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Effect of levofloxacin on liver biochemical parameters following repeated oral administration in dual purpose chicken

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Abstract

Levofloxacin, a third-generation fluoroquinolone, is the S-isomer of ofloxacin and possesses excellent activity against gram-positive, gram-negative and anaerobic bacteria. The experimental birds (35 day old) were randomly allotted into three groups (n=30), Group I birds served as control (Distilled water), Group II and Group III birds were administered with levofloxacin at the dose rate of 10 mg/kg bw and 20 mg/kg bw respectively for five days directly into the crop using a thin plastic tube attached to a syringe for 28 days. The food was withheld for 12 h before oral dosing but not water and water was provided *ad libitum* before the drug administration. The serum samples were used for the determination of AST, ALT and Alkaline Phosphatase biochemical parameters on day 0, 7, 14, 21 and 28. There was a significant increase ($p<0.05$) in AST, ALT and Alkaline Phosphatase enzyme values in Groups III on days 21 and 28 in the experimental birds as compared to control group. In the present study, birds were observed administered with 10 mg/kg through the oral route did not show any variation in the biochemical parameters whereas at the high dose @ 20 mg/kg body showed significant variation in the biochemical parameters causes toxicity on day 21 and 28 in the in dual purpose chicken.

Keywords: Levofloxacin, AST, ALT, alkaline phosphatase, dual purpose chicken

Introduction

Levofloxacin, a third-generation fluoroquinolone, is the S-isomer of ofloxacin and possesses excellent activity against gram-positive, gram-negative and anaerobic bacteria. It also has more pronounced bactericidal activity, particularly against organisms such as *Pseudomonas*, *Enterobacteriaceae* and *Klebsiella* spp. (Klesel *et al.*, 1995) [1]. The bactericidal effect of levofloxacin is achieved through reversible binding to DNA gyrase and subsequent inhibition of bacterial DNA replication and transcription (Fu *et al.*, 1992) [2]. The levofloxacin distributes well to target body tissues, fluids and its uptake makes it suitable for use against intracellular pathogens. However, it penetrates poorly into the central nervous system (Langtry and Lamb, 1998) [3]. The levofloxacin acts by a concentration-dependent killing mechanism, whereby the optimal effect is attained by the administration of high doses over a short period of time (Drusano *et al.*, 1993) [4] followed by a relatively prolonged postantibiotic effect (Aliabadi and Lees, 2001) [5].

Levofloxacin acts by inhibiting two types of topoisomerase II enzymes, namely DNA gyrase and topoisomerase IV. These enzymes are required for DNA repair, transcription, recombination and replication of bacterial DNA (Hawkey, 2003 [6]; Sharma *et al.*, 2009) [7]. The topoisomerases alter the DNA by introducing superhelical twists into double-stranded DNA and also facilitating unwinding of DNA gyrase has two subunits encoded by the GyrA gene, which cause strand breaks on a bacterial chromosome and then reseal the chromosome after supercoiling. The levofloxacin and other fluoroquinolones inhibit A subunits of DNA gyrase resulting in inhibition of bacterial DNA replication and transcription (Yashoda *et al.*, 1993) [8].

The bactericidal effect of drug is through stabilization of a cleavable complex via a cooperative drug binding process to a partially denatured DNA pocket created by the DNA gyrase. The drug also binds to the supercoiled DNA in a manner similar to it binding to the enzyme-DNA complex (Morrissey *et al.*, 1996 [9]; Jacoby, 2005) [10].

Levofloxacin along with other fluoroquinolones such as gatifloxacin, moxifloxacin, grepafloxacin, trovafloxacin offer more favourable pharmacokinetic parameters such as higher

AUC, C_{max} and longer elimination half-life than older compounds such as ciprofloxacin. Levofloxacin is metabolized in the liver to demethyl-levofloxacin and levofloxacin-N-oxide and excreted through the urine (Lubasch *et al.*, 2000) [11]. The drug distributes well to the target body tissues and fluids in respiratory tract, skin, urine and prostate, and its uptake by cells makes it suitable for use against intracellular pathogens (Langtry and Lamb, 1998) [3].

The good bioavailability, large volume of distribution, high C_{max} , AUC and pharmacokinetic-pharmacodynamics hybrid efficacy predictors, adverse effects indicate that administration of levofloxacin at 10 mg/kg bw by different routes may be highly efficacious against susceptible bacteria in turkeys (Aboubakr *et al.*, 2014) [12].

The use of antibiotics in animal farming lead to risk of antibiotic residues in the final food product. To protect the consumer from this risk, regulatory authorities have introduced several legislative initiatives such as the establishment of Maximum Residue Limits (MRLs) and development of other controls measures on food products. With the widespread and inadequate use of fluoroquinolones for animal growth and production, there is lack of recommended withdrawal period for fluoroquinolones so accumulation of drug residues in the animal tissues. To ensure delivery of safe foods to consumers, withdrawal period for drugs must be respected according to the maximum residual limits established by regulatory agencies (Martin *et al.*, 2007) [13].

Fluoroquinolones are frequently used in poultry production and human medicine with safety criteria, including withdrawal periods, doses, and treatment duration, as their misuse and abuse may cause bacterial resistance and the presence of residues in edible tissues. Consequently, the consumption of animal products with fluoroquinolone residues may result in the transmission of resistant bacteria (Gouvea *et al.*, 2015) [14].

Materials and Methods

The experimental birds (35 days old) were randomly allotted into three groups (n=30), Group I birds served as control (Distilled water), Group II and Group III birds were administered with levofloxacin at the dose rate of 10 mg/kg bw and 20 mg/kg bw respectively for five days directly into the crop using a thin plastic tube attached to a syringe for 28 days. The food was withheld for 12 h before oral dosing but not water and water was provided *ad libitum* before the drug administration. The selection of the dosage based on, levofloxacin at 10 mg/kg bw considered as therapeutic dosage in the poultry birds (Banna *et al.*, 2013 [15]; Varia *et al.*, 2009

[16]) Therefore 20 mg/kg of levofloxacin was selected as high dose based on the therapeutic dosage of levofloxacin to see the any adverse effect with respect to serum biochemical analysis.

The serum samples used for the determination of biochemical parameters on day 0, 7, 14, 21 and 28 by using clinical biochemical analyzer - Microlab 300 (Vitalab Scientific, Netherlands). The serum biochemical parameters were estimated using commercially available diagnostic kits from ERBA Mannheim (Transasia Biomedicals Ltd, HP) by following the manufacturer instructions furnished in the leaflet supplied along with the diagnostic kit.

1. Aspartate aminotransferase (AST)
2. Alanine aminotransferase (ALT)
3. Alkaline Phosphatase (ALP)

Statistical analysis

The data were analyzed by using one-way ANOVA. The mean values and standard error of the different groups were compared by Duncan's multiple range test using Statistical Package for Social Sciences (SPSS16, 2010). Data were considered as significant from one another when $P \leq 0.05$.

Results

Serum biochemical parameters

The biochemical parameters (AST, ALT, ALP, were estimated from serum samples obtained on days 0,7,14,21 and 28 of the experiment period after the administration of levofloxacin in dual purpose chicken.

Serum aspartate aminotransferase (AST)

The mean AST values for levofloxacin in Group I (Control), Group II (10 mg/kg bw) and Group III (20 mg/kg bw) of experimental birds were measured at weekly intervals and have been summarized in Table.1 and graphically represented in Fig. 1.

The mean serum AST (U/L) values were 168.40±0.60, 214.24±0.98, 224.30±0.78, 219.58±0.90, 228.86±0.85 U/L for Group I and 172.32±0.65, 218.64±0.80, 230.64±0.64, 250.65±0.90, 267.80±0.68 U/L for Group III and 170.40±0.88, 210.34±0.20, 216.44±0.13, 218.97±0.64, 220.64±0.82 U/L for control group on day 0, 7, 14, 21 and 28 respectively.

There was a significant increase ($p < 0.05$) in AST values in Groups III on day 21 and 28 in the experimental birds as compared to control group.

There was no significant increase ($p > 0.05$) in AST values in Groups II on day 0, 7, 14, 21, 28 and in Groups III on day 0, 7, 14 as compared to control group throughout the experiment.

Table 1: Effect of levofloxacin on Aspartate aminotransferase activity (U/L) in dual purpose chicken

Days	Control	Levofloxacin 10 mg/kg bw (Mean ±SE)	Levofloxacin 20 mg/kg bw (Mean ±SE)
0	170.40±0.88	168.40±0.60 ^a	172.32±0.65 ^a
7	210.34±0.20	214.24±0.90 ^a	218.64±0.80 ^a
14	216.44±0.13	224.30±0.78 ^a	230.64±0.64 ^a
21	218.97±0.64	219.58±0.90 ^a	250.65±0.90 ^b
28	220.64±0.82	228.86±0.85 ^a	267.80±0.68 ^b

Values are mean ± SE n=6a: Nonsignificant ($p > 0.05$) b: Significant ($p < 0.05$)

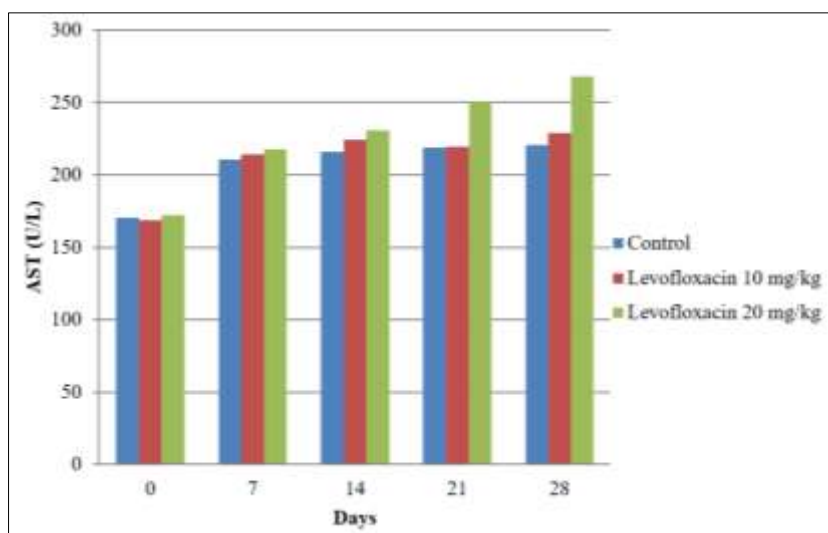


Fig 1: Effect of levofloxacin on Aspartate aminotransferase activity (U/L) in dual purpose chicken

Serum alanine aminotransferase (ALT)

The mean ALT values for levofloxacin in Group I (Control), Group II (10 mg/kg bw) and Group III (20 mg/kg bw) for experimental birds were measured at weekly interval and have been summarized in Table 2 and graphically represented in Fig.2.

The mean serum ALT values were 10.20±0.24, 12.48±0.42, 12.64±0.18, 13.02±0.90, 13.42±0.40U/L for Group II and 10.70±0.78, 13.25±0.80, 14.20±0.64, 16.56±0.62, 17.24±0.92U/L for group III and 9.90±0.27, 12.92±0.52, 12.10±0.47, 12.26±0.28, 12.48±0.73U/L for the control group on day 0, 7, 14, 21 and 28 respectively.

There was a significant increase ($p < 0.05$) in ALT values in Groups III on day 21 and 28 in the experimental birds as compared to control group.

There was no significant increase ($p > 0.05$) in ALT values in Groups II on day 0,7,14, 21, 28 and in Groups III on day 0, 7, 14 as compared to control group throughout the experiment.

Table 2: Effect of levofloxacin on Alanine aminotransferase activity (U/L) in dual purpose chicken

Days	Control	Levofloxacin 10 mg/kg bw (Mean ±SE)	Levofloxacin 20 mg/kg bw (Mean ±SE)
0	9.90±0.27	10.20±0.24 ^a	10.70±0.78
7	12.92±0.52	12.48±0.42 ^a	13.25±0.80 ^a
14	12.10±0.47	12.64±0.18 ^a	14.20±0.64 ^a
21	12.26±0.28	13.02±0.90 ^a	16.56±0.62 ^b
28	12.48±0.73	13.42±0.40 ^a	17.24±0.92 ^b

Values are mean ± SE n= 6 a: Nonsignificant ($p > 0.05$) b: Significant ($p < 0.05$)

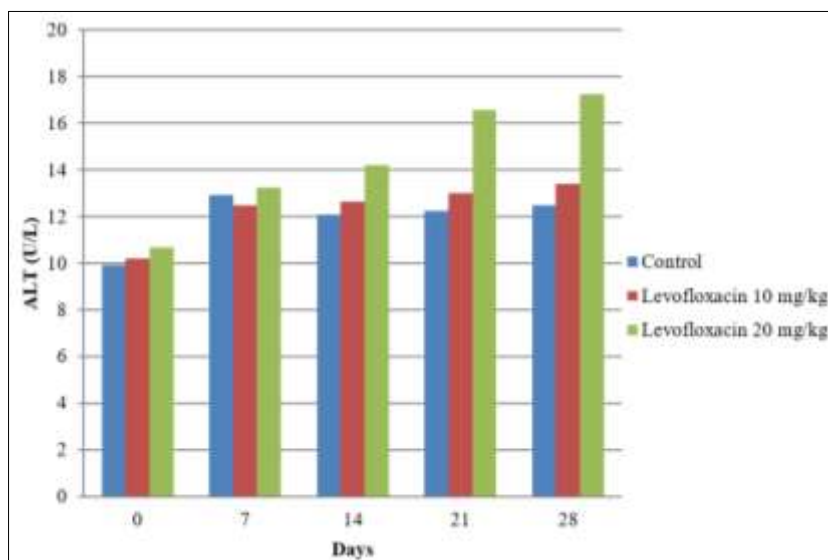


Fig 2: Effect of levofloxacin on Alanine aminotransferase (U/L) in dual purpose chicken

Alkaline phosphatase (ALP)

The mean ALP values for levofloxacin in Group I (Control), Group II (10 mg/kg bw) and Group III (20 mg/kg bw) of experimental birds were measured at weekly interval and have been summarized in Table 3 and graphically represented in Fig. 3.

The mean serum ALP values were 2201±0.24, 2204±0.40,

2216±0.98, 2218±0.87, 2230±0.60U/L for Group II and 2206±0.06, 2210±0.87, 2221±0.97, 2229±0.86, 2243±0.79U/L for group III and 2187±0.22, 2194±0.80, 2200±0.30, 2202±0.96 2218±0.42 IU/L for the control group on day 0, 7, 14, 21 and 28 respectively.

There was a significant increase ($p < 0.05$) in ALP values in Groups III on day 21 and 28 in experimental birds as

compared to control group.

There was no significant increase ($p>0.05$) in ALP values in Groups II on day 0,7,14, 21, 28 and in Groups III on day 0, 7, 14 when compared to the control group throughout the experiment.

Table 3: Effect of levofloxacin on Alkaline phosphatase (U/L) in dual purpose chicken

Days	Control	Levofloxacin 10 mg/kg bw (Mean \pm SE)	Levofloxacin 20 mg/kg bw (Mean \pm SE)
0	2187 \pm 0.22	2201 \pm 0.24 ^a	2206 \pm 0.06 ^a
7	2194 \pm 0.80	2204 \pm 0.40 ^a	2210 \pm 0.87 ^a
14	2200 \pm 0.30	2216 \pm 0.98 ^a	2221 \pm 0.97 ^a
21	2202 \pm 0.96	2218 \pm 0.87 ^a	2229 \pm 0.86 ^b
28	2218 \pm 0.42	2230 \pm 0.60 ^a	2243 \pm 0.79 ^b

Values are mean \pm SE n= 6 a: Nonsignificant ($p>0.05$) b: Significant ($p<0.05$)

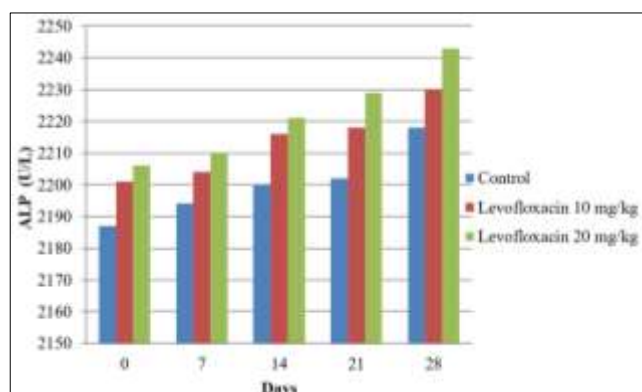


Fig 3: Effect of levofloxacin on alkaline phosphatase (U/L) in dual purpose chicken

Discussion

Aspartate aminotransferase (AST)

In the present study, a significant increase ($p<0.05$) in AST activity in Groups III of the experimental birds on day 21 and 28 as compared to control group.

This finding is supported with Oda *et al.* (2014) [19] who reported a significant increase in serum AST value on first and four weeks after administration of levofloxacin hydrochloride at the dose of 82 mg /kg bw through oral route once daily for four weeks in rabbits.

Elkholy *et al.* (2009) [17] studied an increase in serum AST activities following repeated oral administration of enrofloxacin at 10 mg /kg bw once daily for five consecutive days in laying hens. Fatai *et al.* (2013) [18] reported an increase in AST activity after the administration of ciprofloxacin in rats for a period of five days.

Alanine aminotransferase (ALT)

In the present study, a significant increase ($p<0.05$) in ALT concentration in Group III of the experimental birds on day 21 and 28 as compared to control group.

The present finding is in agreement with findings of Elkholy *et al.* (2009) [17] reported an increase in ALP activities following repeated oral administration enrofloxacin at 10 mg/kg bw once daily for five days in laying hens.

Oda *et al.* (2014) [19] reported that a significant increase ($p<0.05$) in serum ALT activity on first and four weeks after administration of levofloxacin hydrochloride at 82 mg /kg bw through oral route once daily for four weeks in rabbits. An

increased in the ALT activity after the administration of ciprofloxacin in rats for period of five days was reported by Fatai *et al.*, (2013) [18].

Alkaline phosphatase (ALP)

In the present study, a significant increase ($p<0.05$) in ALP activity in Group III of the experimental birds on day 21 and 28 as compared to control group.

The above finding is in accordance with finding of Sureshkumar *et al.* (2013) [20] who reported an increase in alkaline phosphatase activity after administration of enrofloxacin at 10 mg/kg bw via drinking water for five days in birds.

Elkholy *et al.* (2009) [17] reported a significant change in serum ALP activity after repeated oral administration of enrofloxacin at 10 mg/kg bw once daily for five days in laying hens. Fatai *et al.* (2013) noticed that an increase in ALP values after the administration of ciprofloxacin in rats for a period of five days.

Sadariya *et al.* (2010) [21] who reported that ALP values were found to fluctuate within normal range and did not differ significantly in the treatment group compared to control group after the administration of moxifloxacin at the dose of 5 mg/kg bw for 14 days in Wistar rats.

The degeneration of hepatocytes and subsequent leakage of enzymes were the reasons attributed for increase in the levels for ALT, AST and ALP of serum enzymes (Leeson *et al.*, 1995) [22]. The degeneration of skeletal muscles and increase in the osteoblastic activity lead to an increase in the ALP activity (Falconer and King, 1970) [23].

Histological observations such as degenerative and inflammatory changes, vacuolar degeneration of hepatocytes in liver and other organs of the present study uphold the alteration of the serum enzyme values in Group III experimental birds administered with 20 mg/kg bw of levofloxacin in dual purpose chicken.

Conclusion

In the present study, birds observed administered 10mg/kg through the oral route did not show any variation in the biochemical parameters whereas at the high dose @20mg/kg body showed significant variation in the biochemical parameters causing toxicity on day 21 and 28 in the in dual purpose chicken.

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