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Histopathological changes in liver and heart of diclofenac induced visceral gout in broilers and its amelioration with ayurved product

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Abstract

A study was conducted to examine the gross and histopathological changes in the kidney of diclofenac induced visceral gout in day old broiler chicks and its amelioration by an ayurved 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 ayurved product (AV/AUP/16 @ 5ml/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 ayurved product from 1st to 42nd day. Group 5 birds were treated with diclofenac for 14 days followed by ayurved product from 15th day to 42nd day. These birds were sacrificed on 14th, 28th and 42nd day and observed congested, enlarged liver and pericardium along with white chalky deposits of urate crystals on the visceral surface in diclofenac treated groups and presence of light pink coloured urate crystals as radiating pattern in liver parenchyma (H&E stain). These urate crystals were black in color with De Galantha's stain. The histopathological changes were reduced in ayurved product treated groups.

Keywords: visceral gout, diclofenac sodium, De galantha's stain, histopathological changes

Introduction

Visceral gout is a common metabolic disorder characterized by deposition of white urates, which are normally excreted as a white cap on well formed faeces, in various tissues. Improper metabolic processes results in metabolic disorders within birds' body. The abnormal biochemical reactions can be due to improper functioning of the vital organs like the kidney, liver, heart and lung. One of the important metabolic disorders associated with kidney damage in poultry is gout. The increased levels of uric acid in blood leads to deposition of urate crystals on kidney followed by other visceral organs. The organs like liver and heart shows pathological lesions along with kidney in visceral gout affected birds. The study was considered with the objective to study gross and histopathological changes in liver and heart of gout affected birds and also in ayurved treated birds.

Materials and Methods

Drugs and chemicals

1. Diclofenac sodium (Voveran D, Indian Pvt Ltd.,) was procured from Market, Hyderabad.
2. Ayurved product (AV/AUP/16), Ayurved 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 ayurved product (AV/AUP/16 @ 5ml/day/100 birds for 0-2 weeks, 10 ml/day/100 birds for 2-4 weeks,

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20 ml/day/100 birds for 4-6 weeks) upto 42 days. Group 4 were treated with diclofenac for 14 days along with ayurved product from 1st to 42nd day. Group 5 birds were treated with diclofenac for 14 days followed by ayurved product from 15th day to 42nd day.

Ayurved product (AV/AUP/16) composition

Varunachhaal, 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 a 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^oC) 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 [17] and the duplicate sections were cut at 8 μ thickness and stained by De Galantha's for demonstration of urate crystals [16].

Results and Discussion

Gross pathology

Grossly, the birds in Group 1 and 3 revealed normal morphological appearance of liver and heart throughout the experimental study. The birds in group 2 which were treated with diclofenac showed severe congestion and enlargement of the visceral organs like, heart and liver on 28th day of experiment. Liver was found to be enlarged, friable with white chalky urate deposition on the surface of the capsule. In heart, the entire pericardium was seen covered with urate deposits of varying degrees on its serosal surface i.e uric acid pericarditis [32]. Pericardium of heart was firmly adhered to heart. Congestion of viscera might be due to emaciation and dehydration of birds leading to haemoconcentration [28]. The gross observations of the present study were in accordance with earlier workers [1, 2, 4, 5, 6, 9, 10, 15, 19, 20, 21, 23, 25, 26, 27, 29, 30, 32]. Visceral gout was also observed as the main postmortem lesion in vultures treated with diclofenac and was studied by [8, 12, 31].

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 ayurved product against gout. The improvement could be due to active ingredient sunthi in ayurved product that has both antioxidant and anti-inflammatory action.

Histopathology

Liver

In group 2 birds, the liver sections showed, severe sinusoidal congestion, haemorrhages in parenchyma, fatty change and foci of necrotic hepatocytes. Liver parenchyma also had foci of amorphous radiating uric acid crystals mixed with necrotic debris surrounded by a narrow zone of inflammatory cells which were also found by (18). Black coloured urate crystals

were observed by staining with De Galantha's stain [21, 22, 28]. Sections of liver showed congestion, haemorrhages, fatty change and necrosis of hepatocytes were consistently present in diclofenac administered groups of all species. Some authors found sinusoidal and central vein dilatation, bile duct proliferation, degeneration and pyknosis of hepatocytes and cellular infiltration in periportal areas in experimentally induced diclofenac toxicity in male Wistar rats [3, 14]. The results of present study was supported by earlier authors [1, 7, 13, 15, 24]. The lesions in group 4 were less severe than group 2 with leukocytic infiltration in portal triad along with congestion and sinusoidal dilatation on 14th day of experiment indicating protective role of ayurved product. The birds in group 5 showed uric acid crystals in liver parenchyma with severe leukocytic infiltration same as group 2 on 14th day.

On 28th and 42nd day of experiment, there was minimal vacuolar degenerative changes, sinusoidal dilatation along with inflammatory cell infiltration in the liver of birds of group 4 and group 5 when compared with group 2 birds. The milder lesions in group 4 and 5 could be due to the protective effect of antioxidants present in ayurved against free radical induced hepatic injury.

Heart

In the birds of group 2, myocardium of the heart showed urate deposition along with destruction of myocardial cells and infiltration of inflammatory cells. Lesions of heart included myocardial congestion because of severe engorgement of blood vessels and focal to diffuse haemorrhages between muscle fibers. On 28th day heart showed severe infiltration of inflammatory cells along with urates deposition in myocardium and were black in color by De Galantha's stain [28]. The results of present study were in accordance with [18, 21, 24]. The severity of lesion was less in group 4 as compared to group 2 on 14th day of experiment might be due to ayurved product. Thin white chalk like urate salt deposition on the serous membrane and in the subcutaneous connective tissues, heart showed mild to severe deposition of chalky white dried and gritty urate on the pericardium in visceral gout affected birds due to astrovirus infection [11]. The group 5 birds showed urate crystals in myocardium along with leukocytic infiltration and also showed necrotic myofibrils on 14th day.

Group 4 and 5 birds showed only mild congestion in myocardium on 28th and 42nd day of experiment and the lesions were less severe when compared to group 2 birds might be due to ameliorative effect of ayurved product against gout. The protective effect of ayurved was associated with its antioxidant and anti-inflammatory properties which inhibit free radical generation.

Conclusion

In conclusion, the present study shows that diclofenac induced gout altered the biochemical parameters and produced severe pathological changes like uric acid crystal deposition along with leukocytic infiltration in parenchyma of liver and heart, necrosed hepatocytes and myocytes. Supplementation of ayurved improved performance and reduced pathological changes in diclofenac induced gout birds. Further pretreatment of birds with ayurved was more effective than post treatment. The active ingredient in ayurved like sunthi well known antioxidants might contributed to recovery of gout induced birds.

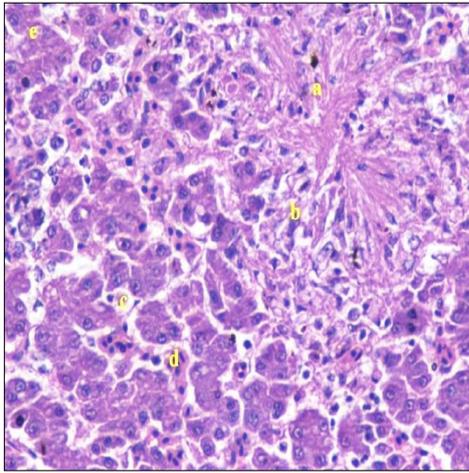


Fig 1: Sections of liver showing urate crystal deposition (a), leukocytic infiltration (b), sinusoidal dilatation (c), congestion (d), necrosed hepatocytes (e) (Group 2, 14th day) H&E X 400

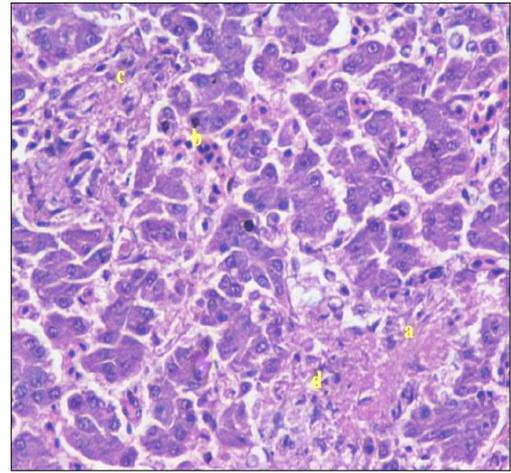


Fig 4: Sections of liver showing urate crystal deposition (a), congestion (b), necrosed hepatocytes (c) and leukocytic infiltration (d) (Group 5, 14th day) H&E X 400



Fig 2: Sections of liver showing black coloured urate crystals (Group 2, 14th day) De Galantha's stain X 400

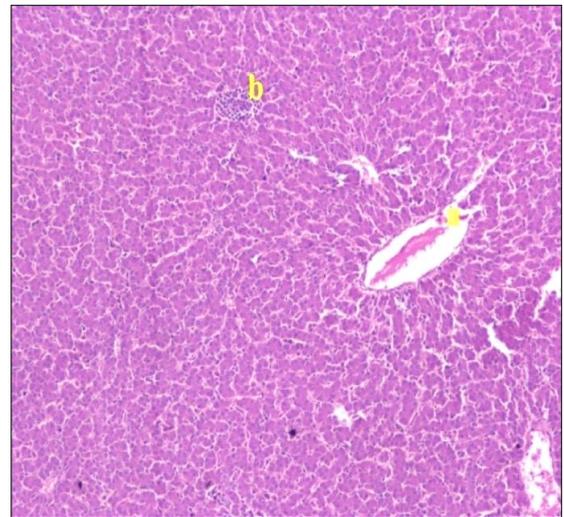


Fig 5: Sections of liver showing slight congestion (a) and mild leukocytic infiltration (b) (Group 4, 28th day) H&E X 100

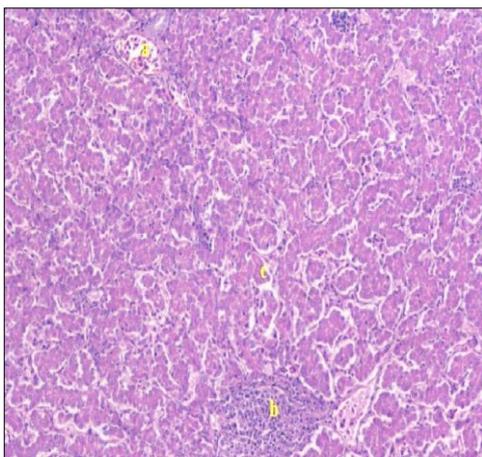


Fig 3: Sections of liver parenchyma showing congestion (a), leukocytic infiltration (b), sinusoidal dilatation (c) (Group 4, 14th day) H&E X 100

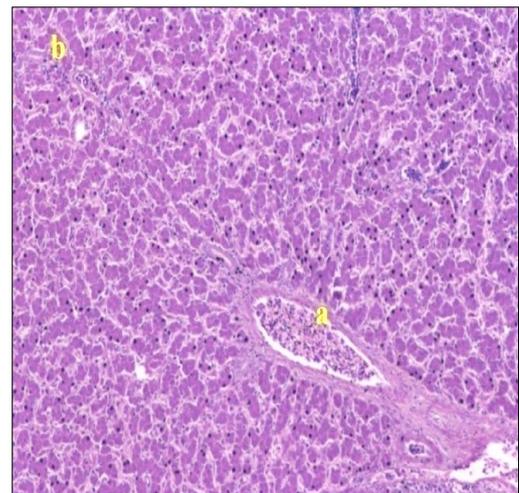


Fig 6: Sections of liver showing mild congestion (a), mild leukocytic infiltration (b), (Group 5, 28th day), H&E X 100

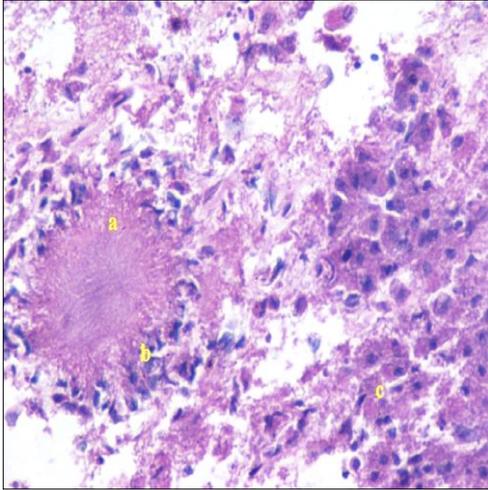


Fig 7: Sections of heart showing urate crystal deposition (a), heavy leukocytic infiltration (b), degenerated myocytes (c). (Group 2, 14th day), H&E X 400

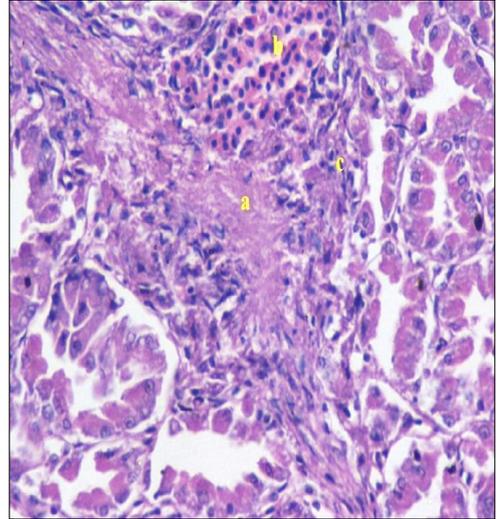


Fig 10: Sections of heart showing urate crystal deposition (a), haemorrhage (b), heavy leukocytic infiltration (c) (Group 5, 14th day), H&E X 400

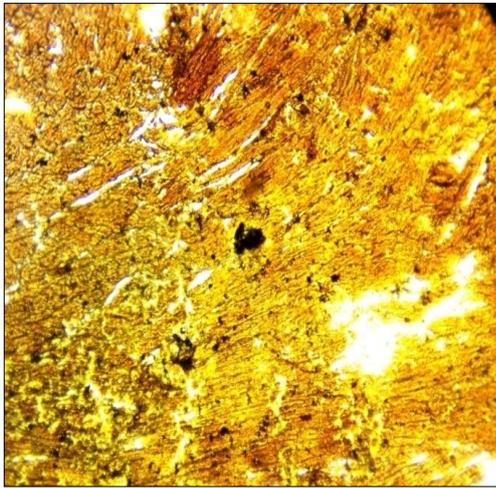


Fig 8: Sections of heart showing black coloured urate crystals (Group 2, 14th day) De Galantha's stain X 100

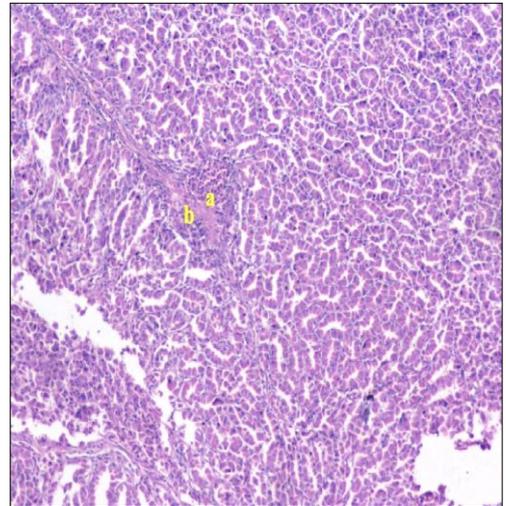


Fig 11: Sections of heart showing urate deposition (a), severe leukocytic infiltration (b), (Group 2, 28th day), H&E X 100

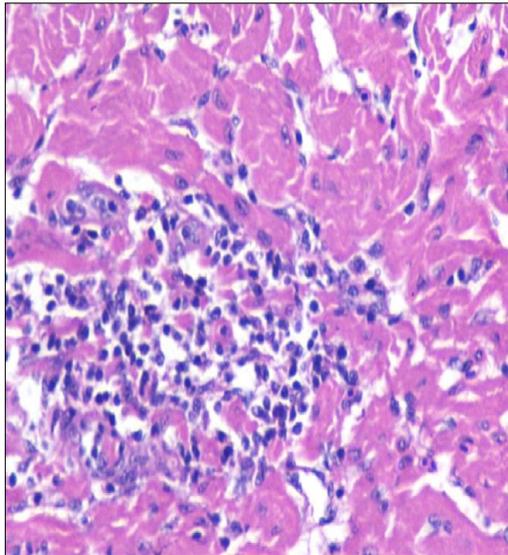


Fig 9: Sections of heart showing heavy leukocytic infiltration and degenerated myocytes (Group 4, 14th day), H&E X 100

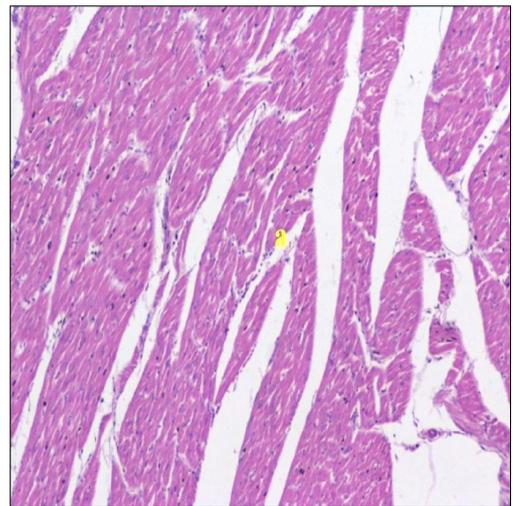


Fig 12: Sections of heart showing mild interfibrillar haemorrhages (a), (Group 4, 28th day), H&E X 100

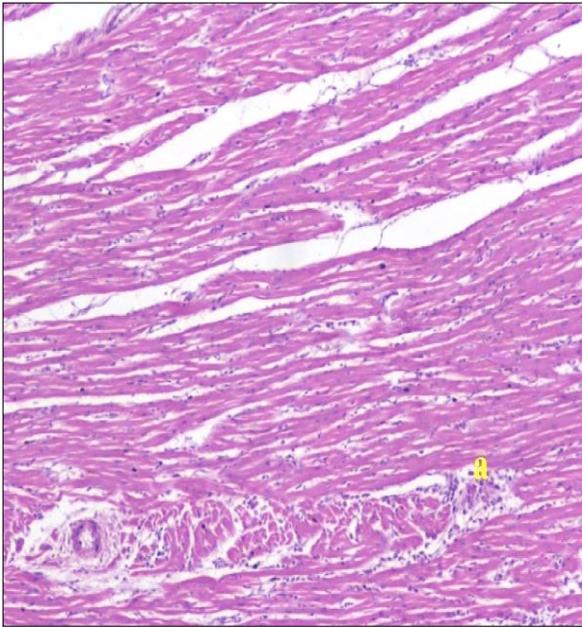


Fig 13: Sections of heart showing mild leukocytic infiltration (a) (Group 5, 28th day), H&E X 100

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