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Study on Ameliorative Effect of Febuxostat on Gout Induced Model in Broiler Chicks

M.K. Patel*, C.J. Dave, R.C. Rathod, B.P. Joshi and D.J. Ghodasara

Department of Veterinary Pathology,

College of Veterinary Science and AH,Anand Agriculture University, Anand-388001

Corresponding Author: maya7.patel@gmail.com

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Abstract

This work was conducted on six groups of day-old Cobb-400 broiler chicks to study the ameliorative effect of febuxostat on gout induced model. Clinical signs were noticed in birds of diclofenac control group II and low dose febuxostat treated group IV. During the study, 27.77% and 22.22% mortality were observed in diclofenac control group II and low dose febuxostat treated group IV, respectively. Febuxostat control group III and febuxostat (medium and high dose) treated groups V and VI had no mortality. Reduction in body weight gain and feed intake was observed in diclofenac control group II as compared to without treatment control group I at the end of every week during the experimental period of 21 days. Reduction in body weight gain and feed intake was observed in low dose febuxostat treated group IV as compared to control group at the end of 1st week. The average FCR was higher in diclofenac control group II (2.54) and low dose febuxostat treated group IV (2.14) as compared to control group (2.00). Kidney: body weight ratio was significantly high in diclofenac control group II as compared to control group at the end of experiment. Gross and microscopic lesions of visceral gout were mainly observed in chicks that died during the experiment from diclofenac control group II and low dose febuxostat treated group IV. The overall lesions showed that diclofenac was nephrotoxic and hepatotoxic in nature. Febuxostat at lower than the therapeutic dose did not prevent nephrotoxicity and hepatotoxicity caused by diclofenac leading to visceral gout. Febuxostat control III and febuxostat (medium and high dose) treated groups V and VI did not reveal any pathomorphological changes. Judicious use of febuxostat is safe in poultry birds between the limit of 4 mg/kg and 6 mg/kg.

Key Words: Ameliorative effect, Febuxostat, Diclofenac, Gout, Broiler chicks.

Introduction

Visceral gout is a common metabolic disorder characterized by high level of uric acid in the blood causing deposition of urates on the surfaces of various visceral organs and is important cause of economic loss to poultry farmers. Diclofenac drugs have been part of our clinical practice since many years. These drugs are used to treat livestock for inflammation and pain relief. A strong correlation was established in the presence of visceral gout and detection of diclofenac residues in body tissues of the affected vultures, (Oaks *et al.,* 2004) so this drug is banned since 2006. Visceral gout was also evident in a study of domestic fowl treated with diclofenac (Naidoo *et al.,* 2007). Several drugs have been proposed for the treatment of gout in birds. In order to find alternative

xanthine oxidase inhibitor to allopurinol, which are safer and efficacious for birds.

Febuxostat is a novel, potent, non-purine selective xanthine oxidase (XO) inhibitor, which in clinical trials demonstrated superior ability to reduce and maintain serum urate levels. The potency of febuxostat is 10-30 times more and well absorbed after oral administration (84% oral bioavailability). It gets absorbed rapidly from the gastro-intestinal tract and excretion of metabolites in the urine and faeces. Keeping in view of alternative therapeutic options that may have a significant impact on the future of successful gout management, it was felt necessary to study the ameliorative effect of febuxostat at different dose level with the known toxic effect of diclofenac sodium in broiler chicks.

Materials and Methods

Experimental birds: The present study was conducted on Cobb-400 broiler chicks. The study plan was approved by the Institutional Animal Ethics Committee (IAEC). A total no. of 108 day-old Cobb-400 broiler chicks were procured from Shakti Hatcheries, Sarsa, Anand, Gujarat.

Experimental design: Day-old chicks were randomly divided into six equal groups,each of 18 chicks. Groups were numbered from I to VI. Group I was kept as control without any treatment. Group II was kept as a diclofenac control and dose of 10 mg/kg b.w. of bird was given through oral gavage daily for 21 days. Group III was kept as a febuxostat control group and dose of 2 mg/kg b.w. of bird was given through oral gavage. Group IV, V, VI, were given diclofenac sodium at the dose rate of 10 mg/kg b.w. of bird with febuxostat given as a graded dose rate of 2 mg/kg, 4 mg/kg, and 6 mg/kg b.w. of bird through oral gavage,respectively. Broiler chicks were observed andweighed daily throughout the experimental period. Mortality and morbidity were recorded throughout the experiment and feed intake was determined by measuring the total amount of feed that each group had consumed taking leftover into consideration during the course of experiment. A detailed postmortem examination of 6 birds each from all six groups was performed and gross lesions were recorded. At the end of experiment, i.e., on 22nd day, all the surviving birds from all 6 groups were subjected to a terminal sacrifice by means of the cervical dislocation technique (Jaksch, 1981). The internal organs,*viz*. kidneys, liver, spleen, heart, proventriculus and lungs were collected and preserved in 10% neutral buffered formalin and absolute alcohol for further histopathological examination.

Statistical analysis: The statistical analysis was carried out by using standard statistical procedures (Snecdecor and Cochran, 1980).

Results and Discussion

Clinical signs

Chicks under group I appeared clinically healthy and did not reveal any remarkable clinical signs. However, birds in the group II and IV showed varying degree of clinical signs. Birds that died from groups II and IV before completion of the experiment had slow growth rate and appeared emaciated, dehydrated, depressed, lethargic and anorectic. Birds showing shifting leg lameness, sitting on their knees and inability to stand revealed involvement of joints. Group III, V and VI treated with febuxostat control, medium and higher dose appeared healthy and did not reveal any clinical signs. Similar clinical signs of diclofenac toxicity were also reported by Ghodasara *et al.* (2014), Akhter and Sarker (2015) and Ramzan *et al*. (2015).

Body weight

There was no statistically significant difference in body weight gain in groups V, VI and III at the end of 1st, 2nd and 3rd week of experiment. However, reduction in body weight gain was observed in group II in comparison with the control at the end of 1st, 2nd and 3rd week. Reduction in body weight gain was also observed in group IV in comparison with the control at the end of 1st week. At the end of 1st week, group II and IV showed a significant ($p < 0.05$) decrease in body weight gain as compared to control. At the end of 2nd and 3rd week, Group II showed highly significant

(p < 0.01) decrease in body weight as compared to control group (Table 1). The previous studies on diclofenac revealed similar results (Seema, 2008; Ghodasara *et al.,* 2014) in broilers.

Table 1: Weekly body weight gain (Mean±SE, g) in broiler chicks under different experimental groups

Day	Experimental Groups					
	I	II	III	IV	V	VI
	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
0	51.90±0.24	51.50±0.43	51. 69±0.25	52.24± 0.24	50.91±0.26	52.09±0.25
7	173.66±4.91	141.33±5.07*	170.33±5.05	150.33±3.56*	170.83±13.12	174.16±5.68
14	423.33±10.77	307.00±31.02**	420.83±10.90	423.5±14.84	413.66±5.15	418.33±14.81
21	758.00±24.99	510.00±18.94**	735.33±11.42	700.51±40.48	739.66±14.54	760.50±13.48

: Significant (p < 0.05)* *: Highly significant (p < 0.01)*

Feed intake

Marginal reduction in feed intake was observed in group II and group IV when compared to control during entire period of experiment (Table 2).The average FCR calculated at the end of experiment was higher in group II (2.54) and IV (2.14) compared to control group I (2.00) (Table 2). This indicated that the diclofenac containing feed reduced the ability of the birds to convert the feed into body mass. Shinde (2007) and Ghodasara *et al.* (2014)also reported similar results in diclofenac toxicity in broiler birds.

Kidney: Body weight ratio

The group II birds treated with diclofenac revealed highly significant (p < 0.01) increase in kidney: body weight ratio as compared to control. Groups III, IV, V and VI did not reveal any change in the kidney body weight ratio as compared to control group (Table 2). This was mainly due to reduced body weight and increased kidney weight because of urate deposition and inflammatory reaction in diclofenac control group. The kidney is a main target organ and its reaction was reflected by increased kidney: body weight ratio. However, febuxostat has better capacity to remove urate deposition and its formation in kidneys did not reveal any change in kidney: body weight ratio in group III, V and VI. Similar observations were recorded by Ghodasara *at al*. (2014).

Table 2: Average feed consumption (g), body weight (g), feed conversion ratio (FCR) and kidney: body weight ratio in chicks under different treatment groups at the end of experiment

Experimental Group	Feed consumption (g)	Body weight (g)	FCR	Kidney: Body weight Ratio ($\times 10^{-3}$) Mean±SE
Group – I	1520.52	758	2.00	9.44±0.38
Group – II	1299.33	510	2.54	13.22±0.72**
Group – III	1504.71	735	2.04	8.33±0.23
Group – IV	1500.00	700	2.14	8.17±0.38
Group – V	1502.90	740	2.03	8.17±0.39
Group – VI	1513.90	760	1.99	8.44±0.33

***Highly significant (p < 0.01)*

Mortality

27.77% mortality was observed in group II, which was noticed from day 3 to day 12 of the experiment. 22.22% mortality was also observed in group IV, which was noticed during the 1st week of the experiment. Groups III, V and VI did not reveal mortality and all the birds were found healthy. Gajera (2006) reported maximum mortality during second week of life which is most vulnerable for the development of gout. Ghodasara *et al.* (2014) reported 25% mortality in diclofenac treated group at dose rate of 15 ppm in feed, which was noticed from day 8 to day 14 of the experiment.

The mortality noticed between 3rd to 12thday of the experiment indicated that older birds were less sensitive to drug and surviving birds were able to resist diclofenac toxicity more efficiently. Mortality in group IV was due to diclofenac toxicity, as low dose of febuxostat did not efficiently reduce the uric acid production, so urate crystal formed on the various visceral organ mainly kidney and liver leading to renal failure.

Gross and Histopathology

The chicks died during the experiment from group II and low dose febuxostat treated group IV revealed gross and microscopic lesions of visceral gout. Both kidneys were pale, enlarged and revealed deposition of urate crystals with dilatation of ureters (Fig. 1). Liver lesions were comprised of presence of chalky white deposits firmly adhered on the surface. Such chalky white deposits were also observed on the surface of heart, proventriculus, gizzard, serosa of intestine and abdominal air sacs (Fig. 2). Most of chicks died during experiment from group II showed gross lesions in joints characterized by white urate deposition in joint capsule (Fig. 3).

Fig. 1. Pale, enlarged and frosted kidney with chalky white urate deposition and dilated ureters filled with unic acid crystals. Fig. 2. Chalky white urate deposits on the surface of pericardium, liver and abdominal air sacs. Fig. 3. Chalky white urate deposiiton on joint capsule.

Histopathological changes in the tissue sections of kidneys were characteristic of visceral gout with deposition of urate crystals in the renal parenchyma with varied severity (Fig. 4). The liver lesions were comprised of mild to severe congestion, hemorrhage, degeneration and necrosis with deposition of urate tophi surrounded by infiltration of inflammatory cells (Fig. 5). Group II birds

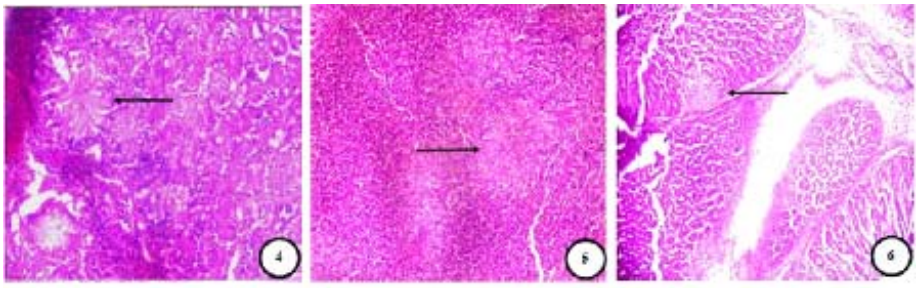

Fig. 4. Section of the kidney showing marked deposition of uric acid crystals in radiating pattern (Arrow) (H&E X 240). Fig. 6. Section of proventriculus showing congestion, hemorrhage, deposition of urate crystals (Arrow) (H&E X 240).

showed congestion, hemorrhage and deposition of urate crystals in proventriculus (Fig. 6). Features of kidney, liver and pericardium lesions recorded in the present study were in accordance with earlier findings (Seema, 2008; Jana *et al*., 2009; Ghodasara *et al.,* 2014; Akhter and Sarker, 2015). Deposition of urate on the proventriculus was also observed by previous authors.

The gross and histopathological lesions observed in different organs indicated that diclofenac at dose levels of 10 mg/kg induces lesions in the form of visceral gout with more or less severity in organs like kidney, liver, heart, proventriculus, gizzard and air sacs. However, birds treated with febuxostat at the dose rate of 4 mg/kg and 6 mg/kg through oral gavage did not reveal any pathomorphological alteration.

From the present study it is concluded that diclofenac at the dose rate of 10 mg/kg bird in diclofenac control and low dose febuxostat treated groups for a period of 21 days continuously, induces toxicity. While medium and high dose febuxostat treated chicks did not reveal any toxicity symptoms. The morbidity and mortality in broiler chicks due to visceral gout reduced feed intake, body weight gain and increased FCR and kidney: body weight ratio in diclofenac control group II. Diclofenac at the dose rate of 10 mg/kg with febuxostat at the dose rate of 2 mg/kg through oral gavage resulted in low mortality in broiler chick due to visceral gout. Diclofenac induced patho-morphological lesions were of vascular and degenerative type changes mainly affecting kidney, liver, heart, proventriculus, gizzard, spleen, lung and intestine and were specific of visceral gout in group II and IV. Febuxostat is safe in poultry birds between the limit of 4 mg/kg and 6 mg/kg which is the therapeutic dose, so it can be used for treatment in birds considering its beneficial effects.

Conflict of Interest: All authors declare no conflict of interest.

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