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Toxic Effects of Norfloxacin on Cartilage Tissues of Broiler Chicken

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ABSTRACT

Background: Norfloxacin is reported to have a wide distribution in the body. It penetrates well into tissues of the genito-urinary tract and crosses the placenta as well and it has relatively high concentrations when it gets into bile. After the withdrawal of enrofloxacin by the U.S. FDA for its use in poultry, the importance of norfloxacin is getting increased and already some veterinary formulations are introduced by authorized companies to the market. Besides, its wide usage for various bacterial infections, new and unrecognized toxicities have emerged; where the most important finding from the pre-clinical evaluation of flouroquinolone was arthropathogenic potential in young animals. This study was conducted to investigate the toxic effects of norfloxacin on the cartilaginous tissues of broiler chickens.

Materials and Methods: The norfloxacin powder was obtained from Trichem laboratories in Bangalore. For better dissolving 1gr norfloxacin was first added to 0.25 ml of acetic acid and 2 ml of 50mmol/L acetate buffer maintained at pH 4.5 was added and mixed until the drug was completely dissolved. The experiment was carried out under hygienic conditions and standard management. One-week-old broiler chickens were procured from a reputed hatchery and divided into five groups; each containing six chickens. The study was conducted for 28 days.

Findings: In the result of the histopathological study, chondrocytes were swollen with degeneration, infiltrated neutrophils, and improper ossification. The chondrocytes degeneration around the blood vessels was also noticed. The lesions were supported by the biochemical finding of ALP (Alkaline phosphatase). On the 21st day of treatment. There was a significant increase in ALP values of groups IV, and V. On the 28th day of treatment the mean serum ALP was significantly increased in groups III, IV, and V birds ($P < 0.05$, $P < 0.01$, $P < 0.01$) respectively. They were compared to group I birds.

Conclusion: It was concluded that norfloxacin has a toxic effect on the cartilaginous tissues of chickens at the dose of 333 and 1100 mg/kg orally.

Keywords: Norfloxacin; Toxicity; Chondrocytes degeneration; Broiler chicken; Cartilage; Alkaline phosphatase

INTRODUCTION

Research into fluoroquinolone antibacterial has led to the discovery of several compounds with greatly improved potency, spectrum, bioavailability, and clinical efficacy (Ball, 1989). Norfloxacin is reported to have a wide distribution in the body. It penetrates well into tissues of the genito-urinary tract and crosses the placenta as well and relatively high concentrations were achieved in bile (Anadón et al., 1995). After the withdrawal of enrofloxacin by the US FDA for its use in poultry, the importance of norfloxacin is getting increased and already some veterinary formulations are introduced by authorized companies to the market.

Besides its wide usage for various bacterial infections, new and unrecognized toxicities have emerged; the most important finding from the pre-clinical evaluation of flouroquinolone was arthropathogenic potential in young animals (Stahlmann et al., 1990). For many years, quinolones induced cartilage toxicity in skeletally immature animals represented indisputable contraindication for the use in young animals. Apart from these, other organs like the kidney and liver have also been stated as the possible targets of quinolone toxicity (Christ, 1990). Corrado et al. (1987) reported toxicosis including cartilage erosion in juvenile dogs and crystalluria in dogs given 50-300 mg/kg norfloxacin for 20 weeks.

Fluoroquinolone antibacterial agents have been reported to induce tendon lesions in juvenile rats. The toxic potentials of 10 fluoroquinolones on the Achilles tendon were compared in juvenile rats. The toxic potential was differentiated: fleroxacin and pefloxacin were the most toxic, with the lowest toxic dose being 100 mg/kg; lomefloxacin, levofloxacin, and ofloxacin were the second most toxic, with the toxic dose being 300 mg/kg; sparfloxacin and enoxacin were the third most toxic, at 900 mg/kg, while norfloxacin, ciprofloxacin, and tosufloxacin showed no toxicity, even at the high dose of 900 mg/kg (Kashida & Kato, 1997). Chyský et al. (1991) reported that there were clear species and drug differences in quinolone effects on cartilage. When individual compounds were compared, pefloxacin caused slightly more transient articular adverse effects than ciprofloxacin Primary injury to juvenile cartilage may be from the direct action of these compounds or metabolites on the chondrocyte. The repression of normally secreted proteolytic inhibitors (Glynn, 1977).

According to Bell (1960) chickens 5-6 weeks of age with no visible lesions, the alkaline phosphatase ranged from 150-520 units. The levels increased in diseases of osseous origin. In the chick, Surendranathan &Nair (1981) reported normal blood glucose and alkaline phosphatase concentration of 153.61±2.15 and 2.08.98 ± 4.32 units respectively.

MATERIALS AND METHODS

A study was conducted to investigate the toxic effect of norfloxacin on the cartilaginous tissue of broiler chickens. The norfloxacin powder was obtained from Trichem laboratories in Bangalore. The norfloxacin, a yellowish white powder was not soluble in water. To make it soluble in water, an acetate buffer (Acetic acid 50 mmol/L and 50 mmol / L of sodium acetate with pH 4.5) was prepared. One g norfloxacin was first added to 0.25 ml of acetic acid and 2 ml of 50mmol/L acetate buffer maintained at pH 4.5 was added and mixed until the drug was completely dissolved. Thus the prepared stock solution was used for further dilution.

The experiment was carried out under hygienic conditions and standard management one one-week-old broiler chicken was procured from a reputed hatchery and divided into five groups each containing sex chickens. The norfloxacin was administered at the doses of 111 mg/kg, 333 mg/kg, and 1100 mg/kg orally for 28 days.

Groups	Dose
Group I (Control)	Distilled water
Group II (Low dose)	0.11 g/kg
Group III (Medium dose)	0.333 g/kg
Group IV (High dose)	1.1 g/kg
Group VI (Vehicle)	Vehicle

The cartilage tissue from the Achilles tendon was collected on day 29 and was subjected to Histopathology and the blood samples were collected from the jugular vein on days 7, 14th, 21st, and 28 for biochemical analysis.

RESULTS

The birds who received med and high doses were showing difficulty in walking during the fourth week. The lesions recorded in chondrocytes for the high dose group were infiltration of neutrophils, improper ossification, degeneration around the blood vessels, and hemorrhage between bone and cartilaginous tissues. The same lesions in mild form were observed in cartilage tissue of broiler chickens received a 333 mg/kg dose of norfloxacin but no lesions were observed in chickens received 111mg/kg of norfloxacin **(Fig. 1).**

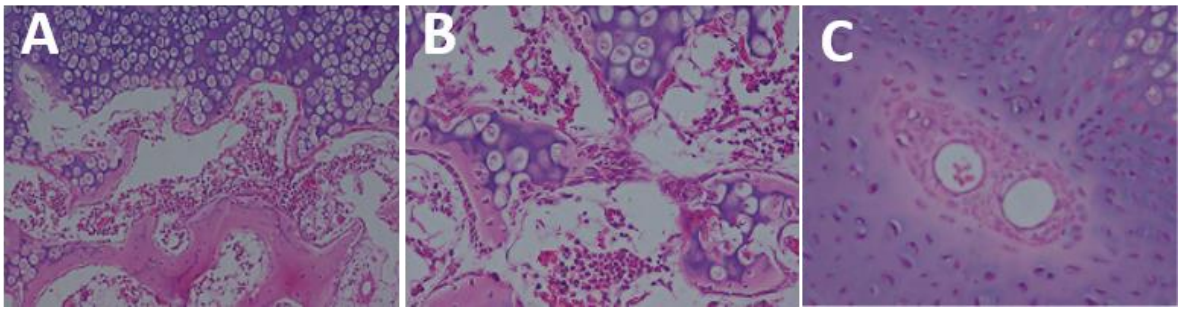

Fig. 1. Effects of norfloxacin on the Histomorphology of cartilage and chondrocytes. The results showed hemorrhage between the cartilaginous plates (Plates1-3) and chondrocytes were swollen with degeneration, infiltration of neutrophils and improper Ossification **(A&B)**. The chondrocytes degeneration around the blood vessels was also noticed **(C)**, (H & E 500X).

The lesions were supported by biochemical finding of ALP (Alkaline phosphatase)

On the 21st day of treatment I, II, III, IV, and V was 229.71±24.40, 221.51±10.00, 321.80±21.16, 635.03±22.35, 802.62±29.93 respectively. There was a significant increase in ALP values of groups IV, and V. On the 28th day of treatment the mean serum ALP level of groups I, II, III, IV, and V were 217.67±25.03, 216.79±32.43, 282.41±18.13, 561.80±32.00, 845.45±39.42 respectively. It was significantly increased in groups III, IV, and V birds (P <0.05, P <0, 01, P <0, 01) respectively compared to group I birds. The ALP levels of control and different treated groups are given in **Table 1.**

Table 1. Effects of Norfloxacin on ALP (U/dl) level of experimental broilers in repeated dose 28 day oral Toxicity study

Groups	Day 7	Day 14	Day 21	Day 28
Group I (control)	222.32±19.87	212.10±7.33	229.71±24.40	217.67±25.03
Group II (Vehicle)	228.03±7.93	259.75±20.51	221.51±10.00	216.79±32.43
Group v (0.11g/kg)	280.82±21.03	332.10±25.10	321.80±21.16	382.41±18.13*
Group IV (0.333g/kg)	460.57±28.13	532.53±32.25*	635.03±22.35***	561.80±32.00***
Group III (1.1g/kg)	528.15±62.43	773.64±54.71*	802.62±29.93***	845.45±39.42***

Data is presented as Mean±SE (n=6).

*, **, ***: Means are different in the same row at significance level of ***P<0, 01, **P<0, 01, *P<0, 05.

DISCUSSION

The results of the study indicate that the birds received med and high doses of norfloxacin were showing difficulty in walking during the fourth week. At the same time, histopathological lesions **(**infiltration of neutrophils, improper ossification, degeneration around the blood vessels, and hemorrhage between bone and cartilaginous tissues) were recorded in chondrocytes of high dose groups. The findings correlate with a finding (Stallmann, 1990): arthropathogenic potential in young animals (Christ et al., 1990): cartilage toxicity in skeletally immature animals Corrado et al. (1987): cartilage erosion in juvenile dogs but our study finding in term of med dose isn't within finding of (Kashida & Kato, 1997) where norfloxacin, ciprofloxacin, and tosufloxacin showed no toxicity, even at the high dose of 900 mg/kg in juvenile rats. This deference may be due to species variation and the opinion is getting supported by the findings of Chysky et al. (1991) who reported that there were clear species and drug differences in quinolone effects on cartilage. In this research, lesions were supported by the biochemical finding of ALP (Alkaline phosphatase) much with the finding of Bell (1960) who show that ALP getting increased in diseases of osseous origin.

CONCLUSION

From the present study it was concluded that the norfloxacin has toxic effect on the cartilaginous tissue of chickens at the dose of 333 and 1100 mg/kg orally.

Conflict of Interest

No, conflict of interest among all authors

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