Study on induced enrofloxacin toxicity in broiler birds

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Abstract— **The present study was conducted with sixty healthy day old broiler chicks to determine the effect of induced enrofloxacin toxicity. The birds were divided into four groups keeping the first group as control. Clinically, the broiler birds in all the treatment groups administered with therapeutic dose of enrofloxacin expressed the clinical symptoms of systemic toxicity. Hematological studies indicated that the haemoglobin concentration, packed cell volume, total erythrocytic count and total leucocytic count showed significant variation (P<0.01) between the treatment groups. Biochemically, all treated groups of birds induced with graded dose level of enrofloxacin, had significantly (P<0.01) decreased levels of total plasma protein with simultaneous reduction in albumen-globulin (A:G) ratio than in controls.**

Keywords— Biochemically, Clinically, Enrofloxacin, Hematological studies, Toxicity.

I. INTRODUCTION

Enrofloxacin is a second generation quinolone derivative which belongs to the group fluoroquinolone. The fluoroquinolones are metabolized in the liver and excreted in urine through the kidney. The liver and kidney develop the highest drug concentration though concentrations in essentially all tissues, including the skeletal and central nervous system reach therapeutic levels. That is why, to detect the pathological alteration in the tissues, the liver and kidneys have been selected for the present investigation.

The present study was carried out to know the clinical signs of toxicity in broiler chicks, to assess the hematological and serum biochemical changes due to toxicity of the drug.

II. MATERIALS AND METHODS

Sixty (60 no.) healthy broiler chicks of either sex at the age group of 2-3 weeks were procured from a commercial hatchery. All the birds were housed in electrically operated battery brooders having thermostatic control. The brooders were cleaned and disinfected properly prior to the experiment. The brooders were kept in a cleaned, well ventilated room. Provision for sufficient light was made with electric lamps. Strict observation regarding ventilation and light was maintained.

The birds were randomly divided into four groups, each group containing fifteen birds. All the birds were maintained under standard conditions of hygiene and maintenance. Floxidin (10% concentration) oral suspension was procured and used in this study.

The birds of Group 1 were treated as control without any drug treatment. The birds of Group 2 containing 15 birds were fed orally with floxidin (10% oral solution) at therapeutic level i.e. 10 mg /kg body weight as recommended by the manufacturer. The birds of Group 3 consisting 15 birds were fed with the drug at double therapeutic dose i.e. 20 mg/kg body weight. The broiler chicks of Group 4 were induced with triple therapeutic dose of the drug from 14 days to 28 days of age.

A. Clinical Signs

From the day of drug treatment to the birds of different groups, the clinical signs, if any due to toxicity were recorded till the end of the experiment.

B. Hematological and Biochemical Studies

The blood was collected from the live birds on 7^{th} and 14^{th} day post treatment (DPT).

The wing vein was selected for collection of blood. Altogether 7 ml of blood was collected from each bird. A part of blood was then transferred in a test tube containing the anticoagulant at the rate of 1 mg per 1 ml of blood. The tube was then placed on the palm of one hand while using the other it was gently rotated to allow the mixing of the blood properly with the anticoagulant. Then the test tubes were kept at room temperature for 10-15 min and then stored in the refrigerator at 4°C till use. This part of blood was utilized for estimation of hematological parameters. Another part of blood was transferred to a wide mouth glass test tube without any anticoagulant and was allowed to clot and the separated serum was utilized for the estimation of different biochemical parameters.

C. Hematological studies

a) Estimation of Hemoglobin (Hb%):

Amount of hemoglobin was estimated by Hellige Sahli's hemoglobinometer and expressed in terms of gram percentage.

b) Packed Cell Volume (PCV%):

It was estimated in Wintrobe's hematocrit tube as per Schalm *et al.* [1]. Readings were taken after centrifuging at 3000 rpm for 30 min. It was calculated as percentage.

c) Total leucocytic and erythrocytic Count (TLC% and TEC%)

As the fowl's erythrocytes are nucleated, the mammalian white blood cell diluting fluids can not be used in total leucocytic count. Because though the erythrocytes may be lysed, the nuclei are left and appear prominently. So, TLC and TEC were evaluated as per the method demonstrated by Sastry [2].

White blood corpuscles and red blood corpuscles were identified by their typical characteristic shape and different in the stain taken. White blood corpuscles were stained violet while the red blood corpuscles were stained greenish.

d) Differential leucocytic count (DLC %):

Differential leucocytic counts were performed by Leishman's method [3]. DLC was calculated as percentage.

D. Biochemical studies

a) Estimation of Total Protein concentration:

Total proteins were estimated colorimetrically by Biruret method [4] and were expressed in gm/dl.

b) Estimation of Serum Albumin concentration:

Serum albumin was observed [4] and the values were expressed as gm/dl.

c) Estimation of Serum Globulin concentration:

Values of serum globulin were calculated by subtracting serum albumin values from total serum protein. The values were expressed in gm/dl.

d) Estimation of Albumin: Globulin (A: G) ratio:

Albumin and globulin ratio was obtained after divided percentage of albumin by percentage of globulin in Serum.

e) Serum Asparate Aminotransferase (AST/SGOT) concentration:

AST was estimated colorimetrically by 2, 4 DNPH method [5] and expressed in IU/dl.

f) Estimation of Alanine aminotransferase (ALT /SGPT) concentration:

ALT was estimated colorimetrically by 2,4 DNPH method [5] and was expressed in IU/dl.

E. Statistical analysis

Data generated by the experiments were analyzed using Student's t-test [6].

III. Results and Discussion

A. Clinical signs

Clinically, the broiler birds administered with therapeutic dose of enrofloxacin (Group 2) and double dose (Group 3) manifested mild diarrhea and depression on 7 days post-treatment (DPT). Birds of Groups 3 and 4 also developed weakness, loss of appetite, unthriftiness and poor growth. Further, Group 4 birds exhibited droopiness, drowsiness, severe depression, respiratory distress, reduction in feed consumption and appreciable decrease in body weight gain. Signs of lameness were only recorded after 12 days post treatment in birds of Groups 3 and 4.

B. Hematological studies

Hematological values of all the groups of bird treated with different doses of enrofloxacin are presented in Table 1. It was observed from the Table that all the treatment Groups 2, 3 and 4 birds showed significant ($P<0.01$) decrease in hemoglobin PCV and TEC values on 7 and 14 DPT indicating anemia which is possibly resulted either from toxic depression

of bone-marrow or suppression of hemopoietic tissues.

C. Biochemical studies

Biochemical indices in this experiment were total plasma protein (gm/dl); albumin, globulin and its A:G, SGOT (IU/dl) and SGPT (IU/dl) of all groups birds treated with graded doses of enrofloxacin are presented in Table 2. Total plasma protein, albumin and its albumin: globulin ratio (A:G) were significantly ($P<0.05$) decreased in birds of Groups 2, 3 and 4 on both 7 and 14 DPT, suggesting hypoproteinamia.

On the other hand, serum enzymatic values were significantly ($P<0.01$) increased in birds of Group 2 (therapeutic dosing) on 14 DPT and also in birds of Group 3 (double dosing) and Group 4 (triple dosing) both on 7 and 14 days post treatment.

Clinical signs in birds dosed with double (Group 3) and triple dose (Group 4) herein were similar to those reported earlier in acute ciprofloxacin toxicity of birds [7], [8], in human [9] and in broiler birds [10]. In this study, birds treated with therapeutic dose had only mild diarrhea and depression which simulated the reports of Norby [11], Sharma *et al*. [9]. Broiler birds of control group had no clinical manifestation at any stage during the experimental period.

Neu [12] and Sharma *et al*. [9] reported anemia in human treated with ciprofloxacin. In the present study, all groups of birds treated with graded doses of enrofloxacin developed leucopenia. The finding of leucopenia was similar to the earlier reports in the broiler birds treated with ciprofloxacin [10]. The development of leucopenia in the enrofloxacin treated broiler birds was mainly contributed to decrease of lymphocytes in the blood. Highly significant ($P<0.01$) variation in heterophil and lymphocyte count on 14 DPT with the control and group 4 birds was also noted (Table 1), which confirmed the findings of Swenson and Reece [12],who reported severe bone marrow depression as an adverse effect of antibiotics causing lymphocytosis and neutropenia.

The resultant hypoproteinemia in the treatment birds irrespective of dose levels was possibly because of its decreased synthesis by the degenerated and/ or necrotic liver resulting to hypoproteinemia. Hypoproteinemia in broiler birds treated with overdoses of ciprofloxacin was reported by Niyogi [10].

Niyogi [10] and Sugawara *et al*. [13] also reported an elevated serum enzymatic activity in broiler birds and monkey respectively after therapy with ciprofloxacin both in therapeutic dose and higher doses of the drug. An increased level of serum enzymatic activity is known to occur in a wide range of inflammatory/degenerative disease conditions particularly in hepatic and nephrotic diseases [14].

IV. Conclusions

Based on clinical signs, hematological and biochemical findings, it was suggested that indiscriminate and injudicious use of second generation fluoroquinolones *viz.*, enrofloxacin produced anemia, leucopenia, hypoglycaemia, hypoproteinemia, increased enzymatic activity and hepatotoxic and nephrotoxic effects in broiler chickens.

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VI. References

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TABLE I

Hematological parameters in broiler birds induced with enrofloxacin toxicity

Parameter	Group 1 (Control)		Group 2		Group 3		Group 4	
	7 DPT	14 DPT	7 DPT	14 DPT	7 DPT	14 DPT	7 DPT	14 DPT
Hb (gm %)	$10.28^{a} \pm 0.14$	$10.19^{a} \pm 0.18$	$9.88^{b} \pm 0.18$	$9.06^{b} \pm 0.18$	$9.12^{b} \pm 0.08$	$8.43^{b} \pm 0.12$	$8.67^{b} \pm 1.14$	$7.60^{b} \pm 0.08$
PCV (%)	$34^{a} \pm 0.05$	$33.5^{a} \pm 0.10$	$33^{b} \pm 0.60$	$31.5^{b} \pm 0.85$	$29.5^{b} \pm 0.10$	$25^{b} \pm 0.68$	$28^{b} \pm 0.10$	$22^{b} \pm 1.57$
TEC($X10^{6}$/cu. mm)	$4.02^{a} \pm 0.03$	$4.01^{a} \pm 0.06$	$3.82^{b} \pm 0.08$	$3.52^{b} \pm 0.07$	$3.16^{b} \pm 0.06$	$2.91^{b} \pm 0.08$	$2.96^{b} \pm 0.09$	$2.62^{b} \pm 0.07$
TLC($X10^{3}$/cu. mm)	$26.88^{a} \pm 0.58$	$24.91^{a} \pm 0.72$	$25.86^{b} \pm 0.67$	28.46 ± 0.54	26.88 ± 0.58	24.91 ± 0.72	25.86 ± 0.67	23.27 ± 0.54
Heterophil (%)	$32^{a} \pm 1.00$	$31.5^{a} \pm 0.5$	$30^{a} \pm 1.00$	$29.5^{b} \pm 0.5$	$26^{b} \pm 1.00$	$24.5^{b} \pm 0.5$	$26^{b} \pm 1.00$	$23.5^{b} \pm 0.5$
Lymphocyte (%)	$58^{a} \pm 1.00$	$58.5^{a} \pm 0.5$	$60^{a} \pm 1.00$	$60.5^{a} \pm 0.5$	$64^{b} \pm 1.00$	$64.5^{b} \pm 0.5$	$65^{b} \pm 1.00$	$68.5^{b} \pm 0.5$
Monocyte (%)	$9.33^{a} \pm 0.62$	$10.00^{a} \pm 0.52$	$7.83^{a} \pm 0.31$	$7.5^{a} \pm 0.43$	$7.00^{a} \pm 0.37$	$6.33^{a} \pm 0.21$	$6.50^{a} \pm 0.50$	$5.50^{a} \pm 0.50$

DPT: days post treatment

The different superscript in a row defer significantly ($P<0.01$)

TABLE 2

Biochemical parameters in broiler birds induced with enrofloxacin toxicity

Parameter	Group 1 (Control)		Group 2		Group 3		Group 4	
	7 DPT	14 DPT	7 DPT	14 DPT	7 DPT	14 DPT	7 DPT	14 DPT
Total plasma protein (gm %)	$5.142^{a} \pm 0.12$	$5.096^{a} \pm 0.127$	$4.825^{b} \pm 0.125$	$4.746^{b} \pm 0.152$	$4.608^{b} \pm 0.147$	$4.517^{b} \pm 0.152$	$4.518^{b} \pm 0.106$	$4.348^{b} \pm 0.215$
Albumin	$2.840^{a} \pm 0.112$	$2.734^{a} \pm 0.125$	$2.414^{b} \pm 0.118$	$2.316^{b} \pm 0.225$	$2.189^{b} \pm 0.198$	$2.103^{b} \pm 0.106$	$2.145^{b} \pm 0.138$	$1.814^{b} \pm 0.212$
Globulin	$2.302^{a} \pm 0.126$	$2.362^{a} \pm 0.212$	$2.401^{a} \pm 0.198$	$2.430^{a} \pm 0.113$	$2.419^{a} \pm 0.168$	$2.414^{a} \pm 0.132$	$2.373^{a} \pm 0.122$	$2.534^{a} \pm 0.212$

A:G	1.234	1.157	1.005	0.955	0.905	0.871	0.904	0.716
SGOT (IU/dl)	166.20 [a] ± 2.19	165.29 [a] ± 2.30	171.23 [a] ± 2.18	176.30 [b] ± 2.33	180.78 [b] ± 2.92	183.08 [b] ± 3.06	184.31 [b] ± 2.86	187.14 [b] ± 2.92
SGPT (IU/dl)	23.13 [a] ± 1.13	23.87 [a] ± 1.25	24.43 [a] ± 1.52	30.26 [b] ±1.46	28.98 [b] ± 1.53	31.67 [b] ± 1.38	32.63 [b] ± 1.62	32.28 [b] ± 1.53

DPT: days post treatment

The different superscript in a row defer significantly ($P<0.01$)