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Haemato-biochemical and anti-oxidant alterations in chicks of visceral gout induced by diclofenac and its amelioration by ayurvet product

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Abstract
Visceral gout assumes prime economic importance in poultry industry due to increased incidence causing production losses and mortality. The present study was carried out to study the efficacy of ayurvet product in gout induced broilers in relation with haemato biochemical and anti-oxidant values. 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 @ 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 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. Haemato biochemical parameters were analyzed on 14th, 28th and 42nd day. Results showed significant ($P<0.05$) decrease in the values of Hb, TEC and PCV whereas TLC count was increased in diclofenac treated groups i.e groups 2, 3 and 4 upto 14 days when compared to control. There was a significant ($P<0.05$) elevated levels of biochemical parameters AST, ALT, ALP, UCCCCric acid and creatinine noticed in diclofenac treated groups indicated liver and renal damage. Antioxidant profile showed decreased GSH and increased TBARS in kidney and liver tissue in group 2 birds. An improvement in respect of haemato-biochemical and antioxidant profile was observed in group 4 and 5 birds.

Keywords: visceral gout, diclofenac sodium, heamato-biochemical parameters, antioxidant profile

Introduction
Gout is a common metabolic disorder that results in abnormal accumulation of urates in domestic birds. Metabolic disorders arise as a result of improper metabolic processes within the birds' body. The abnormal bio-chemical reactions can be due to improper functioning of the vital organs like the kidney, liver, heart and lung. Gout is mostly associated with kidney damage in birds which causes reduced excretion of uric acid leads to its accumulation in blood and body fluids and this subsequently favours its precipitation in various tissues. The kidney damage can arise from infection with certain strains of Infectious bronchitis virus, exposure to some mycotoxins, high levels of vitamin D3, prolonged vitamin A deficiency or inadequate water intake. The study was undertaken to study the alterations in haemato-biochemical and antioxidant levels in diclofenac treated birds and also in ayurvet treated birds to assess the protective effect of ayurvet product in diclofenac 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, Hyerabad).

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

(cbbccAV/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 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.

Methods

Haematological parameters were studied at fortnight intervals. Six birds from each group were sacrificed on 14th, 28th and 42nd day of experiment. Blood (5 mL was collected on the day of sacrifice from wing vein with the help of insulin syringe in an anticoagulant coated vaccutainers {(K3-EDTA tube, 13mm, 4mL (Rapid Diagnostics Pvt. Ltd., Delhi)} to estimate all haematological parameters like Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Haemoglobin (Hb) concentration and Packed Cell Volume (PCV). TEC and TLC were performed as per the method (19) using diluting fluid recommended by Natt and Herick (1952). Hb was estimated by Acid haematin method using Sahli's instrument. PCV was determined by wintrobe method and blood collected in serum vaccutainers, were allowed to clot in clot promoting {(Vit K-coated–clot activator tube–plain–13mm×75mm, 5mL) (Rapid Diagnostic Pvt. Ltd., Delhi)} vacutainers and then centrifuged (Sigma 1-13-bench top laboratory centrifuge, USA) at 20k rpm for 10 minutes. Serum was separated from tube and collected in eppendorf tubes and stored at -20^{0}C and the biochemical parametres like Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP were estimated as per modified International Federation of Clinical Chemistry (IFCC), serum creatinine by Jaffe's method and serum uric acid by Uricase- Trinder reaction. Liver and kidney samples of all groups were collected at the time of sacrifice and tissue homogenate was prepared. In the homogenate of tissue, reduced glutathione (GSH) (17) and thiobarbituric acid reacting substances (TBARS) (21) was estimated.

Statistical analysis

The data of haematological, biochemical parameters and antioxidants were subjected to statistical analysis by applying one way ANOVA using statistical package for social sciences (SPSS) version15.0. The differences between the means were tested by using Duncan's multiple comparision tests and significance level was set at $P < 0.05$ (30).

Results and Discussion

The mean values of HB, TEC, TLC and PCV are given in Table 1. A significant ($P<0.05$) decrease in Hb, TEC and PCV in group 2 birds throughout the experiment and these results concurred with earlier reports of (1, 29). However, significantly ($P<0.05$) higher levels of TLC were observed in group 2 birds and this may be due to metabolic acidosis leading to uremia which may have stimulatory effect on bone marrow leading to leukocytosis (29). Similarly leukocytosis in gout affected birds was reported by earlier scientists (25, 28, 29, 34). In the present study, the subcellular changes observed in kidney might be due to uric acid crystals that cause damage to kidney parenchyma which indirectly effects erythropoiesis. Group 4 birds showed significant ($P<0.05$) decrease in Hb, TEC and PCV than group 1 and 3 and they were higher than group 2 on 14th day implies protective effect of ayurvet against the diclofenac. These values were increased on 28th and 42nd day which were almost comparable with group 3. Similarly TLC values were higher than control group and lower than group 2 on 14th day indicates that some inflammatory reaction was there on 14th day due to urate crystals and these values were normal on subsequent intervals of experiment. The birds in group 3 showed normal values of Hb, TEC, PCV and TLC cas control. Group 5 birds showed significant ($P<0.05$) increase in Hb, TEC and PCV on 28th and 42nd day of experiment when compared to group 2 which indicates ameliorative effect of ayurvet product. These findings were in agreement with the previous report (26). However no significant changes in these blood values were noted in diclofenac treated birds (11, 27, 28).

The levels of AST, ALT and ALP were significantly increased ($P<0.05$) in diclofenac treated groups upto 14 days when compared to control and the mean values are represented in Table 2. Diclofenac causes potential hepatotoxicty that may be associated with the formation of reactive metabolites via hepatic cytochrome P_{450}-catalyzed oxidation and contribute to diclofenac mediated hepatic injury. In the present study diclofenac treated birds (group 2) revealed a moderate to severe hepatic damage due to significant ($P<0.05$) elevation of hepatic enzyme activities (AST and ALP on 14th, 28th and 42nd day and ALT levels on 14th and 42nd day of experiment) in serum when compared to other groups and these results were in accordance with earlier findings (4, 7, 14, 16, 22, 23, 24, 29, 33). In the present study, increased levels of AST, ALT and ALP is due to failure of detoxification of liver due to repeated dose of diclofenac. Group 4 birds showed significant improvement in the values of liver enzymes on 14th, 28th and 42nd day of experimental study as compared to group 2 due to protective effect of ayurvet product against gout. Group 5 birds showed significant ($P<0.05$) decrease in serum hepatic enzymes on 28th and 42nd day of experimental study when compared to group 2 and were almost comparable with group 1 and 3 due. These findings are indicative of protective and therapeutic action of ayurvet product against gout.

In the present study uric acid level can be used to assess renal function, but significant kidney damage must be present before the concentration rises (3). An increase in the serum creatinine levels may be due to a blockade of renal vasodailation due to non-selective inhibition of the COX-2 by diclofenac sodium. In the present study, a significant increase in serum uric acid and creatinine levels were observed in group 2 when compared with other groups throughout the experiment. Elevation of serum urea and creatinine levels are indicators of nephrotoxicty (5). These results were in accordance with earlier findings (1, 2, 4, 7, 8,11, 16, 22, 24, 32) The observations like hyperuricaemia in the toxic groups clearly reflected that repeated use of NSAIDs at therapeutic level for prolonged period may induce marked renal dysfunction (27). Significantly ($P<0.05$) an elevated levels of serum uric acid and creatinine were observed in group 4 on 14th day of experiment when compared to group 3, but these values were reduced on 28th and 42nd day of experiment when compared to Group 2 and were almost comparable with group 1 and group 3. The serum creatinine values of group 5 were insignificant with group 1 and group 3 on 28th and 42nd day of experiment and the serum uric acid values were significantly reduced when compared to group 2 and were comparable with group 1 on 28th and 42nd day of experiment showing ameliorative effect of ayurvet product.

The mechanism of diclofenac-induced mitochondrial injury involves generation of ROS (Reactive Oxygen Species), causing oxidative stress to hepatocytes as proposed by (31).

Diclofenac is metabolized by CYP2C9 to hydroxylated metabolites, which may be further bio activated to reactive iminoquinone intermediates, leads to enhanced superoxide production and increased intracellular Ca^{2+}, results in lethal cell injury (13).

In the present study, significantly (P<0.05) decreased levels of reduced GSH in liver and kidney was observed in group 2 as compared to other groups throughout the experiment and the mean values are given in Table 3. Exposure to xenobiotics caused a depletion of GSH levels in poultry birds (12). Similar findings were reported by earlier workers (7, 12, 20, and 41). Significantly (P<0.05) reduced values of GSH in the present study may be due to ability of diclofenac to induce mitochondrial damage and leads to an increased ROS production which is responsible for oxidative stress. The birds in group 4 shown significantly (P<0.05) lower values of reduced GSH in liver and kidney on 14th day of experiment when compared to group 1 and 3 but higher than group 2 indicating protective action of ayurvet product against gout. The treatment groups (group 4 and 5) showed increase in GSH as compared to group 2 on 28th and 42nd day of experiment indicating ayurvet ability to counteract the oxidative stress. These biochemical results were well corroborated by histopathological observations.

During diclofenac sodium metabolism the number of reactive oxygen species can be increased. These products induce prooxidative damage in renal tissue. The increase in superoxide dismutase levels and MDA activity in renal tissue may indicate peroxidative damage and cause cell damage in kidney tissue (9). MDA assay has been found to be one of the better predictor of oxidative damage (18). In the present study, significantly (P<0.05) an elevated levels of MDA were observed in group 2 throughout the experiment when compared to other groups and the mean values are given in Table 3. The present results are in accordance with (6, 15, and 35). The birds in group 4 showed significantly (P<0.05) higher values of MDA in liver and kidney on 14th day of experiment when compared to group 1 and 3. The birds in group 4 and 5 showed significant reduction in lipid peroxidation status as inferred by the reduced MDA values compared to group 2 on 28th and 42nd day of experimental study. However, marked improvement was noticed in group 4 compared to group 5 indicating pretreatment with ayurvet have improved antioxidant status of the tissue environment and ultimately decreased the peroxidative damages. In present study, significant elevation of LPO level observed in diclofenac induced hepatotoxic group is possibly due to the generation of free radicals which results superoxide catalysed oxidation process. The significant increase in activity of TBARS coupled with reduced levels of GSH in liver in diclofenac-treated groups indicates ongoing peroxidative stress and compromised antioxidant defense mechanisms.

Table 1: Hematological parameters in different groups of birds

Group	Day	HB	TEC	PCV	TLC
Group 1	14	10.88±0.15	3.21±0.09^{b}	29±0.38^{bc}	23.5±1.05^{a}
	28	11.75±0.15	4.22±0.12^{b}	30.5±0.36^{b}	23.6±0.81^{a}
	42	11.88±0.24^{bc}	5.4±0.28	30.17±0.33	27.23±0.44^{ab}
Group 2	14	10.18±0.30	2.41±0.07^{a}	26.83±0.38^{a}	29.72±1^{b}
	28	11.22±0.32	3.62±0.21^{a}	27.5±0.34^{a}	26.2±0.58^{b}
	42	11.05±0.20^{a}	4.42±0.18	27.83±0.48	30.05±0.38^{c}
Group 3	14	11.00±0.28	3.22±0.10^{b}	29.5±1.43^{c}	23.57±0.89^{a}
	28	11.97±0.17	4.5±0.1^{b}	30.5±0.36^{b}	22.8±0.72^{a}
	42	12.17±0.14^{c}	5.15±0.19	30.33±0.42	25.93±0.37^{a}
Group 4	14	10.55±0.21	2.99±0.07^{b}	27.83±0.48^{ab}	25.88±0.74^{a}
	28	11.35±0.18	4.13±0.10^{b}	28.83±0.40^{ab}	23.98±0.49^{a}
	42	11.63±0.15^{bc}	4.85±0.23	28.33±0.44	27.62±0.51^{b}
Group 5	14	10.16±0.26	2.68±0.15^{a}	27.33±0.39^{a}	29.2±0.77^{b}
	28	11.32±0.31	4.23±0.09^{b}	29±0.37^{ab}	23.95±0.46^{a}
	42	11.6±0.33^{b}	5.06±0.09	29.33±0.33	26.75±0.52^{ab}

Values are Mean + SE (n = 6) One way ANOVA

Means with different superscripts differ significantly at P<0.05

Table 2: Biochemical parameters in different groups of birds

Group	Day	AST	ALT	ALP	Uric acid	Creatinine
Group 1	14	211.09±3.73^{a}	14.68±1.85^{a}	260.29±2.54^{a}	5.99±0.32^{a}	0.52±0.01^{b}
	28	215.461±3.69^{a}	20.76±0.42	251.98±9.01^{a}	6.93±0.17^{ab}	0.58±0.05
	42	220.47±3.38^{a}	20.12 ±0.32^{a}	266.82±2.87^{a}	6.36±0.04^{a}	0.56±0.05
Group 2	14	251.38±3.53^{c}	25.43±0.56^{c}	311.05±4.08^{c}	9.95±0.39^{c}	0.73±0.03^{d}
	28	261.06±3.94^{b}	23.41±0.93	327.74±5.20^{b}	8.74±0.10^{d}	0.68±0.02
	42	248.26±3.78^{c}	25.25±0.39^{b}	295.56±4.57^{b}	8.45±0.04^{d}	0.69±0.04
Group 3	14	210.02±2.36^{a}	16.80±1.69^{a}	255.94±5.58^{a}	5.33±0.14^{a}	0.50±0.02^{a}
	28	219.59±7.01^{a}	21.77±O.37	252.91±8.62^{a}	6.37±0.32^{a}	0.53±0.03
	42	216.64±3.25^{a}	19.21±0.65^{a}	263.71±4.65^{a}	6.57±0.04^{b}	0.54±0.04
Group 4	14	239.88±4.70^{b}	20.34±1.84^{bc}	290.38±2.30^{b}	7.21±0.07^{b}	0.65±0.04^{c}
	28	227.55±11.97^{a}	20.93±1.47	269.75±4.18^{a}	7.22±0.30^{bc}	0.61±0.04
	42	229.72±2.53^{b}	21.44±0.38a^{b}	269.75±4.18^{a}	6.79±0.08^{c}	0.61±0.01
Group 5	14	245.71±3.16b^{c}	23.95±0.51^{c}	312.79±5.59^{c}	9.61±0.22^{c}	0.76±0.02^{d}
	28	233.44±9.41^{a}	21.91±0.61	271.79±20.84^{a}	7.84±0.14^{c}	0.62±0.05
	42	231.58±2.34^{b}	19.34±1.81^{a}	272.93±2.02^{a}	6.90±0.02^{c}	0.61±0.04

Values are Mean + SE (n = 6) One way ANOVA

Means with different superscripts differ significantly at P<0.05

Table 3: Antioxidant profile in different groups of birds

Group	Day	GSH Liver	GSH Kidney	Tbars Liver	Tbars Kidney
Group 1	14	0.22±0.00^{c}	0.23±0.00^{ab}	6.43±0.06^{a}	6.32±0.13^{a}
	28	0.27±0.01^{b}	0.25±0.00^{ab}	7.20±0.10^{a}	8.34±0.30^{a}
	42	0.28±0.00^{b}	0.28±0.01^{b}	7.47±0.02	5.69±0.14^{a}
Group 2	14	0.19±0.00^{ab}	0.20±0.00^{a}	10.02±0.09^{b}	10.24±0.12^{d}
	28	0.21±0.00^{a}	0.22±0.00^{a}	9.57±0.08^{d}	9.14±0.09^{c}
	42	0.25±0.00^{a}	0.26±0.00^{a}	8.43±0.03	9.03±0.15^{b}
Group 3	14	0.24±0.01^{d}	0.23±0.01^{c}	6.27±0.06^{a}	7.20±0.10^{b}
	28	0.29±0.01^{b}	0.26±0.01^{c}	7.18±0.09^{a}	8.29±0.09^{a}
	42	0.32±0.01^{c}	0.30±0.01^{c}	7.57±0.07	5.65±0.09^{a}
Group 4	14	0.20±0.00^{b}	0.22±0.00^{b}	8.35±0.06^{c}	9.57±0.09^{c}
	28	0.28±0.00^{b}	0.25±0.00^{ab}	7.53±0.05^{b}	8.41±0.33^{a}
	42	0.32±0.01^{c}	0.30±0.00^{c}	7.55±0.05	5.88±0.11^{a}
Group 5	14	0.19±0.00^{a}	0.19±0.00^{a}	10.19±0.09^{b}	10.18±0.12^{d}
	28	0.27±0.00^{b}	0.24±0.00^{b}	8.19±0.06^{c}	8.61±0.10^{ab}
	42	0.32±0.01^{c}	0.29±0.00^{c}	7.61±0.06	5.99±0.09^{a}

Values are Mean + SE (n = 6) One way ANOVA
Means with different superscripts differ significantly at P<0.05

Conclusion

In conclusion this study shows that diclofenac caused haematological alterations like reduced TEC, Hb and PCV and leukocytosis and also altered the biochemical parameters like AST, ALT, ALP and anti-oxidant levels and produced severe pathological changes indicating liver and kidney damage. Supplementation of ayurvet product improved the performance, heamato biochemical parameters 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 curcumin and sunthi well known antioxidants might contributed to recovery of gout induced birds.

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