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Evaluation of Norfloxacin Acute Toxicity in Five Day old Broiler Chicken

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ABSTRACT

Background: Norfloxacin is a second generation fluoroquinolone, used widely against sensitive organisms. 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 were introduced by pharmaceutical companies in the market. The present study was undertaken to evaluate the acute toxicity of Norfloxacin IP, a member of fluoroquinolone in five day old broiler chicken.

Materials and Methods: Healthy 60 COBB broiler a day old chicks, with an average body weight of 45±5g were acclimatized to the laboratory condition for five days. Six groups; each group consisting 10 experimental birds were used for determining LD_{50} safety value. Clinical observations were made. The tissue samples from concerned organs of all the birds were collected for histopathological study.

Findings: The maximum dose of Norfloxacin, where no mortality occurred was 1.4 g /kg. The dose at which 100 percent mortality observed was calculated to be 1.8 g/kg /body weight. The clinical signs of toxicity observed before death were dullness, excitability, and drowsiness, circling, oozing of fluid from mouth and gasping for breath with beaks wide open. The calculated LD_{50} value for Norfloxacin was found to be 1.55 g/kg for 24 h observation period. The histopathological findings observed in concerned organs were: hemorrhage, congestion and tubular epithelium degeneration and necrosis in kidney, Hemorrhage, edema and infiltration of few inflammatory cells in heart. Congestion of vessels and dilatation of sinusoidal space were observed. Perivascular hepatocytes were degenerated with infiltration of few inflammatory cells on liver and sever lymphocytolysis in spleen.

Conclusion: Norfloxacin can be classified as a moderately toxic drug according to Global Harmonization System (GHS) for classification of toxic compounds as per given protocol.

Key words: $LD_{50;}$ Acute toxicity; Broiler chicken; Lesions; Norfloxacin

INTRODUCTION

Fluoroquinolones a class of antimicrobials which is getting widespread acceptance for the treatment of various bacterial infections due to their broad spectrum antibacterial activity against most of the sensitive gram-positive and gram–negative aerobes, *Mycoplasma* spp. and *Rickettsia* spp at very low concentration (Brown, 1996).

Norfloxacin is a second generation fluoroquinolone, primarily used in the treatment of urinary tract infections, however later clinical studies have shown the efficacy of Norfloxacin for a variety of gram-negative infections including pneumonia, CNS infections, prostatitis and septicemia (Mascellino et al., 1988). Norfloxacin usage in the treatment of gastrointestinal infections caused by *Esherichia coli*, *Salmonella* spp., *Shigella* spp. and *Campylobacter* spp. were also reported (Braunwald et al., 1987). Beside its wide usage for various bacterial infections, new and unrecognized toxicities have emerged; most important finding from pre-clinical evaluation of the flouroquinolone was arthropathogenic potential in young animals due to Ciprofloxacin toxicity (Stahlmann et al., 2000). Apart from these, other organs like kidney and liver have also been stated as the possible targets of quinolone toxicity (Christ, 1988). Norfloxacin is second-generation fluoroquinolone compound featuring a fluorine atom at position-6 and piperazinyl substituent at position-7 of the quinolone nucleus (Wentland, 1990). Clinically, fluoroquinolones are generally well tolerated, but CNS disorders including confusion, hallucination, anxiety, nervousness and seizures have been reported in two per cent of human population (Anastasio et al., 1988; Christ, 1990). Sachan (1998) conducted an acute oral toxicity study of pefloxacin on day old broiler chicks and reported LD_{50} of 1025 mg/kg for pefloxacin and also observed toxic CNS clinical signs such as excitability followed by dullness after single oral administration of pefloxacin. Zou and Wang (2007) reported the oral LD_{50} 3458 mg/kg for Norfloxacin nicotinate in 5-week-old chickens and toxic clinical signs observed in chicks were salivation, diarrhea and nervousness.

The flouroquinolones can cross the blood brain barrier and have been proposed as alternative for the treatment of CNS infections not responding to other drugs (Scheld, 1989). Fluoroquinolone antibiotics were found to induce central nervous system excitatory side effects, including anxiety, nervousness, hallucination, and even seizures on rare occasions (Christ, 1990). Domagala (1994) had pointed out that the substitution at the seventh position of fluoroquinolones greatly influences their efficacies and toxicities. For example, central nervous system effects and interactions with theophylline and nonsteroidal anti-inflammatory drugs were reported to be directly influenced by the substitution at the seventh position in chemical structure. A study was conducted by Zhang et al. (2003) regarding neurotoxicity and toxicokinetics of Norfloxacin on free moving rats in which the epileptiform discharges appeared in all Norfloxacin groups with different latent periods, accompanied with limb twitching and colonic and tonic seizures and relative power of EEG increased. The effect of ciprofloxacin and Norfloxacin treatments on the behavior of rats in the open-field, elevated plus maze, elevated zero maze, feeding latency and social interaction tests were respectively observed. In the result of the ratio between open arm and enclosed arm time and entries also indicated that both ciprofloxacin and Norfloxacin treated rats showed anxious behavior in comparison with control rats in all the parameters studied. Ciprofloxacin and Norfloxacin treatments caused significantly enhanced feeding latencies in comparison to control treatment in the novel environment. The consumption of feed in Norfloxacin treated group was 50.29±1.84 in control group 67.57±1.53 which showed a significant increase when compared to control group (Sen et al., 2007). The quinolones bore both an acidic group (carboxylic acid) and a basic group (tertiary amine). This association gave them amphoteric properties. Their lipid

solubility was low except between pH 6 and 8, within this range they had low water solubility and was proven to precipitate under more acidic conditions. Due to this property crystalluria had been observed in man and animals (Ball, 1986). Corrado et al., (1987) noted crystalluria in dogs after administration of norfloxacin at the dose of 50-300 mg/kg for 20 weeks. As the primary rout for excretion of fluoroquinolones was kidney so for high urine concentration and their poor water solubility at acidic pH had caused the formation of crystals in the urinary tract, where the crystals were thought to be responsible for the renal lesions (Ball, 1986). This reason is based on the fact that lesions of the kidney following fluoroquinolone exposure have never been observed without the presence of crystals but crystals have been observed without evidence of renal lesions. Moreover crystals were also present at lower concentrations than those producing the lesions (Vancutsem et al., 1990). 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 formulation are introduced by reputed pharmaceutical companies in the market. In view of the above facts the present study was undertaken on Norfloxacin in broiler chicken with the following objective. This study was conducted to evaluate the acute toxicity of Norfloxacin IP a member of fluoroquinolone class antimicrobials in broiler chickens.

MATERIALS AND METHODS

Experimental birds: Sixty unsexed COBB breed of broiler day old chicken with an average body weight of 45±5g were procured for the study from a local farm. They were housed in carton boxes. Plastic mesh was used during the primary days of experiment to avoid any discomfort and any annoyance from the insects, mosquitoes etc. Standard management and hygienic conditions were maintained during the period of the experiment. The chicks were allowed to acclimatize with laboratory conditions for 5 days before starting the experiment

Feed and water consumption: Standard feed free from any antibiotics and drugs including coccidiostats procured from is prepared to the birds' ad libitum, Water was given ad libitum.

Drug administration: The norfloxacin yellowish white powder was not soluble in water. One gram norfloxacin was first added to 0.25 ml of acetic acid and 2 ml of 50mmol/L acetate buffer pH 4.5 then stirred until the drug was completely dissolved. The pH of the drug was checked and was solution was pH=4.5. This stock solution was used for further dilution. Fifty mmol / L Acetate buffer was prepared in the following method. Acetic acid 50mmol/L and 50 mmol / L of sodium acetate were prepared then the acetic acid solution was added to the solution of sodium acetate to makeup the pH 4.5. In acute toxicity study the required dose of norfloxacin drug was dissolved in solvent, by addition required amount of distilled water to change the fixed dose concentration and administered through oral rout by using modified infant stomach tube guage No-8. The drug was administered in the solution form directly to the crop of bird through oral rout. For sub-acute toxicity study 1 g norfloxacin IP powder was dissolved in 0, 25 ml of acetic acid and 2ml of acetate buffer that was used as vehicle and the last volume was 3ml that had the concentration of 0.333 g/m. The solution was prepared fresh every day before administering to the birds.

Study procedure: The acute oral toxicity for norfloxacin was conducted in five day old broiler chicken according to the OECD (Organization for Economic Cooperation and Development) guidelines 420.

Preparation of the birds: Healthy 60 COBB breed of broiler day old chicks with an average body weight of 45±5g were adapted to the laboratory condition for five days prior to experiment.

Experiment design: Six groups of birds each group of experimental birds consist 10 experimental birds were used for determining LD_{50} safety value by random selection based on body weight.

Dose selection: The dose to be selected was derived after conduction of preliminary study according to OCDE guideline. Six doses were selected for determining LD_{50} value.

Administration of the dose: Experimental birds used for acute toxicity safety study were given a single oral toxic dose of norfloxacin IP. It was administered directly into the crop using through oral rout by thin modified infant feeding plastic tube gauge No-8 attached to a one ml syringe and the volume of norfloxacin IP solution was maintained up to one ml per experimental bird dilution of with distilled water. Feed was withheld for 12 h before drug administration and offered 6 h after drug administration. Water was provided *adlibitum*

The groups detail dose of norfloxacin IP per kg body weigh concentrations are given in the Table below.

Table 1. The details regarding dose of Norfloxacin administered for each group

No	Groups	No.of Birds	Dose (g/kg)	Concentration after dilution water with distilled
1	Group I	10	1.4	3.1461
2	Group II	10	1.5	3.1761
3	Group III	10	1.6	3.2041
4	Group IV	10	1.7	3.2304
5	Group V	10	1.8	3.2553
6	Group VI	10	1.9	3.2788

Birds general clinical observation: During the observation period birds were carefully observed for effects on skin, face, eyes, mucous membranes, circulatory and respiratory systems, autonomic nervous change as salivation, central nervous system effects as tremors and convulsions, changes in the level of activity, reactivity to handling or sensory stimuli, and altered strength, health conditions, gait, posture, and mortality. The LD_{50} was calculated as per the graphical method described by ProStat software Pearl River. NY 10965. USA.

Collection of organs: The birds, gross morphological changes, were recorded during autopsy. The representative tissue samples from organs such as liver, kidney, spleen, intestine, heart, pancreas, brain of birds were collected for histopathological study. The collected organs were fixed in 10 per cent neutral buffered formalin (Anderson, 1997). The liver, spleen, kidney, heart, lung, intestine, brain of five microns thickness and stained with Haematoxylin and Eosin stain (Luna, 1968).

RESULTS

LD_{50} of norfloxacin: The toxicity study of Norfloxacin was carried out in the present study. From the present study it was found that the maximum dose of Norfloxacin where no mortality occurred was at 1.4 g /kg. The dose at which 100 per cent mortality was observed was calculated to be 1.8 g/kg /body weight.

The calculated LD_{50} value for Norfloxacin was found to be 1.55 g/kg. For confirmation, 10 additional birds were challenged with the dose of 1.55 g/kg and among these birds 50 per cent mortality was found during the 24 h

observation period. Fig 1. The details related to dosing of Norfloxacin, grouping, number of experimental birds and mortality percentage is given in **Table 1.**

Table 1. Dose of Norfloxacin and mortality percentage acute toxicity study

No	Groups	No of birds in each group	No of birds in each group	Concentration after dilution water with distilled	Mortality percentage
1	Group I	10	1.4	3.1461	0
2	Group II	10	1.5	3.1761	30
3	Group III	10	1.6	3.2041	70
4	Group IV	10	1.7	3.2304	70
5	Group V	10	1.8	3.2553	100
6	Group VI	10	1.9	3.2788	100

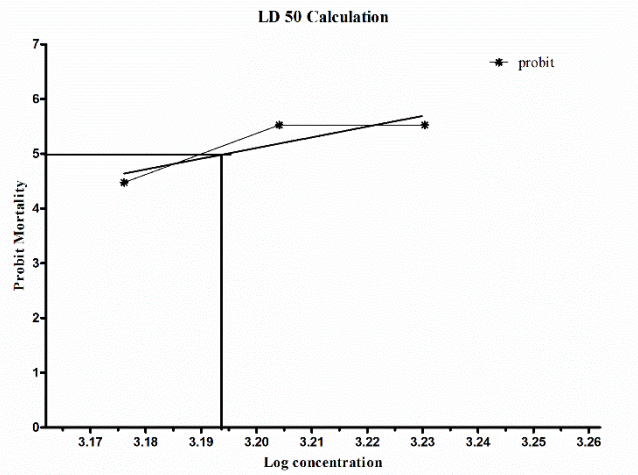

Fig. 1. The graph showing LD_{50} of norfloxacin LD 50 of norfloxacin by probit analysis. The corresponding log 10 dose of probit value 5=3.192. Antilog of 3.192=1549. Therefore, LD 50 is 1549 mg/kg or 1.549/kg

Clinical observations: Some of the clinical signs of toxicity observed before death were dullness, drowsiness, and excitability, circling, oozing of fluid from mouth and gasping for breath with beaks wide open.

Histopathology: Postmortem was conducted on dead experimental birds immediately after death. The histopathological findings observed (acute toxicity 1.8g/kg) in concerned organs were: hemorrhage, congestion and tubular epithelium degeneration and necrosis in kidney (Plate 1), Hemorrhage, edema and infiltration of few inflammatory cells in heart (Plate 2). Congestion of vessels and dilatation of sinusoidal space. Perivascular hepatocytes degeneration with infiltration of few inflammatory cells on liver (Plate, 3) and sever lymphocytolysis in spleen (Plate 4).

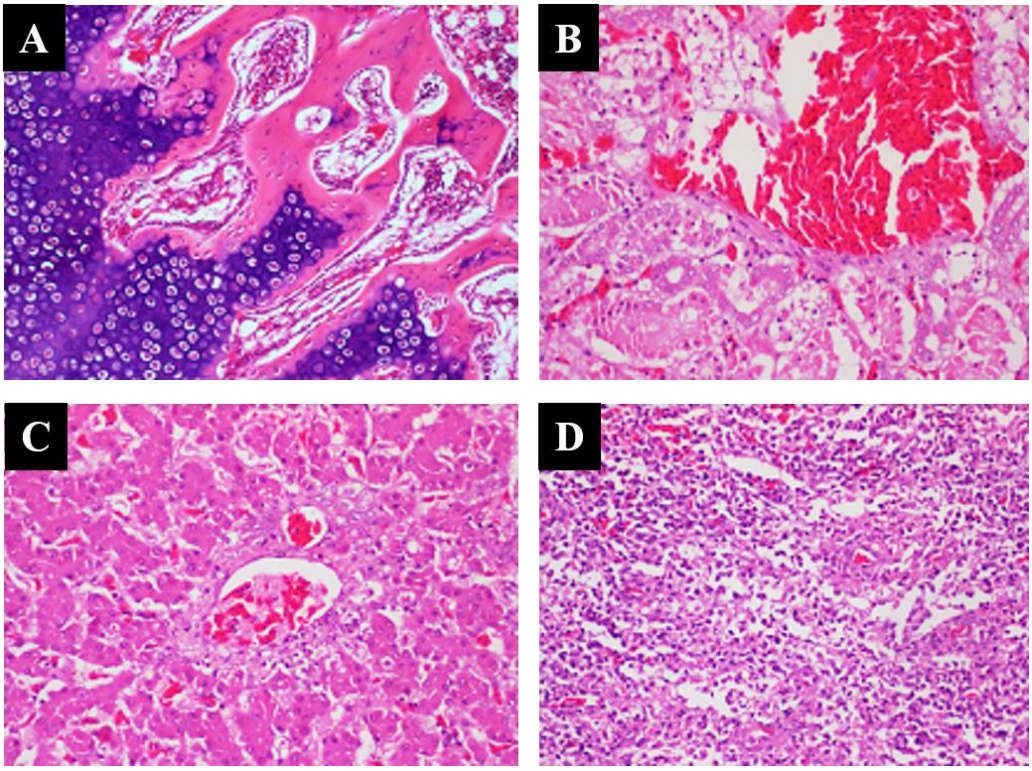

Fig. 1. Plate 1. Acute toxicity (1.8g/kg), Kidney: hemorrhage, congestion and tubular epithelium degeneration and necrosis -H&Ex500 **(A)**. Plate 2. Acute toxicity (1.8g/kg) Heart: Hemorrhage edema and infiltration of inflammatory cells H&Ex500 **(B).** Plate 3.Acute toxicity (1.8g/kg) Liver: Congestion of vessels and dilatation of sinusoidal space. Perivascular hepatocytes degeneration with infiltration of few inflammatory cells - H&Ex500 **(C),** and Plate 4. Acute toxicity (1.8g/kg) Spleen: Sever lympholysis and depletion H&Ex500 **(D).**

DISCUSSION

The results obtained for toxicity studies are discussed here. The literature available on Norfloxacin toxicity is scanty hence, for discussion other fluoroquinolones were also considered. The calculated LD_{50} value of Norfloxacin was 1.55 g/kg body weight. The symptoms of toxicity observed were dullness, drowsiness, excitability making continuous sound, circling, oozing of fluid from mouth and gasping of breath .The dose at which 100 per cent mortality was observed was calculated to be 1.8g/kg and in the maximum dose of 1.4 g /kg body weight where no mortality occurred. So Norfloxacin can be classified as per given protocol a moderately toxic drug according to Global harmonization system (GHS) for classification of toxic compounds. Zhou and Wang, (2007) reported LD_{50} of Norfloxacin nicotinate to be 3458 mg/kg for 5 week old broiler chicken. The symptoms observed were salivation, diarrhea, and nervousness. In the present study the estimated LD_{50} value for Norfloxacin was found to be 2 to 3 fold lesser then the LD_{50} reported by Zhou and Wang, (2007). The observed variation in LD_{50} in the present study has been attributed to the difference in age and body weight of birds. Further, it is also reported that higher values of LD_{50} can be expected in adult birds. the presumption can be further supported by the fact that metabolism and excretion of xenobiotics are compromised by under developed microsomal enzyme system, membrane permeability and hepatic and renal clearance capabilities in young animals

(Clarence et al., 1991). The observed CNS toxicity signs like circling, neuromuscular in-coordination and ataxia in the present acute toxicity study may be due to crossing of flouroquinolones into CNS as Blood brain barrier is also not fully developed in young birds (Scheld, 1989). Further, there are reports where in Fluoroquinolones may induce central nervous system excitatory side effects, including anxiety, nervousness, hallucination, and even seizures on rare occasions in many species (Christ, 1990). Zhang et al. (2003) found neurotoxi effects of Norfloxacin on free moving rats in which the epileptiform discharges appeared in all Norfloxacin groups with different latent periods, accompanied with limb twitching and clonic tonic seizures and the relative power of EEG increased.

CONCLUSION

We can conclude that: the dose at which 100 per cent mortality was observed was calculated to be 1.8g/kg and in the maximum dose of 1.4 g /kg body weight and the calculated LD_{50} value for Norfloxacin was found to be 1.55 g/kg where no mortality occurred. So it can be classified as a moderately toxic drug according to Global harmonization system (GHS) for classification of toxic compounds

Conflict of Interest

No, conflict of interest among all authors

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