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Disease modifying activity of methanolic extract of *Colchicum luteum* against experimental gout in broiler chicken

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Colchicum luteum, a Himalayan herb has been found associated with immense pharmaceutical properties. The present study was aimed to study the protective effect of methanolic extract of *C. luteum* against the haemato-biochemical alterations due to sodium bicarbonate induced gout in broiler chicken. A total of 72 day old broiler chicks of average body weights 178±20.8 g were divided into 6 groups (I to VI, n=12). Group I served as vehicle-treated control and was given drinking water by oral gavage. Group II and III were given sodium bicarbonate @ 2.5 and 5% respectively in drinking water. Group IV and V were provided with 2.5 and 5% sodium bicarbonate along with *C. luteum* extract @ 50 mg/kg body weight respectively. Group VI served as treatment control and was given *C. luteum* extract @ 50 mg/kg body weight alone. Haematological and biochemical analysis revealed a significant increase in haematological parameters (Hb, PCV, TEC, TLC, and heterophil) and biochemical parameters (AST, ALT, uric acid, BUN, creatinine, total protein and albumin) in sodium bicarbonate intoxicated groups. All these parameters however, were comparatively reduced in the birds given *C. luteum* extract. Also, heterophil and monocyte counts were decreased significantly in group VI birds, supplemented with *C. luteum* extract only with no adverse effect on health of birds. The results from the present study establish the protective role of *C. luteum* extract against sodium bicarbonate induced haematological and biochemical alterations.

Keywords: Biochemical analysis, *Colchicum luteum*, Gout, Haematology, Sodium bicarbonate.

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Introduction

In birds, uric acid is end product of both protein and purine metabolism and represents most of the nitrogen excreted[1]. The uricotelic nature of birds, although on one hand is evolutionary advantage providing water conservation, yet on other hand, it predisposes them to gout, which is characterized by hyperuricemia and deposition of calcium-sodium urate crystals in several sites especially kidneys and viscera[2,3]. The combined effects of increased dietary calcium and high protein concentrations on induction of visceral gout in growing birds of layer strain have been established experimentally[4]. Sodium bicarbonate which is commonly used in broilers to combat heat stress and in layer bird diets to improve egg shell quality has also been found to cause visceral gout if provided in excess[5,6]. In Gout, high levels of uric acid accumulates in the blood (hyperuricaemia) leading to urate deposition on the surfaces of various visceral organs or joints particularly hock joint, causing high number of morbidity and mortality in birds[7,8].

Several drugs have been used for the prevention and treatment of gout with marked efficacy. Allopurinol which is commonly used in the treatment of gout in human beings has also been used in poultry and has shown protective effect on diclofenac induced toxicity in domestic chicken[9]. However, herbal and natural products are preferred nowadays because of fewer side effects associated with them. One of the important medicinal plants is *Colchicum luteum*, known as Yellow colchicum in English, Suranjanshirin or Suranjantalkh in Urdu, *Suranjan* in Hindi and *Virkim-posh* in Kashmiri.

Botanical classification of *Colchicum luteum* is Kingdom-Plantae, Division-magnoliophyta, Class-magnoliosida, Family-Liliaceae, Genus-Colchicum and Species-luteum. During spring season, the yellow flowers of *C. luteum* are earliest to blossom in Kashmir. Corms and seeds of this plant have been used by practitioners of traditional medicine for treatment of gout, rheumatism and diseases of liver and spleen[10]. Corms are ovoid, oblong, and flattened

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at base with longitudinal groove on one side. In view of the aforementioned facts, the present study was aimed to investigate the anti-gout potential of *C. luteum* extract against sodium bicarbonate induced gout in broiler chicken.

Materials and Methods

The study has been conducted in the Division of Veterinary Pathology, Faculty of Veterinary Sciences and Animal Husbandry from March 2017 to Jan 2018.

Plant sample collection and identification

Corms of *C. luteum* were procured from Gousia Unani medicines, Budgam Kashmir. The sample was identified by the Department of Unani Medicine, University of Kashmir and whole material was utilized in the study.

Preparation of methanolic extract of *C. luteum*

Corms of *C. luteum* were firstly washed with water and then dried under shade for several days to avoid photo-deterioration. After drying, the corms were chopped and coarsely powdered in a grinder. Air-dried and coarsely powdered material was subjected to methanol extraction for 48 hours in a percolator. The methanolic extract thus obtained was concentrated under reduced pressure using a rotary evaporator to give crude extract.

Experimental birds

A total of 72 day old broiler chicks of FB Ross breed were procured from commercial hatchery. Prior to the procurement of chicks, the room for rearing birds was thoroughly cleaned and every part of the room was fumigated with potassium permanganate and formalin (20 mL formalin ± 14 g potassium permanganate per cubic metre). On the day of procurement, the chicks were given electrol and antibiotic Cipro-TZ @ 1 g/2 L of water and then the chicks received starter mash and normal drinking water. The broiler chicks were kept in the same compartment for 7 days and brooding temperature was correctly maintained. The experiment was carried out in accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the study was approved by the institutional animal ethics committee of F.V.Sc & A.H, SKUAST-Kashmir under no. AU/ FVS/PS-57/6527.

Experimental design

After brooding for 7 days, the birds of average body weight of 178±20.8 g were randomly divided into 6 groups with 12 birds in each group. Group I served as vehicle-treated control and was given drinking water. Group II and III were given sodium Bicarbonate @ 2.5 and 5% respectively in drinking water. Group IV and V were provided with 2.5 and 5% sodium bicarbonate along with oral administration of *C. luteum* extract @ 50 mg/kg body weight (BW) respectively. Group VI served as treatment control and was provided with oral administration of *C. luteum* extract @ 50 mg/kg BW alone. The dose of *C. luteum* extract was selected in accordance with earlier reports[11].

Collection of blood

The blood was collected from the wing vein in all the birds at different intervals (1st, 2nd, and 3rd week post exposure) and was divided into 2 parts. One part was used for haematological studies and other for biochemical studies.

Haematological studies

The blood for haematological studies was collected in vials containing disodium salt of ethylene-diamine-tetra-acetic acid (EDTA) @ 2 mg/mL of blood as an anticoagulant and the following haematological parameters were determined using automatic multispecies haematology analyzer like haemoglobin (Hb), packed cell volume (PCV), total erythrocytic count (TEC), total leucocyte count (TLC) and differential leucocytic count (DLC).

Biochemical studies

Blood for biochemical studies was collected from all the birds in the clot activator vials and then centrifuged at 3000 rpm for 15 min. The serum was collected, stored at -20 °C and analyzed to determine the biochemical parameters by semiautomatic analyzer using commercially available diagnostic kits from Erba Mannheim. Liver function tests like aspartate transferase (AST) - DNPH colourimetric method, alanine transaminase (ALT) - DNPH colourimetric method, total serum protein -biuret method and albumin-BCG dye binding method[12] and kidney function tests like uric acid -quinoneimine dye method, blood urea nitrogen (BUN) –kinetic enzymatic method and creatinine -alkaline picrate method[13] were done.

Statistical analysis

The data from individual groups are presented as mean±standard errors. Differences between groups were analyzed by using one way ANOVA (with significant levels $P \leq 0.05$) followed by Duncan's test with appropriate software like SPSS 20.0.

Results

Haematological studies

The mean values of Hb, PCV, TEC, and TLC of different groups at different intervals was expressed as mean±S.E. as given in Table 1. Birds of group II, III, IV, and V revealed significant increase in Hb concentration and PCV compared to group I and group VI birds at different intervals. Also, the increase in Hb concentration was significantly higher ($P \leq 0.05$) at 3rd week post exposure as compared with corresponding values at 1st and 2nd week in group III and V birds. However, there was significant decrease in the Hb concentration and PCV of group IV and group V compared to group II and III birds, respectively. Also, TEC values were significantly higher ($P \leq 0.05$) in birds belonging to group II and III compared to other groups throughout the experimental period. Lowest TEC was recorded in birds of group VI. Moreover, TEC was significantly higher ($P \leq 0.05$) in group IV and V at 1st week post exposure compared to 3rd week. In case of TLC, the values showed gradual increase in group II and III and were significantly higher ($P \leq 0.05$) towards the end of experiment when compared with values at 1st week post exposure. However, when the values of group IV and V were compared to the corresponding values of group II and III they were significantly decreased. Also, TLC in group VI was significantly decreased when compared with the corresponding values of group I at various intervals.

The mean values of DLC (heterophil, lymphocyte, monocyte, basophil, and eosinophil) of different groups at different intervals were expressed as mean±S.E. as given in Table 2. DLC in this study revealed significant increase ($P \leq 0.05$) in heterophil percentage while decrease in lymphocyte percentage in sodium bicarbonate intoxicated group II and III throughout the experimental period compared control group and *C. luteum* supplemented group IV, V, and VI. Moreover, monocyte, basophil, and eosinophil showed non-significant difference between different treatment groups at different intervals. However, the values of monocyte count of group VI were significantly lower ($P \leq 0.05$) when compared to corresponding values of group I throughout the experimental period.

Biochemical studies

Liver function test

The mean values of AST, ALT, albumin, and total protein of different groups at different intervals was expressed as mean±S.E. as given in Table 3.

AST

Throughout the experimental period, the overall mean AST value was significantly higher ($P \leq 0.05$) in birds of group II and III when compared with birds

Table 1 — Effect of treatment of *C. luteum* on Hb, PCV, TEC and TLC in sodium bicarbonate induced experimental gout in broilers

		Hb (g/dL)	PCV (%)	TEC (10^6/μL)	TLC (10^3/μL)
WEEK 1	Group I	10.17 ± 0.13^{aA}	26.80 ± 0.12^{aA}	3.49 ± 0.03^{aC}	29.97 ± 0.43^{aB}
	Group II	13.40 ± 0.09^{aC}	41.95 ± 0.75^{aC}	4.26 ± 0.01^{aD}	39.07 ± 0.82^{aD}
	Group III	14.90 ± 0.12^{aD}	48.37 ± 0.16^{aE}	4.32 ± 0.03^{aD}	45.42 ± 0.81^{aE}
	Group IV	12.02 ± 0.22^{aB}	36.42 ± 0.65^{aB}	3.31 ± 0.01^{cB}	34.92 ± 0.50^{aC}
	Group V	13.10 ± 0.20^{aC}	43.72 ± 0.43^{aD}	3.30 ± 0.02^{bB}	38.22 ± 0.15^{aD}
	Group VI	10.00 ± 0.14^{aA}	26.72 ± 0.11^{aA}	3.16 ± 0.02^{aA}	24.25 ± 0.45^{bA}
WEEK 2	Group I	10.17 ± 0.11^{aA}	26.57 ± 0.14^{aA}	3.49 ± 0.03^{aB}	30.22 ± 0.50^{aB}
	Group II	13.80 ± 0.08^{aC}	44.32 ± 0.89^{bC}	4.34 ± 0.01^{bC}	42.62 ± 0.99^{bE}
	Group III	14.90 ± 0.12^{aD}	50.00 ± 0.27^{bD}	4.42 ± 0.01^{bD}	47.60 ± 0.26^{bF}
	Group IV	12.05 ± 0.18^{aB}	36.90 ± 0.41^{aB}	3.21 ± 0.01^{bA}	36.15 ± 0.25^{abC}
	Group V	13.62 ± 0.25^{aC}	43.40 ± 0.09^{aC}	3.28 ± 0.02^{bA}	38.77 ± 0.17^{aD}
	Group VI	10.15 ± 0.21^{aA}	26.85 ± 0.16^{aA}	3.22 ± 0.03^{bA}	21.45 ± 0.51^{aA}
WEEK 3	Group I	10.37 ± 0.17^{aA}	26.60 ± 0.12^{aA}	3.49 ± 0.03^{aB}	30.25 ± 0.44^{aB}
	Group II	13.92 ± 0.14^{aC}	47.90 ± 0.14^{cD}	4.45 ± 0.01^{cC}	44.37 ± 0.46^{cE}
	Group III	16.30 ± 0.37^{bE}	55.40 ± 0.24^{cE}	4.44 ± 0.02^{bC}	48.72 ± 0.58^{bF}
	Group IV	12.07 ± 0.25^{aB}	36.25 ± 0.21^{aB}	3.09 ± 0.01^{aA}	36.50 ± 0.19^{bC}
	Group V	14.57 ± 0.19^{bD}	43.62 ± 0.25^{aC}	3.11 ± 0.00^{aA}	39.65 ± 0.31^{aD}
	Group VI	9.85 ± 0.18^{aA}	26.85 ± 0.16^{aA}	3.12 ± 0.01^{aA}	20.82 ± 0.28^{aA}

Means bearing at least one common lowercase superscript between different weeks post exposure and uppercase superscript between different groups does not differ significantly ($P \leq 0.05$)

Table 2 — Effect of treatment of *C. luteum* on DLC (%) in sodium bicarbonate induced experimental gout in broilers

		HET (%)	LYM (%)	MON (%)	BAS (%)	EOS (%)
WEEK 1	Group I	26.75±1.03^{aAB}	61.75±0.75^{aC}	8.75±0.85^{bD}	1.50±0.28^{aA}	1.25±0.47^{aA}
	Group II	32.25±0.47^{aD}	56.50±0.64^{bB}	6.75±0.25^{aBC}	1.00±0.40^{aA}	3.50±0.64^{aB}
	Group III	34.75±0.47^{aE}	52.25±0.75^{aA}	7.50±0.64^{aCD}	1.50±0.50^{aA}	4.00±0.40^{aB}
	Group IV	28.50±0.95^{aBC}	63.00±1.77^{aC}	5.75±0.85^{aABC}	0.50±0.28^{aA}	2.25±0.62^{aAB}
	Group V	29.25±0.47^{aC}	62.50±1.44^{aC}	5.25±0.62^{aAB}	0.25±0.25^{aA}	2.75±0.47^{aAB}
	Group VI	26.00±0.81^{bA}	67.00±1.08^{aD}	4.75±0.25^{aA}	0.25±0.25^{aA}	2.25±0.47^{aAB}
WEEK 2	Group I	28.25±1.18^{aB}	61.50±0.64^{aB}	6.75±0.25^{aAB}	1.00±0.00^{aA}	2.50±0.64^{aAB}
	Group II	33.50±0.28^{aC}	53.50±0.86^{aA}	7.50±0.64^{aB}	1.50±0.50^{aA}	4.00±0.40^{aB}
	Group III	35.25±0.25^{aC}	52.75±0.47^{aA}	6.75±0.25^{aAB}	1.50±0.28^{aA}	3.75±0.47^{aB}
	Group IV	29.50±0.28^{aB}	61.75±0.94^{aB}	6.00±0.91^{aAB}	0.25±0.25^{aA}	2.50±0.64^{aAB}
	Group V	28.50±0.64^{aB}	62.25±1.49^{aB}	5.75±0.47^{aAB}	0.50±0.28^{aA}	3.00±0.40^{aAB}
	Group VI	24.25±0.62^{bA}	68.25±0.75^{aC}	5.25±0.47^{aA}	0.50±0.50^{aA}	1.75±0.47^{aA}
WEEK 3	Group I	27.00±1.22^{aB}	27.00±1.22^{aB}	7.50±0.64^{abBC}	1.25±0.25^{aAB}	2.75±0.47^{aAB}
	Group II	33.25±0.47^{aD}	33.25±0.47^{aD}	6.75±0.25^{aAB}	1.00±0.57^{aAB}	3.00±0.40^{aAB}
	Group III	35.00±0.40^{aD}	35.00±0.40^{aD}	8.50±0.64^{aC}	1.75±0.25^{aB}	3.75±0.75^{aB}
	Group IV	29.50±0.28^{aC}	29.50±0.28^{aC}	5.75±0.85^{aAB}	0.75±0.47^{aAB}	3.00±0.40^{aAB}
	Group V	27.75±0.47^{aBC}	27.75±0.47^{aBC}	5.75±0.47^{aAB}	0.50±0.50^{aAB}	3.50±0.28^{aB}
	Group VI	21.25±0.62^{aA}	21.25±0.62^{aA}	5.50±0.28^{aA}	0.25± 0.25^{aA}	1.75±0.47^{aA}

Means bearing at least one common lowercase superscript between different weeks post exposure and uppercase superscript between different groups does not differ significantly ($P \leq 0.05$)

Table 3 — Effect of treatment of *C. luteum* on Liver function tests in sodium bicarbonate induced experimental gout in broilers

		AST (IU/mL)	ALT (IU/mL)	TP (g/dL)	ALB (g/dL)
WEEK 1	Group I	169.84±0.23^{aA}	11.24±0.32^{aA}	2.22±0.25^{aA}	0.49±0.00^{aA}
	Group II	194.44±6.62^{aB}	28.70±0.29^{aD}	6.41±0.03^{aC}	1.14±0.05^{aC}
	Group III	248.05±14.34^{aC}	37.80±0.71^{aE}	7.32±0.19^{aD}	2.43±0.07^{aD}
	Group IV	183.63±5.66^{bAB}	18.05±0.86^{aB}	3.73±0.34^{abB}	0.92±0.02^{aB}
	Group V	188.45±4.55^{aAB}	22.58±0.65^{aC}	4.30±0.02^{aB}	1.11±0.09^{aC}
	Group VI	169.91±0.24^{aA}	11.30±0.41^{aA}	1.96±0.22^{aA}	0.53±0.03^{aA}
WEEK 2	Group I	170.17±0.20^{aA}	11.33±0.38^{aA}	2.47±0.17^{aAB}	0.51±0.02^{aA}
	Group II	226.61±4.70^{bB}	28.98±0.27^{aD}	6.24±0.12^{aD}	1.68±0.03^{bC}
	Group III	256.40±16.20^{aC}	44.14±0.68^{bE}	7.75±0.50^{abE}	3.23±0.08^{bE}
	Group IV	181.54±3.87^{abA}	20.85±0.54^{bB}	3.07±0.08^{aB}	1.23±0.08^{bB}
	Group V	185.18±6.32^{aA}	25.20±1.80^{aC}	3.94±0.26^{aC}	2.08±0.03^{bD}
	Group VI	170.62±0.38^{aA}	11.32±0.36^{aA}	2.27±0.18^{aA}	0.49±0.06^{aA}
WEEK 3	Group I	170.22±0.19^{aA}	11.37±0.22^{aA}	2.59±0.11^{aA}	0.48±0.03^{aA}
	Group II	262.02±8.03^{cB}	37.76±2.38^{bC}	7.15±0.33^{bC}	1.69±0.03^{bC}
	Group III	292.74±3.09^{bC}	44.01±1.28^{bD}	8.24±0.44^{bD}	3.61±0.08^{cE}
	Group IV	161.42±0.97^{aA}	21.77±0.65^{bB}	4.05±0.34^{bB}	1.03±0.05^{aB}
	Group V	169.57±1.66^{aA}	35.25±1.35^{bC}	4.24±0.05^{aB}	2.17±0.06^{bD}
	Group VI	169.65± 0.39^{aA}	11.45± 0.15^{aA}	2.50± 0.11^{aA}	0.52± 0.05^{aA}

Means bearing at least one common lowercase superscript between different weeks post exposure and uppercase superscript between different groups does not differ significantly ($P \leq 0.05$)

of other groups. However, AST values of group IV and V showed significant decrease ($P \leq 0.05$) in comparison to group II and III respectively at 2nd and 3rd week post exposure.

ALT

There was significant increase ($P \leq 0.05$) in mean ALT values of group II, III, IV, and V compared to group I and VI at different intervals. Also, ALT levels were significantly reduced ($P \leq 0.05$) in group IV and group V birds when compared with values of group II and III respectively. The highest mean values of ALT were revealed by group III followed by group II, V, and IV towards the end of experiment.

Total protein

The overall mean total protein values in group II, III, IV, and V were significantly higher ($P \leq 0.05$)

when compared with group I throughout the experimental period. Also, throughout the experiment, the mean values for total protein were significantly decreased ($P \leq 0.05$) in group IV and V when compared with values of group II and III, respectively. Moreover, within the groups, mean total protein values were significantly higher ($P \leq 0.05$) in group III followed by group II, V, and IV towards the end of the experiment.

Albumin

Throughout the experimental period, the mean values for the albumin were significantly higher ($P \leq 0.05$) in group II, III, IV, and V as compared to group I and VI. However, the values were significantly decreased ($P \leq 0.05$) in group IV and V compared to group II and III, respectively. Also, within the groups, the values were significantly higher ($P \leq 0.05$) in group II, III, and V at 3rd week post exposure when compared with the corresponding values at 1st and 2nd week.

Kidney function tests

The mean values of BUN, creatinine, and uric acid of different groups at different intervals were expressed as mean±S.E. as given in Table 4.

The mean values of BUN were significantly increased ($P \leq 0.05$) in group II, III, and V compared to control group. Throughout the experiment, there was no significant difference ($P \leq 0.05$) in the values of BUN between group II and III.

The CRT values of group II, III, and V were significantly increased ($P \leq 0.05$) at all intervals when compared with the corresponding values of group I, IV, and VI. Overall mean values of serum creatinine revealed significant decrease ($P \leq 0.05$) in group IV and V birds when compared with group II and III throughout the experimental period. The values showed gradual increase in group III and V towards end of the experiment compared to values at 1st week post exposure.

At 1st, 2nd, and 3rd week post exposure, the overall mean uric acid values were significantly higher ($P \leq 0.05$) in group II, III, IV, and V when compared to the values in group I and VI. However, values were significantly lower ($P \leq 0.05$) in group IV and V when compared to values in group II and III, respectively. Moreover, within the groups there was significant increase ($P \leq 0.05$) in mean uric acid levels of group II and III at 3rd week when these values were compared with values at 1st and 2nd week, respectively.

Discussion

Birds of group II, III, IV, and V had significantly increased Hb concentration and PCV compared to group I and VI birds at different intervals. TEC values were significantly higher in group II and III compared to other groups throughout the experimental period. TLC values showed gradual increase in group II and III and were significantly higher towards the end of experiment. The increase in Hb, PCV, TEC, and TLC

Table 4 — Effect of treatment of *C. luteum* on Kidneyfunction tests in sodium bicarbonate induced experimental gout in broilers

		BUN (mg/dL)	CRT (mg/dL)	UA (mg/dL)
WEEK 1	Group I	1.27 ± 0.01^{aA}	0.24 ± 0.01^{aA}	5.49 ± 0.29^{aA}
	Group II	2.50 ± 0.03^{aC}	0.42 ± 0.01^{aB}	11.58 ± 0.43^{aC}
	Group III	2.90 ± 0.03^{aC}	0.59 ± 0.04^{aC}	12.42 ± 0.20^{aC}
	Group IV	1.74 ± 0.35^{aAB}	0.29 ± 0.01^{aA}	7.88 ± 0.25^{abB}
	Group V	1.97 ± 0.04^{aB}	0.29 ± 0.03^{aA}	8.48 ± 0.18^{aB}
	Group VI	1.32 ± 0.02^{aA}	0.25 ± 0.01^{aA}	5.98 ± 0.36^{aA}
WEEK 2	Group I	1.27 ± 0.05^{aA}	0.24 ± 0.01^{aA}	5.63 ± 0.32^{aA}
	Group II	2.29 ± 0.32^{aCD}	0.42 ± 0.02^{aB}	14.37 ± 1.01^{bC}
	Group III	2.55 ± 0.40^{aD}	0.65 ± 0.01^{bC}	16.86 ± 0.36^{bD}
	Group IV	1.58 ± 0.04^{aAB}	0.28 ± 0.01^{aA}	7.68 ± 0.12^{aB}
	Group V	1.83 ± 0.06^{aBC}	0.37 ± 0.02^{bB}	7.81 ± 0.25^{aB}
	Group VI	1.26 ± 0.03^{aA}	0.23 ± 0.01^{aA}	6.04 ± 0.35^{aA}
WEEK 3	Group I	1.31 ± 0.02^{aA}	0.25 ± 0.01^{aA}	5.88 ± 0.26^{aA}
	Group II	2.70 ± 0.03^{aC}	0.48 ± 0.02^{aC}	16.44 ± 0.38^{cC}
	Group III	3.00 ± 0.02^{aC}	0.66 ± 0.01^{bD}	20.27 ± 0.59^{cD}
	Group IV	1.62 ± 0.01^{aAB}	0.26 ± 0.01^{aA}	9.26 ± 0.50^{bB}
	Group V	1.86 ± 0.04^{aB}	0.39 ± 0.01^{bB}	10.52 ± 1.08^{bB}
	Group VI	1.29 ± 0.02^{aA}	0.23 ± 0.01^{aA}	5.83 ± 0.21^{aA}

Means bearing at least one common lowercase superscript between different weeks post exposure and uppercase superscript between different groups does not differ significantly ($P \leq 0.05$).

counts in sodium bicarbonate intoxicated groups might be attributed to increased erythropoietin activity in clinically affected birds[14]. Also, these findings are in agreement with observations of earlier workers[15,16]. However, the finding was inconsistent with others[17] where decreased haemoglobin concentration was reported due to a compromised oxygen-carrying capacity in blood. However, significant reduction in total erythrocyte count and total leucocyte count of *C. luteum* supplemented group VI compared to group I might be ascribed to mitotic inhibitory potential of colchicine and the arrest of cell division at metaphase stage of cell cycle[18].

Significant increase in heterophil percentage while decrease in lymphocyte percentage in sodium bicarbonate intoxicated group II and III throughout the experimental period compared to control group and *C. luteum* supplemented groups might be due to the fact that visceral gout developed as a result of sodium bicarbonate intoxication, is an acute inflammatory condition characterized by the presence of primary inflammatory cells like heterophils. Similar results have been reported by others[19]. However, the decrease in lymphocyte count may be attributed to depletion of lymphoid reserve of spleen as was evident from histopathological examination of spleen and many other organs. Also, significant decrease in differential leucocyte count of birds treated with *C. luteum* extract might be attributed to bone marrow aplasia and pancytopenia induced by oral administration of therapeutic dose of olchicines[20,21]. The increase in AST activity might be attributed to degeneration of cardiac muscles as was evident from gross and histopathological findings in the present study since this enzyme is present in large quantities in cardiac muscles[22]. Comparatively lower values of AST and ALT in *C. luteum* supplemented groups could be ascribed to protective effects of *C. luteum* against sodium bicarbonate toxicosis. While the increase in activity of serum ALT might be due to release of this enzyme from liver as a result of hepatocellular damage as observed in the present study. Since an increase in the activity of serum ALT is considered to be a sensitive indicator of hepatic cell damage and an alteration in permeability of hepatic cell membrane[23]. The earlier reports also showed similar pattern of increase in AST and ALT in gout affected birds[24-26]. Comparatively lower values of AST and ALT in *C. luteum* supplemented groups could be ascribed to the protective effects of *C. luteum* against sodium bicarbonate toxicosis.

The mean value of the total protein revealed significant increase in intoxicated groups compared to treatment groups. This might be attributed to severe damage of kidneys associated with adreno cortical hypofunction, leading to elevated protein concentration. While significant decrease of total protein levels in *C. luteum* treated groups is ascribed its protective effect on kidneys. The significant increase in mean value of albumin in sodium bicarbonate intoxicated groups might be attributed to the fact that gout is a disease process triggered by interactions between monosodium urate crystals and nucleating agents. Albumin has been reported as a possible–nucleating agent that promotes crystallization of monosodium urates[27]. However, the decrease in mean albumin levels in *C. luteum* treated groups may be reduction in uric acid levels and hence reduced crystallization. Increased levels of total protein and albumin in gout affected birds have also been reported by other workers[28-31].

Mean values of serum uric acid, creatinine, and blood urea nitrogen were significantly elevated in sodium bicarbonate, intoxicated groups. This might be attributed to nephrotoxic potential of sodium bicarbonate[32]. Also, high sodium levels increase blood viscosity by reducing red blood cell deformity[15] that may interfere with blood flow through glomeruli. This results in reduced urine out flow and ultimately leads to hyperuricemia and urate deposition in kidneys, resulting in severe kidney damage. Histopathological examination of kidneys showing severe kidney damage in the present study further supported these findings. Similar findings were reported by earlier researchers[33-36]. Significant reduction in serum levels of uric acid, creatinine and BUN in *C. luteum* treated groups in rats have been reported and attributed to its protective effect on kidneys[37]. Also, reduction in uric acid levels after *C. luteum* administration in rabbits has been earlier reported[38].

Conclusion

From this study, it is concluded that *C. luteum* extract has a protective role against sodium bicarbonate induced haematological and biochemical alterations. Thus, *C. luteum* extract can be used in the poultry for amelioration of naturally occurring gout alone or in combination with other herbal medicaments for which further studies are warranted.

Conflict of interest

No conflict of interest between any of the authors.

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