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Ameliorative Potential of Methanolic Extract of *Colchicum luteum* Against Oxidative Stress in Sodium Bicarbonate Induced Gout in Broiler Chicken

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ABSTRACT

Colchicum luteum is an herbal plant and has been found to possess numerous therapeutic properties. In this study, we used methanolic extract obtained from corms of Colchicum luteum against oxidative stress in sodium bicarbonate induced gout in broiler chicken model. A total of 72 day old broiler chicks were divided into 6 groups (I to VI, n12). 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 Colchicum luteum extract @ 50mg/kg body weight respectively. Group VI served as treatment control and was given Colchicum luteum extract @ 50mg/kg body weight alone. The protective effect of Colchicum luteum extract (CLE) against oxidative stress was investigated by assaying lipid peroxidation, Catalase, Superoxide dismutase and Xanthine oxidase. Intoxicated groups revealed oxidative stress which included increased levels of LPO and XO and decreased activity of CAT and SOD. All these parameters however, showed normal activity in the birds given Colchicum luteum extract. The data from the present study establish the protective role of CLE against Sodium bicarbonate induced oxidative damage.

Key words: *Gout, Oxidative stress, Sodium Bicarbonate, Colchicum luteum extract*

INTRODUCTION

Gout is a true crystal deposition disease in which the clinical symptoms are caused by the formation of monosodium urate (MSU) crystals in soft tissues and joints. Uric acid is a weak acid (pKa, 5.8) that exists largely as urate, the ionized form, at physiologic pH. Urate combines with sodium ions to form MSU. As urate and sodium concentration increases in physiologic fluids, the risk for super saturation and crystal formation generally increases.

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Gout is common in birds, some reptiles and man as they lack the ability to produce the enzyme Uricase which converts uric acid (relatively insoluble) to allantoin (highly soluble). Here, the Uricase gene is crippled by 2 mutations that introduce premature stop codons Wu *et al.*[1] The amount of urate in the body depends on the balance between dietary intake, synthesis and the rate of excretion. Hyperuricemia results from urate over production (10%), under excretion (90%), or often a combination of the two. Uric acid is recognized as a marker of oxidative stress. Production of the uric acid induces enzyme Xanthine oxidase and mediates activation of NADPH-oxidase, thereby causes the production of highly reactive superoxide anions by neutrophils Martin-Ventura *et al.*[2] As a by-product ROS have a significant role in the oxidative stress and might be involved in the tissue damage.

Colchicum luteum (Liliaceae family) is a perennial herb found in Himalayas and is widely distributed in China, India, Pakistan and Afghanistan. It has also been reported in hilly areas of Kashmir valley like Bandipora Lone *et al.*[3] Tragbal, Tangmarg and other areas. The herb is known by many names like Yellow colchicum in English, Suranjan shirin or Suranjan talkh in Urdu, Suranjan in Hindi and Virkim-posh in Kashmiri. The Yellow flowers of *Colchicum luteum* are earliest to blossom in Kashmir during spring season. Corms are ovoid, oblong and flattened at base with longitudinal groove on one side (Plate1). Phytochemical investigation of the *Colchicum luteum* have shown the presence of Lumicolchicine, Chlorogenic acid, Colchicines and 3', 4', 5, 7-Tetrahydroxyflavone and is known for its promising antioxidant activity Bashir Ahmad[4]. However, colchicines were the main alkaloids reported Ondra *et al.*[5] The isolation of colchicines from *Colchicum luteum* for the first time was credited to P.S. Pelletier and J. Caventox in the year 1820 Kokate *et al.*[6] and Evans[7]. Approximately 0.25% and 0.45% colchicine has been reported in corms and seeds respectively Chopra *et al.*[8] Based on this information, we designed the present study to investigate the protective effects of methanolic extract of *Colchicum luteum* against Sodium bicarbonate induced oxidative damage in broiler chicken.

MATERIAL AND METHODS

Animals: 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 a potassium permanganate and formalin (20 ml formalin ± 14 g potassium permanganate per cu m³). On the day of procurement the chicks were given electrol and antibiotic Cipro-TZ @ 1g/2 litres 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.

Preparation of methanolic extract of *Colchicum luteum*: Corms of *Colchicum luteum* were brought from Gousia Unani medicines, Budgam Kashmir and 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 design: After brooding for 7 days, the birds 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 *Colchicum luteum* extract @ 50mg/kg body weight respectively. Group VI served as treatment control and was provided with oral administration of *Colchicum luteum* extract @ 50mg/kg body weight alone. The birds were sacrificed at the end of 1st, 2nd and 3rd week post exposure. The dose of *Colchicum luteum* extract was selected in accordance with the report of Nair *et al*.[9].

Post-mitochondrial supernatant

Preparation: Liver of birds were removed readily at time of slaughter, cleaned free of debris and irrelevant material and immediately perfused with ice cold saline (0.85% NaCl). The liver samples were homogenized in chilled phosphate buffer (0.1M, pH 7.4) using a tissue homogenizer. The homogenates were filtered through muslin cloth, and centrifuged at 3000 rpm for 10 min at 4^0C in Remi cooling centrifuge to separate the nuclear debris. The aliquots so obtained were centrifuged at 12,000 rpm for 20 min at 4^0C to obtain post mitochondrial supernatant (PMS), which was used as a source of various enzymes.

Assay for Catalase activity: The Catalase activity was assessed by the method of Claiborne[10]. In brief, the assay mixture consisted of 2·0 ml phosphate buffer (0·1 M, pH 7·4), 0·95 ml H_2O_2 (0·019 M) and 0·05 ml of Post mitochondrial supernatant (PMS) (10 %) in a final volume of 3·0 ml. Changes in absorbance were recorded at **240 nm**. The CAT activity was calculated in terms of nmol H_2O_2 consumed/min per mg protein.

Measurement of Superoxide dismutase activity: The SOD activity was measured by the method of Marklund and Marklund[11]. The reaction mixture consisted of 2·875 ml Tris–HCl buffer (50mM, pH 8·5), pyrogallol (24mM in 10mM HCl) and 100 µl PMS in a total volume of 3 ml. The enzyme activity was measured at **420nm** and was expressed as units/mg protein. Here, one unit of enzyme is defined as the enzyme activity that inhibits the auto-oxidation of pyrogallol by 50 %.

Measurement of Xanthine Oxidase activity: The activity of Xanthine oxidase (XO) was assayed by the method of Stirpe & Della Corte[12]. The reaction mixture consisted of 0·2ml PMS which was incubated for 5 min at 37^0C with 0·8ml phosphate buffer (0·1M, pH 7·4). The reaction was started by adding 0·1ml Xanthine (9mM) and kept at 37^0C for 20 min. The reaction was terminated by the addition of 0·5ml ice-cold perchloric acid (10% (v/v)). After 10 min, 2.4ml of distilled water were added and centrifuged at 4000 rpm for 10 min and mg uric acid formed per min per mg protein was recorded at **290 nm**.

Estimation of protein: Protein content in liver homogenate was determined by the method of Lowry *et al*.[13], using bovine serum albumin as a standard.

Estimation of lipid peroxidation: The assay for membrane lipid peroxidation (LPO) was done by the method of Wright *et al*.[14]. The reaction mixture in a total volume of 3·0ml contained 1·0 ml tissue homogenate, 1·0 ml of TCA (10 %) and 1·0 ml thiobarbituric acid (0·67 %). All the test tubes were placed in a boiling-water bath for a period of 45 min. The tubes were then shifted to an ice-bath and centrifuged at 2500 g for 10 min. The amount of Malonaldehyde (MDA) formed in each of the samples was assessed by measuring the optical density of the supernatant at **532 nm**. The results were expressed as the nmol MDA formed/g tissue by using a molar extinction coefficient of $1.56\times10^5\ M^{-1}\ cm^{-1}$.

Statistical analysis: The data from individual groups are presented as means with their standard errors. Differences between groups were analyzed by using ANOVA followed by the Tukey–Kramer multiple comparison test. The minimum criterion for statistical significance was set at $P < 0{\cdot}05$ for all comparisons.

RESULT AND DISCUSSION

In the present study we have seen the protective effect of *Colchicum luteum* extract against oxidative stress in sodium bicarbonate induced gout in broiler chicken. Throughout the experimental period the Catalase activity and Superoxide dismutase activity was significantly reduced in Sodium bicarbonate intoxicated group II and III. However, the

activity was restored in *Colchicum luteum* extract supplemented group IV and V. Also, the Catalase activity revealed significant decrease in group III but significant increase in group IV and V birds at 2nd and 3rd week of exposure compared to 1st week. The values of Catalase varied from 180.6 ± 31.64 to 204 ± 29.37 in group II, 129.3 ± 26.44 to 152.6 ± 27.37 in group III, 309 ± 29.49 to 374.6 ± 48.63 in group IV and 304 ± 44.27 to 338.3 ± 46.34 in group V (Table 1, Fig. 1) and the values of SOD varied from 121.6 ± 4.47 to 135.6 ± 5.09 in group II, 116.6 ± 5.76 to 122.6 ± 6.18 in group III, 151.0 ± 5.22 to 159.0 ± 4.76 in group IV and 144.7 ± 5.62 to 150.7 ± 5.73 in group V. Also, there was no significant change in SOD activity between group II and III (Table 1, Fig. 2).

Since, SOD and CAT are the most important enzymes that prevent oxidative damage Finkel and Holbrook, 2000[15]. Their decreased activity in intoxicated groups might be attributed to high levels of uric acid which mediates activation of NADPH-oxidase and causes the production of highly reactive superoxide anions by neutrophils Martin-Ventura *et al.*[2]. While restored levels may be due to promising antioxidant or free radical scavenging activity of crude *Colchicum luteum* extract as reported by Bashir Ahmad[4].

The XO activity was significantly increased in Sodium bicarbonate intoxicated group II and III compared to control group. However, XO activity was significantly decreased in *Colchicum luteum* extract treated group IV and V when compared with group II and III respectively. Xanthine Oxidase activity was expressed in terms of µg of uric acid/min/mg protein. Mean values varied from 0.530 ± 0.02 to 0.549 ± 0.02 in group II, 0.582 ± 0.01 to 0.593 ± 0.01 in group III, 0.492 ± 0.02 to 0.505 ± 0.02 in group IV and 0.441 ± 0.01 to 0.460 ± 0.01 in group V (Table 1, Fig. 3).

Since, Xanthine oxidase is superoxide producing enzyme, the increase in its activity in intoxicated groups might be in response to oxidative stress caused by hyperuricemia. However, the decreased activity in *Colchicum luteum* extract supplemented groups might be attributed to antioxidant activity of the extract Bashir Ahmad[4], reduction in uric acid levels Mohammad *et al.*[16] or inhibition of Xanthine Oxidase.

Throughout the experiment, the level of Malonaldehyde (MDA) was significantly increased in sodium bicarbonate intoxicated group II and III compared to control group. While treatment with *Colchicum luteum* extract (50mg/kg Bwt) significantly normalized the level of Malonaldehyde in group IV and V when compared with group II and III respectively. Lipid Peroxidation is expressed as nmoles MDA formed/ g tissue. Highest Malonaldehyde level was revealed by group III (4.10±0.26 at 1st week, 4.52±0.276 at 2nd week, 5.41±0.43 at 3rd week). There was no significant difference in Malonaldehyde levels in Group VI as compared to Group I (Table 1, Fig. 4).

An elevated level of Malondialdehyde (MDA), a lipid peroxidation product, in Sodium bicarbonate intoxicated groups might be attributed to oxidative damage of liver by uric acid crystals. Similar finding have been reported by Peng *et al.*[17]. However, normalization of MDA levels in *Colchicum luteum* extract treated groups might be attributed to significant enzyme inhibition activity of the crude methanolic extract and its fractions against Lipoxygenase Bashir *et al.*[18].

CONCLUSION

The results of the present study demonstrate that oxidative stress is closely associated with Sodium bicarbonate induced toxicity and methanolic extract of *Colchicum luteum* shows the protective efficacy against Sodium bicarbonate induced toxicity, possibly via attenuating the oxidative stress. However, there is a further need for molecular studies in this regard before it can be taken for clinical trials.

Table 1: Effect of Treatment of *Colchicum luteum* on lipid peroxidation and antioxidant enzyme activity in Sodium Bicarbonate induced experimental gout in broiler

		LPO (nmoles MDA formed/mg Tissue)	Catalase (nmoles H_2O_2 Consumed/min/mg protein)	SOD (U/mg)	XO (µg of Uric acid/min/mg protein)
WEEK 1	*Group I*	1.06±0.15	424.8±48.96	170.8±3.58	0.410±0.03
	Group II	3.43±0.86**	180.6±31.64**	121.6±4.47***	0.549±0.02**
	Group III	4.10±0.26***	152.6±27.37***	116.6±5.76***	0.593±0.01***
	Group IV	1.24±0.23$^{\#\#}$	309.9±29.49$^{\#\#}$	151±5.22$^{\#}$	0.505±0.02$^{\#\#}$
	Group V	1.46±0.11$^{\#\#}$	304.1±44.27$^{\#}$	144.7±5.62$^{\#\#}$	0.460±0.01$^{\#\#}$
	Group VI	0.97±0.13	424.8±50.37	171.5±6.78	0.412±0.02
WEEK 2	*Group I*	1.03±0.15	414.2±52.16	180.8±3.58	0.401±0.03
	Group II	3.90±0.776***	180.6±31.68***	135.6±5.09***	0.530±0.02***
	Group III	4.52±0.276***	141.0±24.27***	122.6±6.18***	0.589±0.01***
	Group IV	1.35±0.16$^{\#\#\#}$	369.8±41.14$^{\#}$	159.0±4.76$^{\#}$	0.499±0.02$^{\#\#\#}$
	Group V	1.62±0.13$^{\#\#\#}$	338.3±46.34$^{\#}$	150.7±5.73$^{\#\#}$	0.459±0.01$^{\#\#\#}$
	Group VI	0.90±0.10	408.9±46.44	177.5±3.32	0.412±0.02
WEEK 3	*Group I*	1.02±0.13	403.6±59.6	174.8±5.49	0.396±0.03
	Group II	4.52±0.72***	204±29.37**	131.6±3.49***	0.543±0.02***
	Group III	5.41±0.43***	129.3±26.44***	120.6±5.80***	0.582±0.01***
	Group IV	1.93±0.48$^{\#\#}$	374.6±48.63$^{\#}$	153.0±3.02$^{\#}$	0.492±0.02$^{\#\#}$
	Group V	2.06±0.43$^{\#\#\#}$	333.5±45.37$^{\#}$	144.7±6.62$^{\#}$	0.441±0.01$^{\#\#\#}$
	Group VI	0.90±0.10	414.2±41.64	171.5±2.90	0.403±0.02

Results represent mean ± SE of birds per group per week. Results obtained are significantly different from Control group (**P < 0.01) and (***P < 0.001) and are also significantly different from Sodium Bicarbonate intoxicated groups ($^{\#}$P < 0.05), ($^{\#\#}$P < 0.01) and ($^{\#\#\#}$P < 0.001). Group I: Normal Control, Group II: Sodium Bicarbonate (2.5 %), Group III: Sodium Bicarbonate (5 %), Group IV: Sodium Bicarbonate (2.5 %) + *Colchicum luteum* (50 mg/kg Bwt), Group V: Sodium Bicarbonate (5 %) + *Colchicum luteum* (50 mg/kg Bwt), Group VI: *Colchicum luteum* (50 mg/kg Bwt).

Plate 1: Corms of *Colchicum luteum*

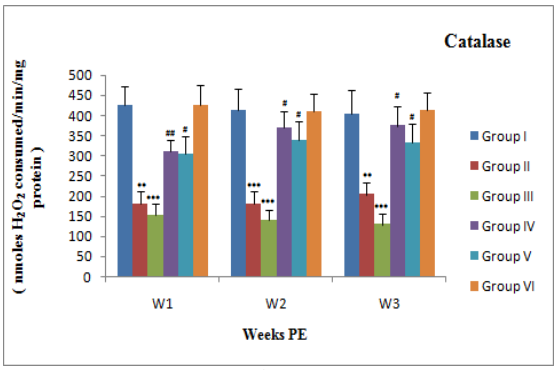

Figure 1

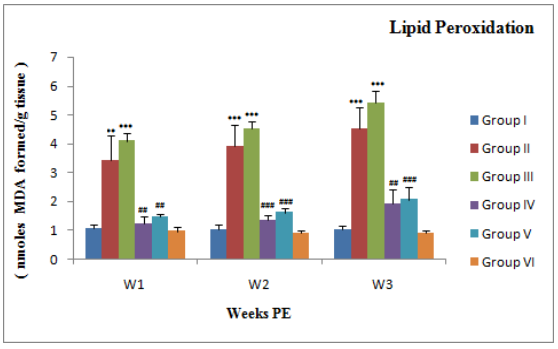

Figure 2

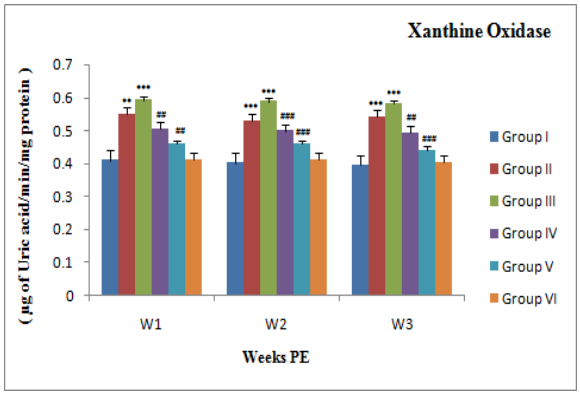

Figure 3

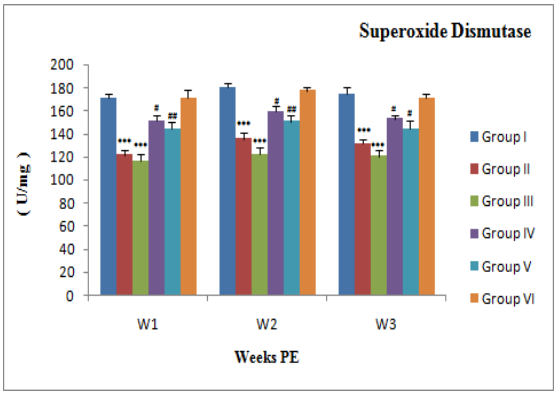

Figure 4

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