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Toxicity of levofloxacin on kidney following repeated oral administration in dual purpose chicken

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Abstract
The study was conducted in 30 to 40 day old healthy dual purpose chicken Indian Rock -3 (IR-3), a strain of White Plymouth Rock The safety evaluation of levofloxacin following the repeated oral administration was conducted in dual purpose chicken. The experimental birds were administered with levofloxacin at the dose rate of 10 mg/kg bw and 20mg/kg bw respectively directly for 28 days in dual purpose chicken. There was a significant increase in creatinine, urea concentration and significant decrease in total protein and albumin values in the Group III administered with high dose of levofloxacin at 20 mg/kg bw for 28 days suggestive of producing the toxic effect which were supported by the gross and histopathological observation in kidney tissue samples.

Keywords: Dual purpose chicken, levofloxacin, kidney, histopathology

Introduction
Levofloxacin, a third-generation fluoroquinolone, is the S-isomer of ofloxacin and possesses excellent activity against gram-positive, gram-negative and anaerobic bacteria (North *et al.* 1998) [12]. It also has more pronounced bactericidal activity particularly against organisms such as *Pseudomonas*, *Enterobacteriaceae* and *Klebsiella* spp (Klesel *et al.* 1995) [9]. 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) [6]. The levofloxacin acts by a concentration-dependent killing mechanism, whereby the optimal effect is attained by administration of high doses over a short period of time (Drusano *et al*. 1993) [3] followed by a relatively prolonged postantibiotic effect (Aliabadi and Lees, 2001) [1].
The levofloxacin and other fluoroquinolones inhibit A subunits of DNA gyrase resulting in inhibition of bacterial DNA replication and transcription (Yashoda *et al.* 1993) [17]. 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 (Jacoby, 2005) [8]. Ibrahim *et al*. (2011) [7] who reported a significant increase in creatinine, urea and decrease in total protein and albumin level following the administration of enrofloxacin at 10 mg/ kg bw for five days in birds. Fatai *et al*. (2013) [5] reported an increase in creatinine and urea activity after the administration ciprofloxacin at 14.35 mg/kg bw for a period of five days in rats. There was a significant ($p<0.05$) decrease in the total protein and albumin values after the administration of levofloxacin at 60 mg/kg bw in croiler chicken (Suman *et al.* 2009) [14] who reported that There was a congestion, tubular degeneration, and areas of hemorrhage in kidney after administered with enrofloxacin at 10 mg/kg bw through drinking water for five days in broiler chicken (Ellakany *et al*. 2007) [4]. Sureshkumar *et al*. (2013) [15] reported that degeneration of tubular epithelial cells with haemorrhagic areas, cytoplasm of swollen tubular epithelial cells and filled with eosinophilic material were observed in the toxicity of enrofloxacin administered at 10mg/kg bw via drinking water for five days in poultry birds However, the data on safety of repeated oral administration of levofloxacin in poultry are lacking. Therefore, the present study was planned to evaluate safety of levofloxacin following multiple oral dose administration in dual purpose chicken.\

Materials and method
Experimental animals
The study was conducted in 30 to 35 days old (n= 210) healthy dual purpose chicken Indian Rock-3(IR-3), a strain of White Plymouth Rock developed by Karnataka Veterinary Animal

and Fisheries Sciences University, Bidar. The study was performed at the Department of Poultry Science, Veterinary College, Hebbal, Bengaluru. The birds were kept under observation for one week prior to commencement of experiment and subjected to clinical examination in order to exclude the possibility of disease. The birds were provided antibiotic-free standard broiler ration for fourteen days. The animal house was maintained at room temperature (25 ± 2^0C) and at 45 to 65 per cent relative humidity. Food and water were supplied *ad libitum* and standard managemental practices were followed to keep the birds free from stress. The prior approval of the Institutional animal Ethics Committee (IAEC) was obtained before the commencement of the experiment (LPM/IAEC/181/2014, Date: 10/01/2014).

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 selection of the dosage based on, levofloxacin at 10 mg/kg bw considered as therapeutic dosage in the poultry birds (Varia *et al*., 2009; Banna *et al*. 2013) [16, 2]. 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, gross and histopathological examination.

The serum samples used for the determination of biochemical parameter son 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. The following parameters were done for the estimation of the toxicity on the kidney in the dual purpose chicken; Creatinine (Cr), Blood Urea Nitrogen (BUN), Total Protein and Serum Albumin.

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 $P \leq 0.05$.

Gross and histopathological examination

After the collection of the blood for serum biochemical analysis, six birds from each group were randomly selected and sacrificed at weekly interval on day 0, 7, 14, 21 and 28 during the study period. The birds were subjected to a detailed post mortem examination and gross lesions if any were recorded. The samples of liver, kidney and heart were collected, washed with normal saline and then collected in 10 percent neutral buffered formalin (NBF). They were processed through routine paraffin embedding technique. All the organs were processed for histopathology by cutting sections of 4µm thickness and stained with Haematoxylin and Eosin (Luna, 1968) [10]. The histolopathological lesions were observed in sections of various organs were systematically recorded and photomicrographs were taken using Zeiss AxioCam Erc5s microscope.

Results and discussion

Creatinine (Cr)

The mean creatinine 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.1 There was no significant (P>0.05) increase in creatinine values in Group II on day 0,7,14, 21, 28 and in Group III on day 0 and 7 as compared to the control group throughout the experiment. There was a significant (P<0.05) increase in creatinine values in Groups III on day 21 and 28 in the experimental birds as compared to control group. There was a significant increase (P<0.05) in creatinine values in Groups III of the experimental birds on day 14, 21 and 28 as compared to control group.

Table 1: Effect of levofloxacin on serum creatinine (mg/dl) in dual purpose chicken

Days	Control	Levofloxacin 10 mg/kg bw (Mean ±SE)	Levofloxacin 20 mg/kg bw (Mean ±SE)
0	0.57±0.04	0.58±0.09[a]	0.60±0.03[a]
7	0.48±0.07	0.52±0.04[a]	0.56±0.16[a]
14	0.54±0.05	0.61±0.16[a]	0.70±0.04[a]
21	0.70±0.06	0.73±0.08[a]	0.92±0.12[b]
28	0.72±0.10	0.85±0.13[a]	1.04±0.06[b]

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

The present finding was in accordance with findings of Ibrahim *et al*. (2011) [7] who reported a significant increase in creatinine level following the administration of enrofloxacin at 10 mg/ kg bw for five days in birds. Fatai *et al*. (2013) reported an increase in creatinine activity after the administration ciprofloxacin at 14.35 mg/kg bw for a period of five days in rats. Elkholy *et al.* (2009) [4] found that an increasedin serum creatinine value following repeated oral administration of enrofloxacin at 10 mg /kg bw once daily for five days in laying hens. Sureshkumar *et al.* (2013) [15] reported that an increase in creatinine values after administration of enrofloxacin at the therapeutic dose of 10mg/kg bw via drinking water for five successive days in birds.

The elevated levels of creatinine observed in this study indicated the kidney damage caused by high dose of levofloxacin at the dose rate of 20 mg/kg bw which drew support from the histopathological observations made in Group III of the experimental birds which included tubular epithelial cell degeneration, desquomation, congestion, hemorrhages, necrosis along with infiltration of inflammatory cells in the interstitium of renal tubular epithelium towards the end of the experiment in kidney.

Urea

The mean urea 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.2.

Table 2: Effect of levofloxacinon urea (mg/dl) in dual purpose chicken

Days	Control	Levofloxacin 10 mg/kg bw (Mean ±SE)	Levofloxacin 20 mg/kg bw (Mean ±SE)
0	3.28±0.31	3.31±0.54[a]	3.38±0.74[a]
7	3.29±0.90	3.34±0.62[a]	3.40±0.43[a]
14	3.30±0.65	3.48±0.34[a]	3.50±0.29[a]
21	3.46±0.76	3.50±0.67[a]	3.60±0.46[b]
28	3.48±0.92	3.54±0.68[a]	3.64±0.52[b]

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

There was no significant increase (P> 0.05) in urea values in Group II on day 0,7,14, 21, 28 and in Group III on day 0, 7, 14 as compared to the control group throughout the experiment. In the present study, there was a significant increase (P<0.05) in the urea activity in Group III of the experimental birds on day 21 and 28 as compared to control group.

Similar finding was observed by Fatai *et al*. (2013) [5] they reported an increase in the urea activity after administration of ciprofloxacin at 14.35 mg/kg bw for a period of five days in rats. Sadariya *et al*. (2010) [13] reported an increase in urea values after administration of moxifloxacin at 5 mg/kg bw for 14 days in wistar rats.

The elevated levels of urea observed in this study indicated the kidney damage caused by levofloxacin which drew support from the histopathological observations made in Group III administered with 20 mg/ kg b.w of levofloxacin were tubular epithelial cell degeneration, desquomation, congestion, hemorrhages, necrosis along with infiltration of inflammatory cells in the interstitium of renal tubular epithelium towards the end of the experiment.

Total protein and Serum albumin

The mean serum protein 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.3 and 4.

Table 3: Effect of levofloxacin on Total protein (g/dl) in dual purpose chicken

Days	Control	Levofloxacin 10 mg/kg bw (Mean ±SE)	Levofloxacin 20 mg/kg bw (Mean ±SE)
0	2.87±0.48	2.24±0.18[a]	2.64±0.07[a]
7	3.32±0.43	3.20±0.78[a]	3.16±0.20[a]
14	3.52±0.45	2.84±0.68[a]	2.52±0.86[b]
21	3.59±0.24	2.73±0.92[a]	2.49±0.17[b]
28	3.63±0.44	2.60±0.72[b]	2.43±0.25[b]

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

Table 4: Effect of levofloxacin on serum albumin (g/dl) in dual purpose chicken

Days	Control	Levofloxacin 10 mg/kg bw (Mean ±SE)	Levofloxacin 20 mg/kg bw (Mean ±SE)
0	1.85±0.23	1.88±0.25[a]	1.86±0.13[a]
7	1.84 ±0.35	1.87±0.61[a]	1.83±0.28[a]
14	1.88±0.25	1.88±0.19[a]	1.62±0.09[b]
21	2.17±0.14	2.14±0.29[a]	1.58±0.26[b]
28	2.15±0.52	1.86±0.01[b]	1.42±0.06[b]

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

There was no significant decrease (P>0.05) in the serum protein and serum albumin values in Group IIon day 0,7,14, 21 and in Group III on day 0 and 7 respectively comparedto the control group throughout the experiment. There was significant decrease(P<0.05) in the serum protein and serum albumin values in Groups II on day 28 and in Group III on day 14, 21, 28 in the experimental birds as compared to control group.

In the present study, a significant (P<0.05) decrease in serum protein and albumin values on day 28 and day 14, 21, 28 in Group IIand Group III of the experimental birds respectivelywhen compared to control group.

The present finding is in accordance with findings of Suman *et al.* (2009) [14] who reported that significant (p<0.05) decrease in the total protein and albumin values after the administration of levofloxacin at 60 mg/kg bw in croiler chicken.

Ibrahim *et al*. (2011) [7] reported a significant decrease in total protein and albumin values following the administration of enrofloxacin 20% at 10 mg/kg bw for five days in experimental birds when compared to the control birds. Fatai *et al*. (2013) [5] reported decreased in total protein and albumin values after administration ciprofloxacin at 14.35 mg/kg bw for a period of five days in rats. On the contrary, Sadariya *et al*. (2010) [13] estimated the mean total protein and albumin concentration in rats after administration of moxifloxacin for 14 days in male and female wistar rats and were found that there was no significant change (p < 0.05) from corresponding control values.

The hypoproteinaemia and hypoalbuminaemia observed in Group II and III could be attributed to the reduction in feed consumption, inactivation of biosynthetic enzymes and impairment of protein synthesis by high dose of levofloxacin administered in dual purpose chicken. In addition, liver being the main organ of protein synthesis especially albumin, the hepatic damage observed in the present trial could be the other contributing factor for lowered serum total protein and albumin (Kaneko *et al.*, 1997).

Gross pathology

In the present study, gross pathological changes were observed in the kidney of dual purpose chicken on weekly interval on day 0, 7, 14,21 and 28 of the experimental study. The Group I (control) birds showed no gross

pathomorphological changes in all the organs examined during necropsy. The gross pathological lesions were observed on Group II and Group III on day 28 and day 21, 28 respectively in kidney samples.

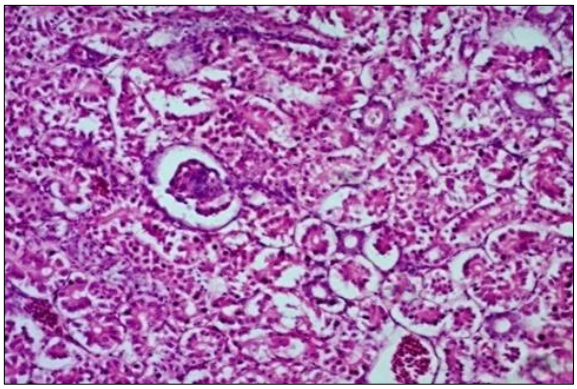
In Group II, levofloxcain at 10 mg/kg bw, there was no gross pathological change observed on day 0,7,14 and 21 days. On day 28, kidney was slightly enlarged and congested compared to the control group. In Group III, levofloxcain administered at 20 mg/kg bw, there was no gross pathological changes observed on day 0,7 and 14. The kidneys were moderately enlarged and congested and enlarged, severely congested kidney on day21 and 28 respectively.

Histopathology

The Group I, section of kidney showed normal architecture of renal tubular epethelial cells sections of kidney in Group II did not reveal any histopathological changes on day 0,7, 14 and in group III on day 0, 7 throughout the experimental period compared to the control group (Fig.1). The section of kidney showed mild tubular epithelial cell degeneration (Fig.2) and epithelial cell degeneration with infiltration of inflammatory cells in the interstitium (Fig.3) on day 21 and 28 respectively in group II of experimental birds as compared to control group.

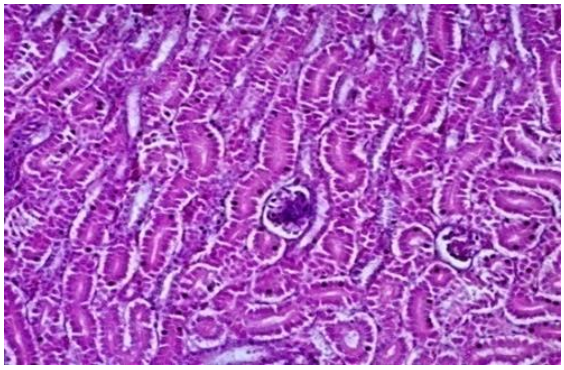
In group III, section of kidney showed tubular epithelial cell degeneration, necrosis with glomerulus atropy on day 14 (Fig.4). The congestion, tubular epithelial cell degeneration, desquomation (Fig.5) and severe hemorrhages in interstitium, tubular epithelial cell degeneration, necrosis along with infiltration of inflammatory cells (Fig.6) on day 21 and 28 respectively compared to the control group.

Histopathology



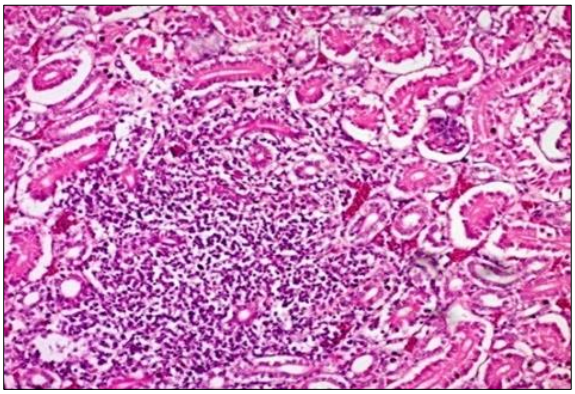
(a)

Fig 1: Normal architecture of renal tubular epethelial cells



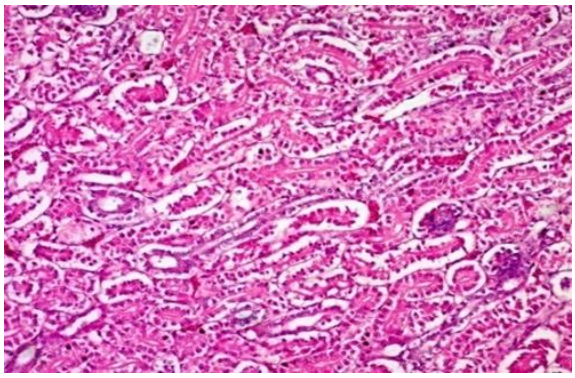
(b)

Fig 2: Mild tubular epithelial cell degeneration



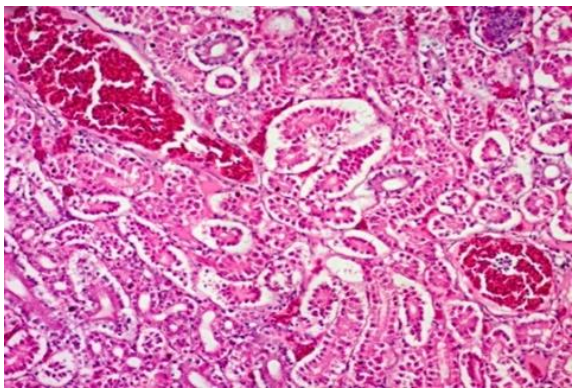
(c)

Fig 3: epithelial cell degeneration with infiltration of inflammatory cells in the interstitium



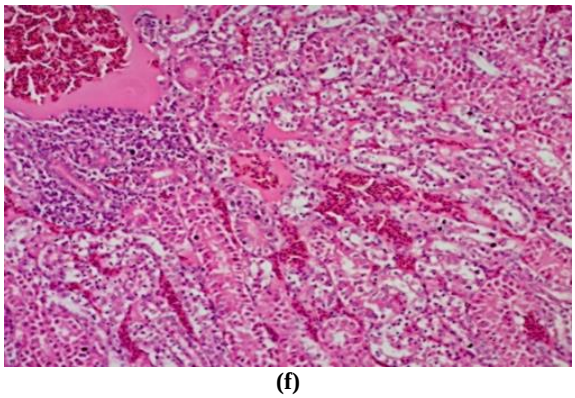
(d)

Fig 4: kidney showed tubular epithelial cell degeneration, necrosis with glomerulus atropy



(e)

Fig 5: congestion, tubular epithelial cell degeneration, desquomation



(f)

Fig 6: severe hemorrhages in interstitium, tubular epithelial cell degeneration, necrosis along with infiltration of inflammatory cells

The above findings are in accordance with Ellakany *et al*. (2007) [4] who reported that congestion, tubular degeneration, and areas of hemorrhage in kidney after administered with enrofloxacin at 10 mg/kg bw through drinking water for five days in broiler chicken. Sureshkumar *et al*. (2013) [15] reported that degeneration of tubular epithelial cells with haemorrhagic areas, cytoplasm of swollen tubular epithelial cells and filled with eosinophilic material were observed in the toxicity of enrofloxacin administered at 10 mg/kg bw via drinking water for five days in poultry birds. Nada and Shawi (2012) [11] reported that nephrosis, cell swelling of the epithelial lining and coagulative necrosis of renal tubules observed after the administration of ciprofloxacin at25 mg/ kgand 50 mg/ kg bw in rats.

Conclusion

The experimental birds 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 forfive days directly into the crop using a thin plastic tube attached to a syringe for 28 days. There was a significant increase in creatinine, urea concentration and significant decrease in total protein and albumin values in the Group III administered with high dose of levofloxacin at 20 mg/kg bw for 28 days. The birds were observed for clinical signs of toxicity, which were supported by increase in serum biochemical parameters and histopathological changes in the Kidney of dual purpose chicken. This is suggestive of administration of high dose of levofloxacin causes toxicity in dual purpose chicken.

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