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HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *CELOSIA ARGENTEA* LINN. SEEDS IN RATS

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The hepatoprotective activity of 70% ethanolic extract of *Celosia argentea* Linn. seeds was investigated against carbon tetrachloride (CCl_4) induced hepatic damage in rats. Administration of extract (200 and 400 mg/kg b.wt.) orally for 7 days produced significant lowering of serum marker parameters like AST, ALT, ALP, total bilirubin, and an increase in serum total proteins and albumin. Furthermore, the extract caused a significant reduction in lipid peroxidation (TBARS) and an elevation in antioxidant defense parameters as compared to CCl_4 treated control rats. Thus, the results indicate hepatoprotective activity of *C. argentea* in CCl_4 intoxicated rats.

Keywords : Antioxidative activity; CCl_4; *Coelosia argentea*; Hepatoprotective; lipid peroxidation.

Introduction

Liver is the most important organ concerned with the biochemical activities in the human body. It has great capacity to detoxicate toxic substances and synthesize useful principles. Therefore, damage to liver inflicted by hepatotoxic agents is of grave consequences. Inspite of the tremondous advances made in allopathic medicine, no effective hepatoprotective medicine is available. Plant drugs are known to play a vital role in prevention and management of liver diseases. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activities[1,2].

Celosia argentea (Family-Amaranthaceae), commonly known as cock comb, grows as a weed during rainy season throughout India and other tropical regions of the world, such as Srilanka, South Asia, Africa and America[3]. Traditionally, the plant has been used as herbal medicine in treating a wide variety of the diseases. The seeds of *C. argentea* have been traditionally used as a therapeutic drug for hepatic disease, diabetes, inflammation, metrorhagia, gonorrhoea, aphrodisiac, healing of wounds and injuries[4-6]. Experimental studies have shown that the extract of seeds possesses antidiabetic[7], antimetastatic and immunomodulatory activities[8,9]. Phytochemical studies of seeds of this plant have shown the presence of bicyclic peptides, like celogentins A-C[10], moroidin[11] and celosian, acidic polysaccharide[8]

Since the scientific studies on its use in hepatoprotection are scanty, therefore, the present investigation was undertaken to evaluate hepatoprotective effect of 70% ethanolic extract of *C. argentea* seeds against carbon tetrachloride (CCl_4) induced liver injuries to validate its use in folklore against hepatocellular damage.

Materials and Methods

Plant Collection and Extraction : C. argentea plants were collected from the fields around Jaipur, during September - October months and authenticated at the Herbarium, Department of Botany, University of Rajasthan, Jaipur. The seeds were collected from mature plants, shade dried, powdered and subjected to soxhlet extraction for 30 h using 70% ethanol as solvent. The extract was filtered and concentrated to dryness under reduced pressure and low temperature (40°C). The so obtained viscous extract was used for experimental study.

Animal : Colony bred, adult Wistar strain male rats (150-175g) were used for this investigation. All the rats were maintained under controlled standard animal house conditions with free access to pallet food (Lipton, India Pvt. Ltd.) and tap water.

Experimental Protocol : The rats were divided in to four groups of seven animals each. Liver damage was induced by administration of a single dose of carbon tetrachloride (CCl_4) 1 ml/kg b. wt in olive oil (1:1) intraperitoneally, a day prior to the start of treatment. Animals of group I served as normal control, received vehicle for 7 days. Animals of group II served as CCl_4 treated control. Animals of group III and IV received 70% ethanolic extract of *C. argentea* seeds at the doses 200 and 400 mg/kg b. wt/day, orally, respectively for 7 days in addition to prior single CCl_4 treatment.

Autopsy : On the 8th day, all animals were sacrificed under light ether anesthesia. Blood was collected directly from the cardiac puncture. Serum was separated and stored at -20°C for biochemical analysis. Liver was excised immediately, cleaned, in ice cold normal saline, weighed and frozen at -80° C for the estimation of lipid peroxidation (TBARS)[12], glutathione (GSH)[13], catalase[14] and ascorbic acid.[15]

Serum Analysis : Serum samples were used for the estimation of the activities of aspartate transaminase (AST) and alanine transaminase (ALT)[16], alkaline phosphatase

Table 1. Effect of 70% ethanolic extract of *C. argentea* seeds on serum biochemical parameters of CCl_4 intoxicated rats.

Treatments	AST (U/L)	ALT (U/L)	ALP (KA units)	Total bilirubin	TP (mg/dl)	Albumin (mg/dl)
Normal	52.65 ± 2.24	35.23 ± 1.16	10.80 ± 0.65	0.52 ± 0.02	6.86 ± 0.27	3.51 ± 0.21
CCl_4 (1ml/kg b.wt)	121.20 ± 5.15^{b}	104.34 ± 6.62^{b}	28.12 ± 1.84^{b}	1.28 ± 0.06^{b}	5.21 ± 0.29^{a}	2.37 ± 0.15^{a}
CCl_4 + *C. argentea* extract (200 mg/kg b.wt.)	91.45 ± 4.80**	82.60 ± 3.24*	20.37 ± 0.86**	0.88 ± 0.04***	6.25 ± 0.24*	2.84 ± 0.12*
CCl_4 + *C. argentea* extract (400 mg/kg b.wt.)	70.32 ± 3.68***	58.46 ± 3.14***	16.60 ± 1.22***	0.72 ± 0.04***	6.73 ± 0.31**	3.32 ± 0.16**

Values are mean ± SEM

Levels of Significance

^{a} $P < 0.01$; ^{b} $P<0.001$ when compared to normal rats,

* $P < 0.05$ '; ** $P < 0.01$; *** $P<0.001$ when compared to CCl_4 treated rats.

Table 2. Effect of 70% ethanolic extract of *C. argentea* seeds on relative liver weight, lipid peroxidation (TBARS) and antioxidant defense system in CCl_4 intoxicated rats.

Treatments	Relative liver weight (mg/100g. b. wt.)	TBARS (n moles MDA/mg protein)	GSH (n moles/mg tissue)	Catalase (CAT) (n moles H_2O_2 decomposed/ min/mg of protein)	Ascorbic acid (mg/g)
Normal	3240 ± 160	0.72 ± 0.05	3.10 ± 0.036	59.00 ± 3.40	1.25 ± 0.070
CCl_4 (1ml/kg b.wt)	4038 ± 210^{a}	2.56 ± 0.09^{c}	2.12 ± 0.051^{c}	36.24 ± 4.12^{b}	0.84 ± 0.052^{b}
CCl_4 + *C. argentea* extract (200 mg/kg b.wt)	3620 ± 110	1.80 ± .11***	2.37 ± 0.07**	48.84 ± 2.65*	1.08 ± 0.043**
CCl_4 + *C. argentea* extract (400 mg/ kg b.wt)	3360 ± 95*	1.28 ± 0.13***	2.89 ± 0.09***	54.25 ± 2.36**	1.18 ± 0.071**

Levels of Significance

^{a} $P < 0.05$; ^{b} $P <0.01$; ^{c} $P < 0.001$ when compared to normal rats Values are mean ± SEM

* $P < 0.05$; ** $P<0.01$; *** $P < 0.001$ when compared to CCl_4 treated rats

(ALP)[17], and levels of total bilirubin[18], total protein[19] and albumin (Standard Kit from Ranbaxy Laboratories, Delhi).

Statistical Analysis : All the results are expressed as mean ± SEM. Statistical comparisons were made by means of Students 't' test and $P<0.05$ was considered as significant.

Results and Discussion

A significant ($P<0.001$) increase in the serum levels of AST, ALT, ALP, total bilirubin and a significant decrease in serum total proteins ($P<0.01$) and albumin ($P<0.01$) was observed in CCl_4 treated control rats as compared to normal rats. Administration of *C. argentea* seeds extract (70% ethanolic) showed a significant dose dependent restoration of all those altered biochemical parameters towards normal value when compared with CCl_4 treated control rats (Table 1).

The level of lipid peroxide (TBARS) was significantly ($P<0.001$) elevated while glutathione (GSH) ($P<0.001$) catalase ($P<0.01$) and ascorbic acid ($P<0.01$) were significantly lowered in CCl_4 treated rats when compared to normal rats. Administration of extract in CCl_4 intoxicated rats significantly reversed these changes dose dependently (Table 2).

Liver injury induced by CCl_4 is the best characterised system of the xenobiotic induced hepatotoxicity and is a commonly used model for the screening of the antihepatotoxic / hepatoprotective activity of drugs[20].

Assessment of liver function can be made by estimating the activities of serum AST, ALT and ALP, which are enzymes originally present in higher concentration in cytoplasm[21]. When there is hepatocellular damage, these enzymes leak in the blood circulation[22]. Elevated levels of marker enzymes (AST, ALT, ALP), total bilirubin in serum of CCl_4 treated control rats corresponds to extensive liver damage induced by hepatotoxins [23, 24].

Decline in the concentration of total protein and albumin in serum of CCl_4 treated control rats might be due to adverse effect of hepatotoxin on the capacity to synthesize proteins and ablumin[25].

Treatment of *C. argentea* seeds extract markedly prevented CCl_4 induced elevation of hepatic marker enzymes (AST, ALT, ALP), total bilirubin and enhanced the levels of total proteins and albumin in serum indicating improvement of functional status and cellular integrity of hepatocytes.

Mild increase in relative weight of liver in CCl_4 treated control rats might be due to accumulation of fat, which was restored to normal in rats receiving 400 mg/kg b. wt extract.

The result of the present study demonstrate significant elevation in the levels of lipid peroxidation (TBARS) and depletion in glutathione (GSH), catalase and ascorbic acid in liver of CCl_4 exposed control rats suggesting enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanism to prevent formation of excessive free radicals. The decreased levels of glutathione, ascorbic acid and catalase enzyme activity may be due to increased utilization in trapping the oxyradicals[26]. The lipid peroxidate degradation of biomembrane caused due to trichloromethyl radical ($CCl_3^{\cdot}$), an active metabolite of CCl_4, is one of the principal causes of hepatotoxicity of CCl_4[27].

C. argentea extract treatment in CCl_4 intoxicated rats cuased significant decrease in lipid peroxidation (TBARS) and increase of glutathione (GSH), ascorbic acid contents and catalase activity in liver, suggests decrease in oxidative stress and improvement of antioxidant defence system. Thus, 70% ethanolic extract of *C. argentea* seeds owe it's hepatoprotective effect either by inhibition of biotransformation of CCl_4 to active free radical ($CCl_3^{\cdot}$) or by impairment of CCl_4 induced lipid peroxidation or due to its ability to restore the activity of antioxidants. Many plants having antioxidant activities have shown hepatoprotecive effect in CCl_4 intoxicated animals[28-31].

The result of present study indicates that 70% ethanolic extract of *C. argentea* possesses hepatoprotective activity. Further studies are, however, needed to isolate specific components responsible for it and to establish its mechanism of action.

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