plasma concentrations of enrofloxacin and its metabolite, ciprofloxacin, in rats after the completion of treatment period, were significantly higher in rats receiving higher dose enrofloxacin. Co-administration of Se did not produce any significant effect on plasma concentrations of the two fluoroquinolones.

### 3.5. Testicular antioxidant status

Results for the effect of repeated administration of enrofloxacin on testicular antioxidant status are presented in Table 4. When compared to the control group, the activity of SOD in the groups treated with high dose enrofloxacin, both alone and in combination with Se, was significantly lower. However, the latter group had a