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ANTIOXIDANT EFFECT OF *COSCINIUM FENESTRATUM* IN CARBON TETRACHLORIDE TREATED RATS

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Abstract : Antioxidant effect of methanol extract of *Coscinium fenestratum* stem powder was examined using carbon tetrachloride-intoxicated rat liver as the experimental model. Hepatotoxic rats were treated with the methanol extract for 90 days (daily, orally at the dose of 60 mg/kg body weight). Lipid peroxidation in carbon tetrachloride-administered rats was evidenced by a marked elevation in the levels of thiobarbituric acid reactive substances and diene conjugates, and also a profound diminution in glutathione content in the liver. Rats co-administered with the methanol extract retained an almost normal level of these constituents. The decreased activities of antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in carbon tetrachloride-intoxicated rats, and its retrieval towards near-normalcy in the methanol extract co-administered animals revealed the effectiveness of *Coscinium fenestratum* in combating oxidative stress due to hepatic damage. The findings provide a rationale for further studies on isolation of active principles and its pharmacological evaluation.

Key words : *Coscinium fenestratum* carbon tetrachloride lipid peroxidation antioxidant enzymes rat liver

INTRODUCTION

It has been stated that one of the principal causes of carbon tetrachloride (CCl_4) – induced hepatopathy is lipid peroxidation by $CCl_3^{\bullet}$, a free radical derivative of the toxin (1). The antioxidant activity or the inhibition of the generation of free radicals is important in providing protection against such hepatic damage. An antioxidant effect has been reported to play a crucial role in the hepatoprotective ability of many plants (2–6). Search for crude drugs of plant origin with antioxidant activity has become a central focus for study of hepatoprotection today. This may ensure a possible solution for the increasing incidence of tissue damages seen in organisms as a consequence of exposure to toxins of extrinsic or intrinsic origin.

Anti-hepatotoxic activity of *Coscinium fenestratum* was unearthed by our previous study (7). The objective of the present work

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is to explore the antioxidant activity of this plant against CCl_4-induced hepatopathy in rats.

Coscinium fenestratum (Gaertn). Colebr of family Menispermaceae is a woody climbing shrub found in Western Ghats in Tamil Nadu and Kerala. The stem portion of this plant is suggested to have thermogenic, anti-inflammatory, antiseptic and tonic effects, and is used against ophthalmopathy, inflammations, ulcers, jaundice and general debility (8). Singh et. al. (9) revealed the hypotensive activity of stem extracts of this plant in experimental dogs, rats and guinea pigs. This study is in furtherance to our previous investigation regarding the anti-hepatotoxic activity of the stem powder of *C. fenestratum*.

METHODS

Plant material

Stem part of *Coscinium fenestratum* was collected from Mannarghat area of Palghat district, Kerala during April-May. It was chopped, air dried at 35–40°C for a week and pulverized in an electric grinder. The powder obtained was successively extracted in petroleum ether (60–80°C), benzene, chloroform, methanol and distilled water. The extracts were then made to powder by using rotary evaporator under reduced pressure. The stem powder yielded 0.8, 1.2, 2.8, 2.6 and 3.1% w/w powdered extract with petroleum ether, benzene, chloroform, methanol and distilled water respectively. Experiment with the different powdered extracts revealed the methanolic extract offering the maximum hepatoprotection (Fig. 1). The powdered methanolic extract of *C. fenestratum* stem (MEC) was stored in freezer for further use.

Experimental animals

Twenty four male albino rats of Sprague-Dawley strain weighing 80–100g were purchased from Small Animals' Breeding Centre of Kerala Agricultural University, Mannuthy, Trichur. The animals were housed in polypropylene cages maintained in controlled temperature and light cycle. They were fed with Amrut Laboratory Animal Feed (Nav Maharashtra Chakan Oil Mills Ltd, Pune). Food and water were provided *ad libitum*. They were given a week's time to get aCClimatized with the laboratory conditions.

Experimental design

Body weight of animals was recorded and they were divided into 3 groups of 8 animals each as follows: Group I animals served as control and received sc administration of liquid paraffin (LP) twice a week at the dose of 3 ml per kg body weight of each animal. Group II animals constituted the hepatotoxic group, which received administration of LP + CCl_4, sc, twice a week at the dose of 1 ml/kg body weight of CCl_4 in double the volume of LP at lower abdomen on every first and fourth days of the week. Group III animals received sc administration of LP+CCl_4 twice a week as mentioned above. They also received MEC daily at the dose of 60 mg/kg body weight (effective dose) of each rat in a suspension of 1 ml water, orally by intubation. (A dose dependent study with MEC revealed that it evoked hepatoprotection at doses ranging 30–80 mg/kg body weight of animal. 60 mg of

MEC was found to be the effective dose) Animals were maintained at laboratory conditions for a period of 90 days.

Animals were fasted overnight on the 89th day. On the next day, after recording body weight, the animals were sacrificed by decapitation and blood was collected by incision of jugular vein. The liver was dissected out, blotted off blood, rinsed in phosphate buffered saline (pH 7.4) and immediately proceeded for biochemical estimations. Serum was prepared from the collected blood.

Biochemical Estimations

The measurement of thiobarbituric acid reactive substances (TBARS) (10) was done as an index of peroxidation of lipids. Superoxide dismutase (SOD) and catalase (CAT) activities were determined by the methods of Marklund and Marklund (11), and Aebi (12) respectively. Diene conjugates (CD) content was measured in tissue lipid extracts by method of Klein (13). Reduced glutathione (GSH) content was determined after deproteinisation by the method of Beautler and Kelley (14). Glutathione peroxidase (GPX) was assayed by the method of Rotruck et al. (15). Glutathione transferase (GTS) and glutathione reductase (GRD) were assayed by the methods of Habig et. al (16), and Horn and Burns (17) respectively. Protein was estimated by the method of Lowry et. al. (18).

Statistical analysis

The results were presented as the mean ± SEM. Student's 't' test was used to analyse statistical significance. P values less than 1% were considered significant.

RESULTS

Elevated levels of TBARS and CD were observed in liver of CCl_4–treated rats, as compared to normal control animals. (Table I). These constituents attained a near normal level in the liver of CCl_4 + MEC - administered rats. GSH content in liver decreased in Group-II animals when compared with the Group-I controls. GSH content in liver retained near-normalcy in Group-III animals.

TABLE I: Effect of *Coscinium fenestratum* on the antioxidant status of liver in rats.

Parameters	*Group I*	*Group II*	*Group III*
Thiobarbituric acid reactive substances (μ mol/mg protein)	0.64±0.05	0.98±0.06*	0.74±0.04†
Diene conjugates (μ mol/100 g tissue)	0.38±0.04	0.72±0.05*	0.32±0.04†
Reduced glutathione – GSH (μ mol/100 g tissue)	402.8±13.7	226.4±10.1*	383.1±10.8†

Values are mean ± SEM of 8 animals in each group.
*P<0.01 as compared to Group I.
†P<0.01 as compared to group II.

TABLE II: Effect of *Coscinium fenestratum* on activity of antioxidant enzymes.

Parameters	*Group I*	*Group II*	*Group III*
Superoxide dismutase (U/mg protein)	13.47±0.51	6.94±0.38*	12.91±0.58†
Catalase (U/mg protein)	8.36±0.33	3.96±0.31*	7.88±0.43†
Glutathione peroxidase (U/mg protein)	0.74±0.05	0.46±0.04*	0.68±0.05†
Glutathione reductase (U/mg protein)	6.43±0.31	3.01±0.38*	6.04±0.42†
Glutathione transferase (μ mol/mg protein)	6.94±0.61	13.93±0.71*	6.47±0.49†

Values are mean ± SEM of 8 animals in each group
*P<0.01 as compared to Group I.
†P<0.01 as compared to Group II.

Activities of antioxidant enzymes are presented in Table II. The levels of SOD, CAT, GPX and GRD recorded a significantly less value in CCl_4-treated rats as compared to normal controls. In CCl_4+MEC-treated rats, the levels of these enzymes registered a near-normalcy. However, the activity of GTS was significantly high in CCl_4-treated group, which was brought towards near-normalcy in CCl_4+MEC-treated rats.

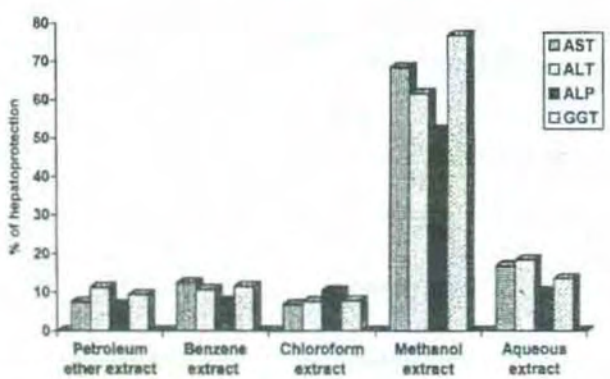

Fig. 1: Percentage of hepatoprotection offered by *Coscinium fenestratum* stem extracts in respect of different biochemical parameters.

AST - Aspartate aminotransferase
ALT - Alanine aminotransferase
ALP - Alkaline phosphatase
GGT - Gamma glutamyl transpeptidase

DISCUSSION

The body has an effective mechanism to prevent and neutralize the free radical-induced damage. This is accomplished by a set of endogenous antioxidant enzymes, such as SOD, CAT, GPX, and GRD etc. When the balance between ROS (reactive oxygen species) production and antioxidant defences is lost, 'oxidative stress' results, which through a series of events deregulates the cellular functions leading to various pathological conditions (19). Any compound, natural or synthetic with antioxidant properties might contribute towards the partial or total alleviation of this type of damage.

In the present study, elevated level of TBARS and CD observed in CCl_4-treated rats is a clear manifestation of excessive formation of free radicals and activation of lipid peroxidation system resulting in hepatic damage. The significant decline in the concentration of these constituents in the liver tissue of CCl_4 + MEC–treated animals indicates antioxidant effect of *C. fenestratum*.

GSH is a major non-protein thiol in living organisms which plays a central role in co-ordinating the body's antioxidant defence processes. Perturbation of GSH status of a biological system can lead to serious consequences. Decline in GSH content in the liver of CCl_4 intoxicated rats, and its subsequent return towards the near-normalcy in CCl_4 + MEC-administered group also expose anti-lipid peroxidative effect of *C. fenestratum*. Explanations of the possible mechanisms underlying the hepatoprotective properties of drugs include the prevention of GSH depletion (20) and destruction of free radicals (21). These two factors are believed to attribute to the hepatoprotective property of *C. fenestratum*.

SOD, CAT and GPX constitute a mutually supportive team of defence against ROS. SOD is a metalloprotein and is the first enzyme involved in the antioxidant defence by lowering the steady-state level of $O_2^{-\bullet}$. CAT is a hemeprotein, localized in the peroxisomes or the microperoxisomes. This enzyme catalyses the decomposition of H_2O_2 to water and oxygen and thus protecting the cell from oxidative damage by H_2O_2 and $^{\bullet}OH$. GPX is seleno-enzyme two third of which (in liver) is present in the cytosol and one third in the mitochondria. It catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide. In our study, decline in the activities of these enzymes in CCl_4 – administered animals and attainment of near normalcy in CCl_4 + MEC-treated rats revealed that lipid peroxidation and oxidative stress elicited by CCl_4 intoxication had been nullified due to the effect of *Coscinium fenestratum*. This observation perfectly agrees with those of Lin et. al (2) who demonstrated hepatoprotective and antioxidant activities of *Boehmeria nivea*.

GTS plays an essential role in liver by eliminating toxic compounds by conjugating them with glutathione. GRD is concerned with the maintenance of cellular level of GSH (especially in the reduced state) by effecting fast reduction of oxidised glutathione to reduced form. The activities of these two enzymes were found to be in the reverse order. In CCl_4 – administered rats, level of GTS was found to be significantly high whereas that of GRD was low. However, these enzymes restored an almost normal activity in CCl_4 + MEC-treated rats. All these results unravel the antioxidant efficacy of *Coscinium fenestratum*.

Natural antioxidants strengthen the endogenous antioxidant defences from ROS ravage and restore the optimal balance by neutralizing the reactive species. They are gaining immense importance by virtue of their critical role in disease prevention. In this context, *C. fenestratum* can rightly be mentioned as a plant with antioxidant activity. Efforts are in progress here to isolate and purify the active principle involved in the antioxidant activity of this plant.

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