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Evaluation Of Antioxidant Activity Of Aerial Part Extract Of Coleus spicatus. Benth On Chromium(VI) Induced Oxidative Stress In Albino Rats

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Abstract: Chromium(VI) is a strong oxidant which causes cytotoxicity through oxidative stress in tissue systems. The present evaluation reports the antioxidant activity of ethanolic extract of aerial parts of *Coleus spicatus*. Benth, family labiatae, on chromium induced oxidative stress in male albino wistar rats. Oxidative stress was induced in the rats by force-feeding of potassium dichromate equivalent to a dose of 30mg/kg body weight of chromium(VI) for 30 days. The antioxidant effect of the extract were studied through lipid peroxidation assay(LPO), superoxide dismutase activity(SOD), catalase activity (CAT), estimation of reduced glutathione(GSH), determination of AST and ALT. Difference doses of the ethanolic extract of aerial part of *Coleus spicatus* were evaluated for the protection against the chromium induced oxidative stress. The report shows that the extract at a concentration of 200mg/kg and 400mg/kg body weight protected animals from the chromium induced oxidative injury significantly.
Key words: Antioxidants; *Coleus spicatus*; Chromium; Oxidative stress.

1.Introduction

Chromium is a naturally occurring heavy metal found commonly in the environment in the trivalent Cr(III) and hexavalent Cr(VI) forms. It exists as oxo species such as CrO_3 and CrO_4^{2-} that are robustly oxidizing[1], and result in excessive cytotoxicity which might lead to dermal damage, renal failure, liver damage, gastro intestinal bleeding, intra vascular hemolysis, coma and even death[2]. Cr(VI) compounds have been declared as potent occupational carcinogenesis among workers in chrome plating, stainless steel and pigment industries. Cr(VI) compounds are well known oxidizing agents capable of directly inducing tissue damage and possess carcinogenic and teratogenic potency. Cr(VI) are easily taken up by the cells and are subsequently reduced to Cr(III) species. This reduction generates free radicals which play a major role in the adverse biological effects of these compounds[3].

Coleus spicatus Benth (labiatae) is an important plant in Indian system of medicine. It is a perennial fleshy herb, in arid places on rocky ground among bushes found in Salem and Coimbatore district of Tamil Nadu, India[4]. It grows upto 50cm in height with branchlets hispid in nature[5]. Traditionally this plant is used as a stimulant, treatment of cough[6], diuretic, cytotoxic, and various phytoconstituents viz coleon S and T, α – amyrin tormentic acid, flavones, kumata kinin, 3,7-dimethyl quercetin and sitosterol were isolated from aerial parts of the plant[7]. In spite of numerous medicinal uses attributed to this plant, there are no pharmacological evidences that support the use of this medicinal species yet. In an earlier study[8], we reported that the aerial part extract has a significant antioxidant activity, in vitro studies. To

confirm whether the antioxidant activity obtained from in vitro applies to in vivo also, a study has been carried out to evaluate the antioxidant activity of the aerial part extract against the chromium induced oxidative injury using wistar albino rats as a model system.

2.Methodology

2.1 Plant material

The aerial parts of *Coleus spicatus* were collected from various places of Salem district, Tamil Nadu, India and identified by Dr.V.Chelladurai, Ex. Professor Medicinal plant survey for Siddha, Government of India. Tirunelveli, Tamil Nadu, India. A voucher specimen (HS 034) was kept in the herbarium of the Department of Pharmacognosy, Ezhuthachan College of pharmaceutical sciences, Marayamuttom, Thiruvananthapuram, Kerala, India.

2.2 Animals

The study were conducted on male albino wistar rats weighing 180-220gm maintained at 20-25^{o}C ± 2^{o}C with food and water adlibitum and rats were maintained on a 12 hour light dark cycle.

2.3 Preparation of extracts

The shade dried aerial parts of *Coleus spicatus* were powdered coarsely and about 1kg of this powder was macerated with ethanol 70%(v/v) during 72h. The obtained extract was filtered and concentrated in a rotary evaporator under reduced pressure. The yield was 26.8 gm w/w.

2.4 chromatographic studies

TLC and HPTLC studies were carried out with EECS prescribed the standard methods. A Camag automatic TLC sampler 4 (ATS 4), a camag TLC scanner 3 and Win CATS-4 software for interpretation of data were used for the HPTLC studies. Aluminum plates (10×10cm) precoated with silicagel 60F_{254} (E Merck) as an absorbent were used. All the solvents were used. All the solvents used were of HPLC grade obtained from MERCK. The plates were developed using Toluene : Ethyl acetate : Formic acid : Methanol (30 : 30 : 7.5 : 7.5) after 12 h saturation.

2.5 Treatment

The normal control group was maintained on saline in a dose of 10ml/kg(G_1). The toxic control group was administered Chromium(VI) 30mg/kg for 30 days(G_2), and standard group was administered silymarin in a dose of 75mg/kg orally(G_3). The treatment control groups(G_4 and G_5) was administered EECS orally in dose of 200mg/kg and 400mg/kg respectively. Groups G_3, G_4 and G_5 was given two doses of EECS one hour prior to the administration of Chromium(VI).

2.6 Oxidative stress

Oxidative stress was induced for all rats except normal control group by force-feeding of 1ml potassium dichromate equivalent to a dose of 30mg/kg body weight of Chromium(VI) for 30 days. Food and water intake by the animals was monitored daily and body weight was measured weekly. The animals were sacrificed by euthanasia method and weight of various organs of the body was determined.

2.7 Determination of biochemical parameters

Superoxide dismutase (SOD) was estimated by the method of Kono.Y[9]. Lipid peroxidation assay (LPO) was determined by the method of Ohkawa et al.[10]. Reduced glutathione (GSH) was determined in the blood by method of Ellman[11] and catalase activity (CAT) was determined by the method of Luck[12]. Alanine amino transferase (ALT) and aspartate amino transferase (AST) from the liver in the blood serum was assayed by the method of Reitman et al[13].

2.8 Statistical analysis

The results expressed as mean ±SEM. The evaluation of the data was done using oneway ANOVA followed by Newman Keuls multiple range tests. Probability values less than ($P < 0.01$) were considered significant.

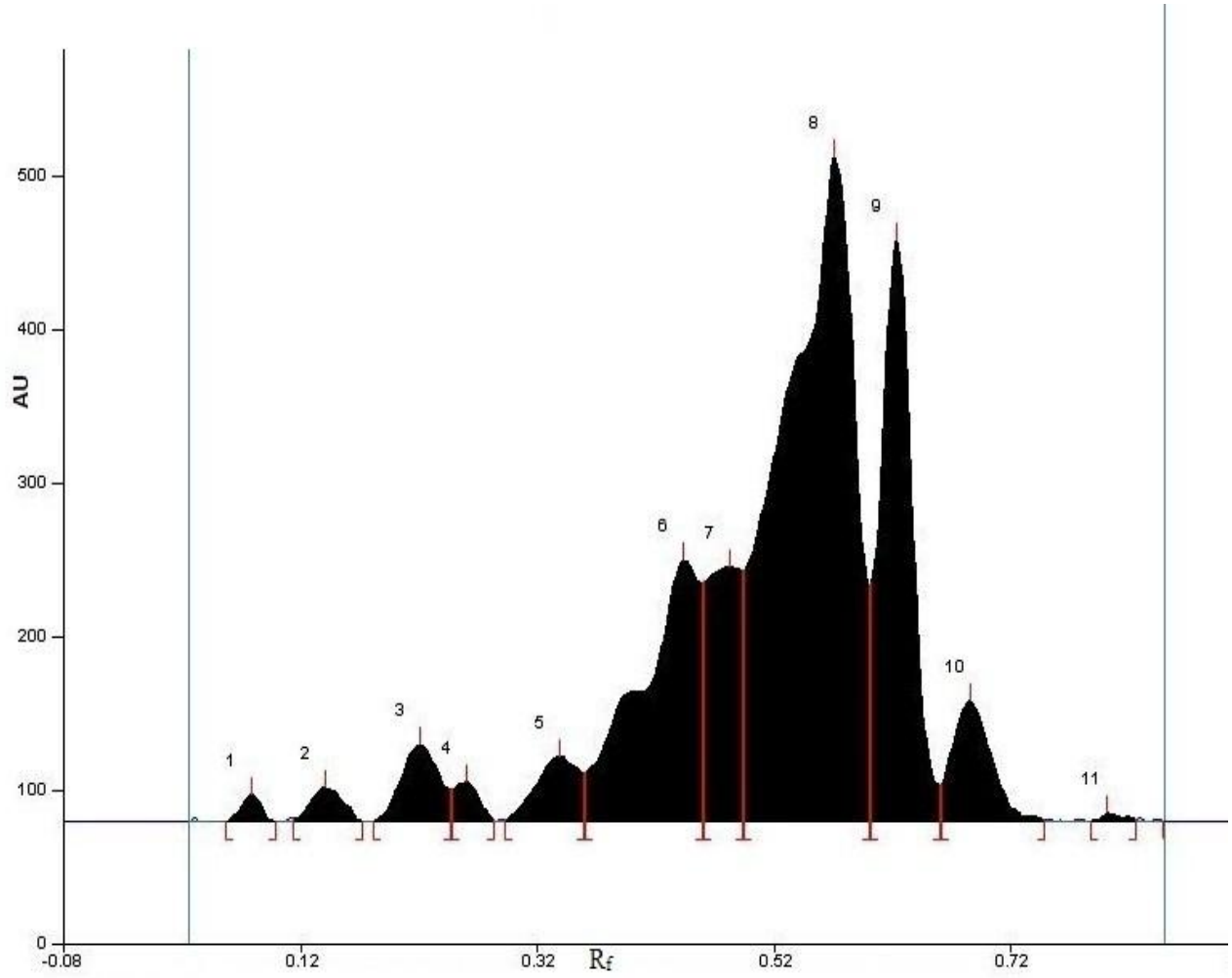
3. Results

3.1 Chromatographic studies

The HPTLC profile of ethanolic extract of *Coleus spicatus* was determined. Viewed under light at 366nm, the EECS contained 11 phytocontituents at R_f values of 0.05, 0.11, 0.18, 0.25, 0.29, 0.36, 0.46, 0.49, 0.60, 0.66 and 0.79 **(fig. 1)**

3.2 Body weight and food and water consumption.

The effect of EECS on body weight changes during the chromium induced oxidative stress is shown in **table. No.1.** chromium feeding resulted in a significant decrease in the body weight with the duration of treatment; however, in animals fed with both the EECS and chromium, there was no significant change as compared to the control group. Administration of chromium did not cause any significant change in the food and water intake.



Peak	Start R_f	Start height	Max R_f	Max height	Max %	End R_f	End height	Area	Area %	Assigned substance
1	0.05	0.1	0.08	18.1	1.3	0.10	0.2	223.1	0.52	Unknown*
2	0.11	2.3	0.14	22.3	1.61	0.17	0.1	453.8	1.07	Unknown*
3	0.18	0.3	0.22	49.9	3.59	0.24	21.0	1187.9	2.79	Unknown*
4	0.25	21.1	0.26	25.7	1.85	0.28	0.6	409.4	0.96	Unknown*
5	0.29	1.3	0.34	42.7	3.07	0.36	31.3	1149.9	2.71	Unknown*
6	0.36	32.0	0.44	170.1	12.25	0.46	155.4	6400.7	15.06	Unknown*
7	0.46	155.9	0.48	165.9	11.94	0.49	163.6	3578.6	8.42	Unknown*
8	0.49	163.9	0.57	432.1	31.10	0.60	153.3	19474.1	45.82	Unknown*
9	0.60	154.8	0.62	378.5	27.24	0.66	24.0	7594.1	17.87	Unknown*
10	0.66	24.5	0.68	78.5	5.65	0.75	1.2	1957.9	4.61	Unknown*
11	0.79	0.3	0.80	5.5	0.40	0.82	1.6	74.6	0.18	Unknown*

Fig. 1. Chromatogram of the ethanolic extract of *Coleus spicatus* at 366 nm

Table .1 Effect of body weight of normal and experimental animals in each group.

Groups	Initial Body Weight	Final Body Weight
G_1	215.5 ± 5.70	222.60 ±4.60
G_2	218.5 ±6.40	170.45 ±3.30*a
G_3	222.5 ±7.30	232.6 ±5.50
G_4	212.5 ±5.30	220.10 ±4.65
G_5	205.10 ±5.25	210.35 ±5.85

a* Values were significantly different from Initial Body Weight of G_2 at $P < 0.01$

Table 2 Effect of ethanolic extract of *Coleus spicatus* on chromium induced free radicals in rats

Groups	SOD U/L	Catalse	Reduced GSH mg/dl	Lipid peroxidation nmoles/ml	AST U/L	ALT U/L
G_1	35.55±2.08	280.8±4.52	110.42±2.55	174.32±3.40	195.48±3.40	87.78±2.48
G_2	32.35±1.90	190.32±2.20*a	67.38±1.45*a	260.35±5.38 *a	332.15±7.55*a	207.22±5.18*a
G_3	30.65±1.72	240.35±3.42*b	96.7±1.98*b	225.6±2.35*b	236.24±4.68*b	127.50±3.12*b
G_4	32.50±1.88	210.25±3.12*b	80.44±1.22*b	206.42±2.42*b	264.70±5.28*b	160.48±2.22*b
G_5	37.80±2.28	222.30±1.65*b	86.40±1.35*b	216.35± 2.10*b	250.72±4.45*b	147.82±2.12*b

a* Values were significantly different from Normal control (G_1) at $P < 0.01$
b ** Values were significantly different from toxic group (G_2) at $P < 0.01$

3.3 Effect EECS on SOD and catalase levels.

Administration of chromium caused a significant increase ($p<0.01$) in the liver tissue catalase levels but did not affect SOD levels (Table 2).The EECS in a dose of 200mg/kg and 400mg/kg body weight was able to restore the catalase levels to that of control values.

3.4 Effect of EECS on reduced GSH and MDA levels

The liver tissue GSH levels were significantly decreased following the chromium treatment, whereas significant increase in plasma MDA levels was observed. (Table 2). Administration of EECS in a dose of 200mg/kg and 400mg/kg body weight, reverted the GSH and MDA levels to that of control values.

3.5 Effect of EECS on AST and ALT levels

AST and ALT levels were increased ($p<0.01$) in all the animals treated with chromium (Table No: 2). Administration of 200mg/kg and 400mg/kg body weight doses of EECS significantly inhibited the chromium induced increase in enzyme levels and restored to that of control values.

4 Discussion

One of the most important early events in cell degeneration leading to necrosis is the Lipid per oxidative damage that occurs mainly in the cell membrane. In addition, lipid peroxidation represents one of the most reaction resulting from free radicals attack on biological structures Cr(VI) and Cr(V) are both able to yield ROS[14,15]. The majority of oxidative stress studies in rat have used TBARS as a tissue damage indicator[16,17] . In addition there was no study relating EECS with chromium intoxication. Therefore in this study was undertaken to evaluate for the antioxidant activity against the chromium (VI) induced oxidative stress in male albino rats. The results of the present study demonstrate that the EECS at a concentration of 200mg/kg and 400mg/kg body weight protected the animals significantly from the chromium induced oxidative damage.

Oral feeding of chromium resulted in a significant decrease in body weight. Chromium (VI) Compounds are well known oxidizing agents capable of directly inducing tissue damage and possess carcinogenic, mutagenic and teratogenic potency[18]. Chromium (VI) compounds are easily taken up by cells and are subsequently reduced to Cr(III) species. This reduction generates free radicals, which play major role in the adverse biological effect of these compounds[19]. Administration of chromium significantly increases the lipid peroxidation as evident by the increase in MDA levels. To cope with the oxidative stress, there was a significant decrease in reduced glutathione (GSH) and catalase level in the liver tissue. No significant change in the SOD activity was observed in the Chromium-treated animals and our results fall in confirmation with earlier studies[20].Besides activating the oxidative stress, Chromium also caused a marked increase in AST and ALT levels suggesting that the Chromium treatment also causes hepatic damage. Many workers have also demonstrated the hepato-toxic effect of Chromium(VI) [21,22], which is mainly due to the lipid peroxidation. These adverse effects of Chromium(VI) could be significantly curtailed by pretreating the animals with the EECS.

In animals fed with both doses of EECS significant protection was observed against the chromium induced oxidative stress. The EECS inhibited the chromium induced increase in MDA levels and restores the intracellular antioxidant. Like GSH and catalase levels to that control. The EECS also protected the animals significantly from the hepatotoxicity induced by chromium is revealed by the decreased AST and ALT activity compared to the chromium (VI) treated animals.

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