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Norfloxacin-Repeated Dose Toxicity on Liver of Broiler Chickens

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ABSTRACT

Background: Norfloxacin is a second generation fluoroquinolone, primarily used in the treatment of urinary tract infections. However, later clinical studies have documented the efficacy of norfloxacin for a variety of Gram-negative infections including pneumonia, CNS/CSF infections, prostatitis and septicemia. The present study was conducted to investigate the toxic effect of norfloxacin during 28 day repeated dose toxicity on the liver tissue of broiler chickens.

Materials and Methods: The experimental birds acclimatized to laboratory housing condition were assigned as vehicle, control, treatment, and satellite groups (each group contained 6 birds). Stellate group, without administering Norfloxacin solution with partial control group was maintained for extra two weeks after the 28-day period.

Findings: Gross lesions in the liver of group IV and V observed were fatty change, enlargement, and increase of size and petechial hemorrhagic. Hepatocytes were swollen with mild vacuolar changes with granular cytoplasm. Focal areas of hepatocytes with degeneration infiltration of mononuclear cells, dilation of sinusoid, perivascular infiltration of inflammatory cells, bile duct epithelial hyperplasia and perivascular infiltration were noticed in the present study. The organ/body weight ratio percentages of liver for groups I, II, III, IV and V on 29th day were 2.81, 3.53, 3.54, 3.98 and 5.03 respectively. There was significant ($P<0.001$) increase in organ/body weight percentage ratio of group V as compared to the control group. On day 21 of treatment the serum ALT concentration was significantly increase in group V ($P < 0.001$) when compared to group I birds. On day 28 of treatment the mean serum ALT concentration was significantly ($P < 0.01$) higher in group V birds compared to group I birds. On 21st day of treatment the serum AST level of group V was significantly increased ($P < 0.001$) when compared to group I birds. On 28th day of treatment the mean serum AST was significantly high in-group V ($P < 0.001$) birds when compared to group I birds. These findings were accordance with finding of other authors.

Conclusion: According to gross pathology, histopathology and biochemical findings in the present study it was concluded that the norfloxacin has toxic effect on the liver tissue of chickens at the dose of 333 and 1100 mg/kg orally.

Keywords: Norfloxacin, Sub-acute toxicity; liver degeneration; Broiler chicken; liver; AST, ALT.

INTRODUCTION

Norfloxacin is a second generation fluoroquinolone, primarily used in the treatment of urinary tract infections. However, later clinical studies have documented the efficacy of norfloxacin for a variety of Gram-negative infections including pneumonia, CNS/CSF infections, prostatitis and septicemia (Mascellino et al., 1986). Norfloxacin usage in the treatment of gastrointestinal infections caused by Esherichia coli, Salmonella spp., Shigella spp. and Campylobacter spp. are also reported (Braunwald et al., 1987), which are the common disease causes of poultry.

Fluoroquinolones, including trovafloxacin, ciprofloxacin, ofloxacin, enoxacin, norfloxacin, and, gatifloxacin, have been associated with hepatotoxicity causes hepatocellular necrosis, which results in elevated ALT and AST concentrations (Coleman et al., 2002). Elevation in levels of hepatic enzymes occurred in 1.8%-2.5% of the patients florquinolones in clinical doses and these effects were reversible after the withdrawal of drug (Halkin, 1988). According to Hess (2016) SGPT being freely soluble in cytoplasm, was released from the hepatic cells even with very slight damage, which may not be detectable morphologically. Unlike SGPT, SGOT did not readily leak out from liver cells unless there was extensive injury to the liver. Gonzalez and Henwood (1989) observed elevated SGOT and SGPT concentration in patients undergoing treatment with pefloxacin. The most commonly reported abnormal laboratory finding in human patients treated with ciprofloxacin were increased glutamic pyruvic transaminase and glutamic oxaloacetic transaminase (Matsuno et al., 1995). Cornelius (1980) was the first to examine activity of glutamic oxaloacetic transaminase (SGOT) in fowls. The concentration of SGOT expressed was on the basis of the amount of pyruvate liberation per ml of plasma. On 28th day the amount of SGOT was 226.7 units in poultry. The fluoroquinolone antimicrobials norfloxacin and enrofloxacin were found to inhibit hepatic microsomal cytochrome P-450 monooxygenases in the livers of broiler chickens using dosages as given in commercial flocks (Shlosberg A, et al., 1997) impaired metabolism of a number of drugs has been associated with fatty liver. These findings suggest an association between increased lipid deposition and impaired CYP enzymes. (Gomez et al., 2009) Fluoroquinolones may accumulate, when they are repeatedly administered (Regmi et al., 2005). The present study was conducted to investigate the toxic effect of norfloxacin on the liver tissue of broiler chickens.

MATERIALS AND METHODS

Experimental birds acclimatized to laboratory housing condition were assigned as vehicle, control, and treatment groups, consisting of six groups of 6 birds in each group. Satellite group was given with high dose 1.1g /kg as high dose group and maintained along with the control group. Stellate group, without administering Norfloxacin solution with partial control group was maintained for extra two weeks after the 28-day period.

The norfloxacin powder was obtained from Trichem laboratories 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 prepared stock solution was used for further dilution.

The experiment was carried out under hygienic condition and standard management one-week-old broiler chicken 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 a period of 28 days based on LD_{50}= 549mg/kg.

Table 1. Details of group and doses administered in sub-acute liver toxicity.

Group	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 V (Vehicle)	Vehicle
Group VI (Satellite)	1.1 g/kg

The histopathological findings were based on histopathologic lesion in liver, sections in birds taken high doses and med doses of the drug. The satellite group shown recovery according to hematological, biochemical and histopathologic finding.

The liver for organ/body weight ratio percentage and tissues from liver of treated chickens were collected on day 29 for Histopathology. The blood samples were collected from jegulare vein on day 7, 14th, 21st and 28 for biochemical analysis (ALT, AST).

RESULTS

Gross lesions in the liver of group IV and V observed were fatty change, enlargement, and increase of size and petechial hemorrhagic.

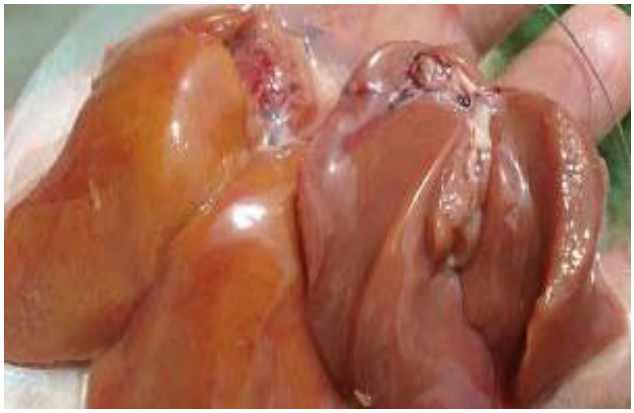

Fig. 1. Sub-Acute toxicity (High dose) Gross pathology liver showing fatty degeneration (compared with low dose)

The organ/body weight ratio percentages of liver for groups I, II, III, IV and V on 29th day were 2.81, 3.53, 3.54, 3.98 and 5.03 respectively. There was significant ($P<0.001$) increase in organ/body weight percentage ratio of group V as compared to the control group. The other groups didn't show any significant increase in liver weight.

Table. 2. Table shows effects of norfloxacin (IP) on organ/body (%) weight in experimental broiler chickens in sub-acute toxicity study.

	Group I (control)	Group II (Vehicle)	Group III (0.11g/kg)	Group IV (0.333g/kg)	Group V (1.1g/kg)	Group VI (1. 1g/kg)
Liver/BW	0.028100	0.035300	0.035400	0.039800	0.050300	0.05470

In histopathological finding birds received high dose hepatocytes were swollen with mild vascular changes and granular cytoplasm. Focal area of hepatocytes degeneration was also seen with infiltration of leukocytes.

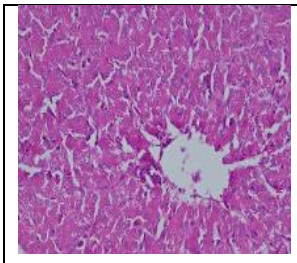	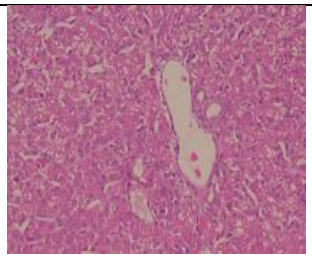	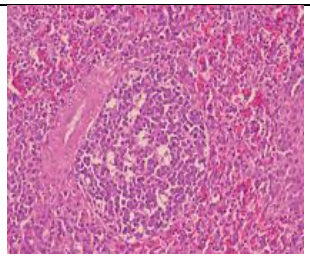	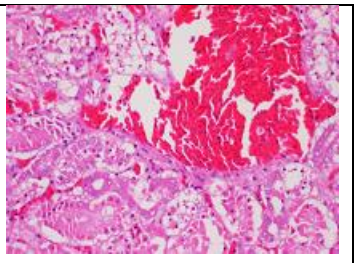
Plate 2. Hepatocytes were swollen with mild vascular changes in granular cytoplasm. Degeneration of focal area of hepatocytes -H&Ex500.	Plate 3. Infiltration of leukocytes, dilation of sinusoid, perivascular infiltration of inflammatory mononuclear cells -H&Ex500	Plate 4. Bile duct epithelial hyperplasia and perivascular infiltration -H&Ex500	Plate 5. Congestion of vessels and dilatation of sinusoidal space. Perivascular hepatocytes degeneration with infiltration of few inflammatory cells.

In addition, dilation of sinusoids, perivascular infiltration of inflammatory cells and bile duct epithelial hyperplasia was noticed. (Plate. 1-3)

The same lesions in mild form were observed in liver tissue of broiler chicken received 333 mg/kg dose of norfloxacin but no lesions were observed in chickens received 111-mg/kg norfloxacin. Satellite group showed lesions: Congestion of vessels and dilatation of sinusoidal space. Perivascular hepatocytes degeneration with infiltration of few inflammatory cells (Plate 5). The histopathological lesions were simultaneously supported by biochemical findings. On day 21 of treatment, the serum ALT concentration of group I, II, III, IV and V were 9.23±1.78, 8.98±1.41, 11.81±1.44, 15.16±2.09 and 21.02±2.03 U/dl respectively. Significant increase in ALT concentration was observed in group V ($P < 0.001$) when compared to group I birds. On day 28 of treatment the mean serum ALT concentration of group I, II, III, IV and V were 9.66±3.08, 14.95±5.46, 22.91±5.84, 40.06±10.7 and 43.89±4.05 U/dl respectively. The serum ALT concentration was significantly ($P < 0.01$) higher in group V birds compared to group I birds.

Table 3. Effects of norfloxacin on ALT level of experimental broiler chickens

Groups	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Group I (control)	7.23±1.05	9.54±2.36	9.23±1.78	9.66±3.08	43.89±4.05	43.89±4.05
Group II (Vehicle)	8.40±1.84	8.98±3.35	8.98±1.41	14.95±5.46		
Group III (1.1g/kg)	8.41±1.44	14.76±2.55	11.81±1.44	22.91±5.84		
Group IV (0.333g/kg)	8.45±1.43	21.20±3.23	15.16±2.09	40.06±10.7		
Group v (1.1g/kg)	9.60±1.04	20.78±3.63	21.02±2.03***	43.89±4.05***	31.97±5.94**	29.86±3.88***

Values are Means ±SE

***P<0,001, **P<0, 01, *P<0, 05, the values on 35 and 42 pertain to satellite group.

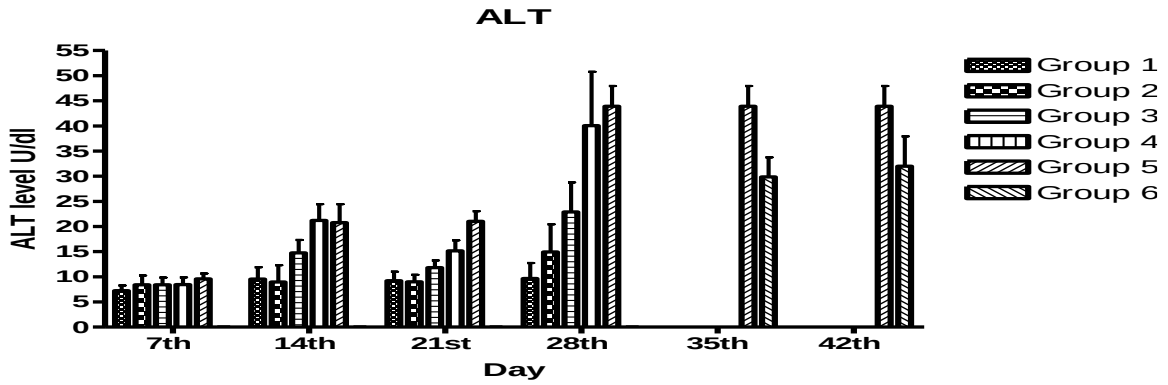

Fig. 1. Effects of norfloxacin on ALT level (U/dl) of experimental broiler chickens

On 21st day of treatment I, II, III, IV and V were 227.16±2.07, 234.83±8.20, 231.16±13.28, 281.50±19.04, 324.5±24.34 U/dl respectively. The serum AST level of group V was significantly increased ($P < 0.001$) when compared to group I birds. On 28th day of treatment the mean serum AST level of group I, II, III, IV and V were 224.83±10.08, 249.50±11.18, 246.33±7.20, 269.83±21.81, 330.83±14.70 U/dl respectively. It was significantly high in-group V ($P < 0.001$) birds when compared to group I birds.

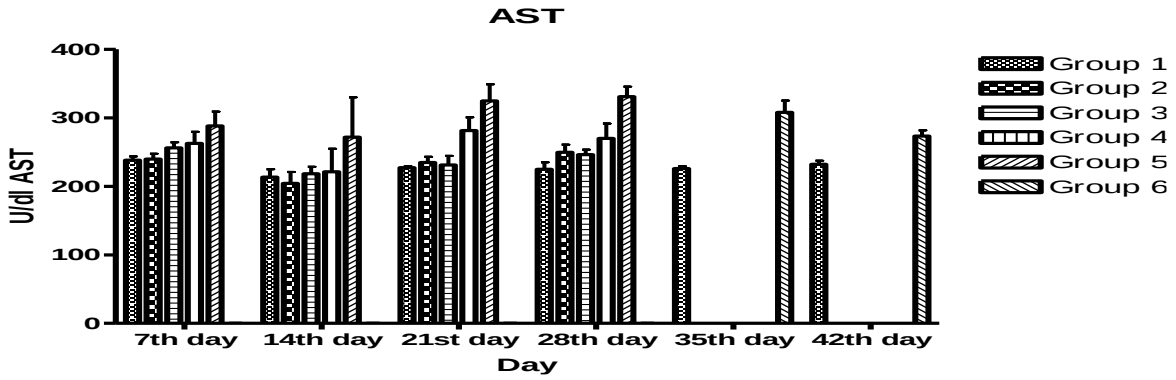

Fig. 2. Effects of norfloxacin on AST level (U/dl) of experimental broiler chickens in repeated dose sub-acute toxicity study

Table 4. Effects of norfloxacin on AST level of experimental broiler chickens in repeated dose sub-acute toxicity study

Groups	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Group I (control)	238.16±5.44	213.33±11.59	227.16±2.07	224.83±10.08	330.83±14.7	330.83±14.7
Group II (Vehicle)	239.66±7.89	204.33±16.54	234.83±8.20	249.5±11.18		
Group III (0.11g/kg)	256.16±8.142	218.33±10.29	231.16±13.28	246.33±7.2		
Group IV (0.333g/kg)	262.66±16.83	221.16±33.65	281.5±19.04	269.83±21.81		
Group v (1.1g/kg)	288.16±20.90	271.66±58.21	324.5±24.34**	330.83±14.7**	307.83±17.3**	273.33±8.43***

Values are Means ±SE, ***P<0,001, **P<0, 01, *P<0, 05. The values on 35 and 42 pertain to satellite group

DISCUSSION

The fatty change in liver, according to (Gomez et al. 2009), is impaired metabolism of a number of drugs, associated with fatty liver, which suggests an association between increased lipid deposition and impaired CYP enzymes. In the meanwhile, (Shlosberg et al. 1997) reported that fluoroquinolone antimicrobials norfloxacin and enrofloxacin were found to inhibit hepatic microsomal cytochrome P-450 monooxygenases in the livers of broiler chickens using dosages as given in commercial flocks or may be correlated with finding of (Regmi et al,. 2005) who observed accumulation of Fluoroquinolones when they were repeatedly administered. These are accordance with finding of (Coleman. C et al. 2002) who reported that Norfloxacin have been associated with hepatotoxicity causes hepatocellular necrosis and results in elevated ALT and AST concentrations.

The increase in ALT and AST values in the present study correlate with the finding of Hess (2016), who reported that SGPT being freely soluble in cytoplasm, was released from the hepatic cells even with very slight damage, which may not be detectable morphologically. Unlike SGPT, SGOT did not readily leak out from liver cells unless there was extensive injury to the liver. In the same time these findings are in accordance with observations of Amerson, which denotes that fluoroquinolones increased alanine transaminase, alkaline phosphatase, aspartate

transaminase activities the norfloxacin potential hepatotoxicity can be supported by an increase in serum ALT and AST concentration coupled with histopathological changes in the liver of broiler chicken (Amerson, 1982).

Conclusion

According to gross pathology, histopathology and biochemical findings in the present study, it was concluded that the norfloxacin has toxic effect on the liver tissue of chickens at the dose of 333 and 1100 mg/kg orally

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