Original Research

Kidneys: Histopathological Changes in Diclofenac Induced Visceral Gout in Broilers and its Amelioration with Natural Herbals

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Abstract

A study was conducted to examine the gross and histopathological changes in the kidneys of diclofenac induced visceral gout in day old broiler chicks and its amelioration with an ayurvet product. A total of 125 healthy day-old male broiler chicks (Vencobb strain) were divided into 5 groups consisting of 25 birds in each. The group 1 birds served as control and group 2 served as diclofenac toxic control (@ 30 ppm in feed) for 14 days. Group 3 birds were treated with ayurvet product (AV/AUP/16 @ 5 mL/day/100 birds for 0-2 weeks, 10 mL/day/100 birds for 2-4 weeks, 20 mL /day/100 birds for 4-6 weeks) upto 42 days. Group 4 were treated with diclofenac for 14 days along with ayurvet product from 1st to 42nd day. Group 5 birds were treated with diclofenac for 14 days followed by ayurvet product from 15th day to 42nd day. These birds were sacrificed on 14th, 28th and 42nd day and observed pale colored, enlarged kidneys in diclofenac treated group birds and presence of light pink coloured urate crystals as radiating pattern in kidney parenchyma (H&E stain). These urate crystals were black in color with De Galantha's stain. The histopathological changes were reduced in ayurvet product treated groups.

Key words: Diclofenac Sodium, De Galantha's Stain, Histopathological Changes, Kidneys, Visceral Gout, Vencobb Strain

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Introduction

Visceral gout is a common metabolic disorder characterized by high level of uric acid in the blood causing deposition of urates on the surface of various visceral organs and is responsible for high morbidity and mortality in broilers. This condition can occur as an individual problem at any age but outbreaks are seen in young chicks in the first or second week of life (which is called as baby chick nephropathy) and could be of infectious origin also. The non- infectious causes are connected with nutritional or managemental problems (Mycotoxin contamination of the feed, excess protein, vitamin A deficiency, lack of water supply,

cold stable temperature etc.,) and may be called as pre-renal causes (Eldaghayes *et al*., 2010). The study was considered with the objective to study gross and histopathological changes in kidney of gout affected birds and also in ayurvet treated birds.

Materials and Methods

Drugs and Chemicals

1. Diclofenac sodium (Voveran D, Indian Pvt Ltd.,) was procured from Market, Hyderabad.
2. Ayurvet product (AV/AUP/16), Ayurvet Ltd, Himachal Pradesh.

Experimental Birds

In the present study a total 125 healthy day-old male broiler chicks (Vencobb strain) weighing 45-60g were procured from Venkateshwara Hatcheries Pvt, Ltd., Hyderabad. The experiment was carried out according to the guidelines and prior approval of the Institutional Animal Ethics Committee (IAEC) (No.35-2018/IAEC, CVSc, Hyderabad).

Experimental Design

The group 1 birds served as control and group 2 served as diclofenac toxic control (@ 30 ppm in feed) for 14 days. Group 3 birds were treated with ayurvet product (AV/AUP/16 @ 5 mL/day/100 birds for 0-2 weeks, 10 mL /day/100 birds for 2-4 weeks, 20 mL /day/100 birds for 4-6 weeks) upto 42 days. Group 4 were treated with diclofenac for 14 days along with ayurvet product from 1st to 42nd day. Group 5 birds were treated with diclofenac for 14 days followed by ayurvet product from 15th day to 42nd day.

Natural Herbals (Ayurvet Product)

Varuna chhaal, Gokshura, Punarnava, Sunthi, Haridra.

Pathological Studies

Birds were sacrificed on 14th, 28th and 42nd day of experiment. Six birds from each group were subjected to detailed and systematic necropsy examination. Gross pathological lesions observed during necropsy were recorded and respective tissue samples were collected for histopathology in suitable preservatives.

Histopathology

The tissues samples of heart, liver, kidneys were collected and fixed in 10% neutral buffer formalin (NBF) soon after sacrifice. After fixation the collected tissue samples were processed and embedded in paraffin (58 – 62^{0}C) and were sectioned at 3-5 µ thickness and stained with routine hematoxylin and eosin (H&E) for histopathological examination as per the standard procedure (Luna, 1968) and the duplicate sections were cut at 8 µ thickness and stained by De Galantha's for demonstration of urate crystals (Luna, 1968).

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Results and Discussion

Gross Pathology

Grossly, the birds in group 1 and 3 revealed normal morphological appearance of kidney throughout the experimental study. The birds in group 2 which were treated with diclofenac showed severely congested and enlarged kidneys (Fig. 1).

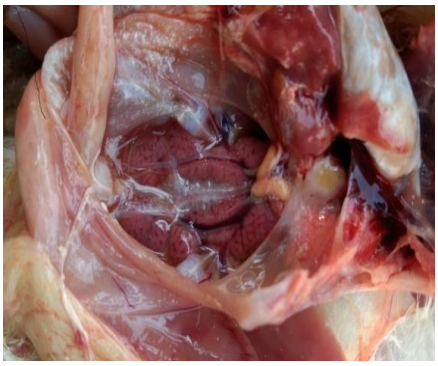

Fig. 1: Group 2 bird on 14th day showing pale and congested kidneys

They appeared frosted due to accumulation of urate crystals, minute pin point haemorrhages along with chalky white deposits of urate crystals on the serosal surface. The gross observations of the present study were in accordance with Rahamathulla and Mohiyuddeen (1973); Chandra *et al*. (1984); Reddy Mohan *et al*. (1984); Chang and Fun (1992); Gajera (2006); Reddy *et al*. (2006); Patel *et al*. (2007); Jana *et al*. (2009); Sharma and Vegad (2010); Yewale (2010); Feizi *et al*. (2011); Sultana *et al*. (2012); Patel *et al*. (2014); Nitin and Ghosh (2014); Amaravathi *et al*. (2015); Akter and sarker (2015); Behtari and Feizi (2015) and Ramzan *et al*. (2015). Visceral gout was also observed as the main postmortem lesion in vultures treated with diclofenac and was studied by Gilbert *et al*. (2002); Cunningham *et al*. (2003) and Swan *et al*. (2006). On 14th day of experiment, decreased severity of lesions was observed in group 4 compared to group 2. From 28th day onwards improvement observed in the gross lesions in group 4 and 5 when compared to group 2 indicating ameliorative effect of ayurvet product against gout. The improvement could be due to active ingredient curcumin in ayurvet product that has both antioxidant and anti-inflammatory action.

Histopathology

The kidneys collected from group 2 on 14th day revealed changes characterized by marked congestion, hameorrhages and focal to diffuse degenerative changes in tubular epithelium like vacuolar degeneration, desquamation and necrosis of tubular epithelial cells and infiltration of mononuclear cells (MNC's) along with heterophils in the interstitial spaces. Hyaline casts were also present in tubular lumen of few sections. Kidney parenchyma exhibited aggregates of uric acid crystals characterized by needle shaped urate crystals as pink radiating amorphous material surrounded by a narrow zone of inflammatory cells (Fig. 2). Similar

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histological lesions were also reported in gout affected birds by Rahamathulla and Mohiyuddeen (1973); Reddy mohan *et al*. (1984); Guo *et al*. (2005); Patel (2005); Hedau *et al*. (2008); Jana *et al*. (2009); Yewale (2010); Muhammed *et al*. (2012); Bulbule *et al*. (2013) and Akter and sarker (2015). These uric acid crystals were observed in black color by De Galantha's stain (Fig. 3) which was also observed by Rahamathulla and Mohiyuddeen (1973); Jana *et al*. (2009); Yewale (2010); Mohan *et al*. (2012); Patel *et al*. (2014) and Patil *et al*. (2015). Chandra and Balwant (1980) described intratubular and interstitial uric acid depositions surrounded by inflammatory reactions as the characteristic feature of gouty nephritis and also observed that the extent of damages to kidney was directly correlated to the degree of urate deposition. Ghodasara *et al*. (2014) observed moderate, multifocal, periglomerular and interstitial mononuclear cells infiltration in the kidney of visceral gout affected birds due to astrovirus infection. Group 4 birds showed mild degenerative changes, necrosed tubular epithelium along with leukocytic infiltration. (Fig. 4). The kidneys of group 5 showed urate crystals as radiating pattern in parenchyma same as group 2 birds (Fig. 5).

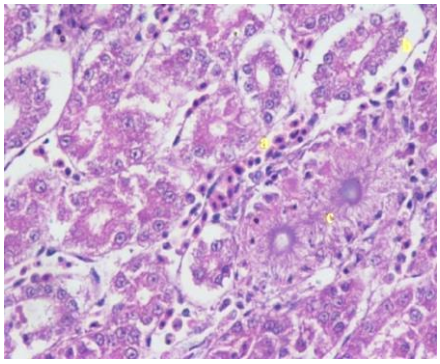

Fig. 2: Section of kidney showing intertubular haemorrhages (a), desquamation of tubular epithelium (b), urate crystal deposition in the tubules as radiating pattern (c) (Group 2, 14th day) H&E X 400

Fig. 3: Section of kidney showing black colored urate crystals (Group 2, 14th day) De Galantha's stain X 100

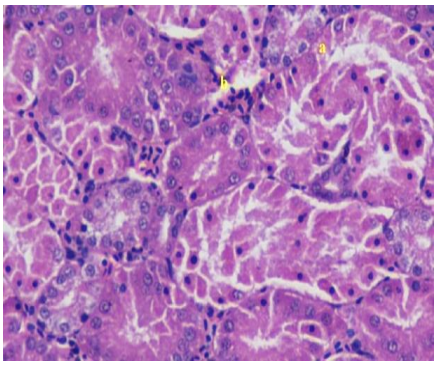

Fig. 4: Section of kidney showing degenerative changes in tubules (a), vacuolated changes within the tubules, congestion of blood vessels (b- Group 4, 14th day) H&E X 400

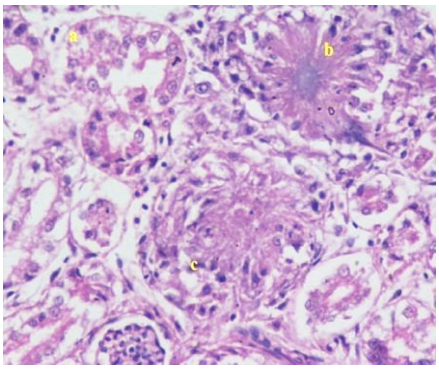

Fig. 5: Section of kidney showing necrosed renal tubules (a), urate crystal deposition within the tubules (b) and leukocytic infiltration(c) (Group 5, 14th day) H&E X 400

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On 28th day, group 2 birds showed vacuolar degeneration, distorted tubules, desquamation of tubular epithelium, multiple hyaline casts in the lumen of tubules (Fig. 6). The birds of group 4 showed varying type of minimal to mild degenerative changes, mild congestion and occasional infiltration of inflammatory cells without uric acid crystals deposition in parenchyma (Fig. 7). The birds in group 5 showed multiple foci of leukocytic infiltration and desquamated tubular epithelial cells (Fig. 8). The lesions in group 4 birds were less pronounced than group 5 birds on 14th, 28th and 42nd day because of protective effect of ayurvet product in group 4 birds. The lesions in the birds of group 5 were less severe on 28th and 42nd day of experiment when compared with group 2 because of ameliorative action of ayurvet product against diclofenac induced gout (Fig. 9). The improvement in kidney function test (KFT) observed in group 4 and 5 were also in tandem with these findings, thereby affirming the beneficial effect of ayurvet against the gout.

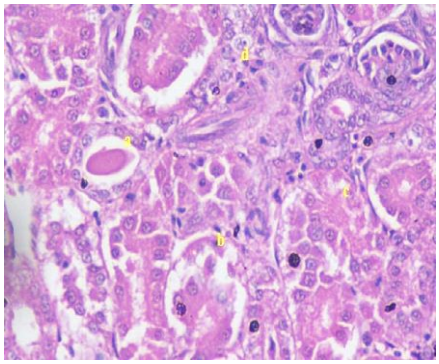

Fig. 6: Section of kidney showing hyaline cast (a), desquamation of tubular epithelium (b), necrotized tubules (c) and mild leukocytic infilteration (d) (Group 2, 28th day), H&E X 400

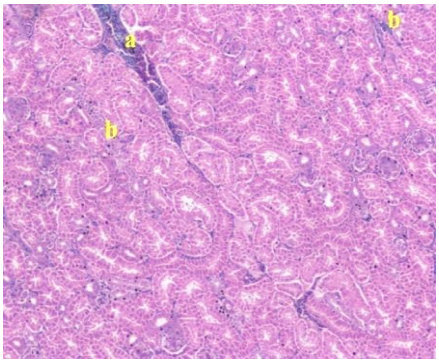

Fig. 7: Section of kidney showing congestion (a) and mild leukocytic infiltration (b) (Group 4, 28th day), H&E X 100

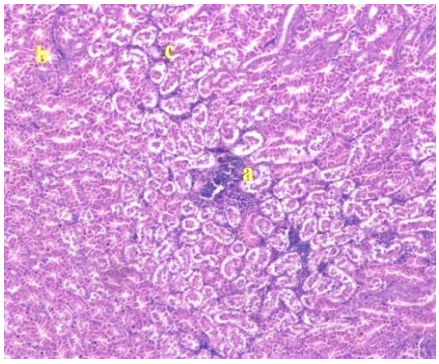

Fig. 8: Section of kidney showing multiple foci of leukocytic infiltration (a), degenerated tubules (b) and desquamation of tubular epithelium (c) (Group 5, 28th day), H&E X 100.

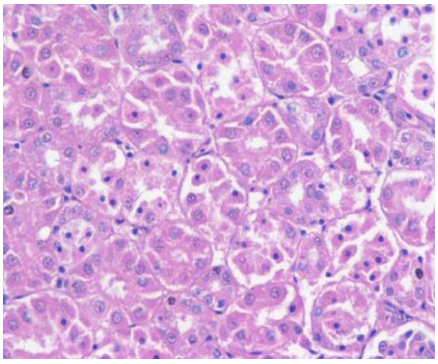

Fig. 9: Section of kidney showing normal tubules (Group 5, 42nd day) H&E X 400.

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Conclusion

In conclusion, diclofenac induced gout altered the renal specific biochemical parameters and produced severe pathological changes in kidneys. Supplementation of ayurvet improved performance and reduced pathological changes in diclofenac induced gout birds. Further, pretreatment of birds with ayurvet was more effective than post treatment. The active ingredient in ayurvet like sunthi well known antioxidants might be responsible for recovery of gout induced birds.

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