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HEPATOPROTECTIVE EFFECT OF *HYGROPHILA SPINOSA* AND *CASSIA OCCIDENTALIS* ON CARBON TETRACHLORIDE INDUCED LIVER DAMAGE IN EXPERIMENTAL RATS

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ABSTRACT

The present study was undertaken to analyze the levels of some known antioxidant (both enzymic and non enzymic) activities in the roots of Hygrophila spinosa *and* Cassia occidentalis *also to find out the hepatoprotective effect of the same in carbon tetrachloride induced liver damage in albino rats. The roots were found to be rich in antioxidants. Liver damage in rats were induced by carbon tetrachloride . To find out the hepatoprotective activity, the aqueous extract of the plant root samples were administrated to rats for 15 days. The serum marker enzymes Aspartate transaminase, Alanine transaminase and Gama Glutamyl were measured in experimental animals. The increased enzyme levels after liver damage with carbon tetrachloride were nearing to normal value when treated with aqueous extract of the root samples. Histopathological observation also proved the hepatoprotectivity of the root samples.*

KEY WORDS

Hepatoprotective effect, Antioxidants, Liver damage, Liver marker enzymes.

INTRODUCTION

The World Health Organization has defined traditional medicine as comprising therapeutic practices that have been in existence for hundreds of years (1) The traditional preparations comprise medicinal plants, minerals and organic matter. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy (2). Ayurvedic medicine is essentially promotive and preventive in therapeutic approach. Many Ayurvedic medicines are used for treating liver disorders. Thus search for crude drugs of plant origin with antioxidant activity has become a central focus of study of hepatoprotection (3).

Hygrophila spinosa belongs to Acathaceae family. Its common name is Nirmulli or gokulakanta. The root contains an alkaloid named hygrosterol(4). The plant is used as demulcent, aphrodisiac, diuretic, urinary tonic and hepatoprotective substance. The aerial parts and the roots are used in herbal preparations (5).

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Cassia occidentalis is a medicinal plant belongs to Leguminosae family, Its common name is Ponnavarai. The parts used are roots, leaves and seeds. It is used for fever, menstrual problems, tuberculosis, diuretic anemic, liver complaints, and as a tonic for general weakness and illness (6).

Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effect of carbon tetrachloride (CCl_4) are largely due to its active metabolite, trichloromethyl radical (7) The administration of CCl_4 in rats enhances hepatic protein oxidation and results in the accumulation of oxidized proteins in the liver (8).

The present study was conducted to evaluate the hepatoprotective effect of the roots of *Hygrophila spinosa* and *Cassia occidentalis* on carbon tetrachloride induced liver damage in experimental rats.

MATERIALS AND METHODS

The study was carried out in two phases. Phase I involved the assessment of antioxidant activity of the samples. In Phase II hepatoprotective effect of the samples were studied.

Assessing the antioxidant activity of the samples : The samples were analysed for the non enzymic antioxidants,

Ascorbic acid by the method of Roe and Keuther (9), a-tocopherol by Rosenberg (10) Reduced glutathione by Moren *et al.* (11) Total carotenoids by Zakaria (12), Total phenols by Malick and Singh (13), Flavonoids (14) and Tanin (15) .

Preparation of the aqueous extract of the sample : 1 g of the root sample was extracted in 10ml of water using mortar and pestle, centrifuged and collected the supernatant (1ml contains 100mg). The weight of each animal was approximately 100g. The collected supernatant was used for the animal experiment. The concentration was found out by trial and error method. It was given in the concentration of 20 mg/100g body weight of the animal given orally. (0.2 ml of the supernatant given daily for a period of 15 days).

Selection and grouping of animals : Female albino rats of Wistar strain weighing 90-150g were selected for the study. They were purchased from small animal breeding station, Thrissur and were acclimatized to the laboratory conditions in a light and temperature controlled room for 15 days before starting the experiments. The animals received standard diet and water throughout the study.

The animals were divided into five main groups (6 animals in each group).

Group I : Control, normal healthy rats.

Group II : CCl_4 control. Rats injected with CCl_4 in paraffin oil (1:1), 2ml/kg. Bodyweight intraperitoneally (i.p).

Group III : Paraffin oil control. Rats injected with paraffin oil (2ml /kg body weight) i.p.

Group IV : Aqueous extract control. Rats administered with aqueous extract of the drug orally (200 mg/kg body weight) for 2 weeks.

Group V : Induction and treatment. CCl_4 was injected i.p and treatment with aqueous extract orally was started a day after the injection for a period of 1 week. This was followed for another 1 week.

Animal study was carried out for a period of 2 weeks and all the groups of the animals were sacrificed at the end of the study.

Preparation of the sample for biochemical studies : All the animals were anaesthesized with chloroform. The blood was collected by inner canthus and kept for 30 minutes without disturbing. The clot was then centrifuged for 15 – 20 minutes at 2000 rpm to separate serum and used for biochemical analysis. The liver was removed for histopathology.

Assessment of serum marker enzymes : To find out the hepatoprotective effect of the sample, the following serum marker enzymes were analyzed. Alanine aminotransferase (ALT) (16), Aspartate aminotransferase (AST) (16)γ - glutamyl transferase (GGT) (17)

Histopathological studies : A Portion of liver samples from each groups were fixed in 10 per cent formaldehyde and stained with hematoxylin and eosin for histopathological observation (18)·

Statistical analysis : One way ANOVA was carried out to find out the significant difference between the control and treatment groups.

RESULTS AND DISCUSSION

Studies on antioxidant enzymes and phytochemical analysis : Table 1 indicates the levels of non-enzymic antioxidants in the roots of *Hygrophila spinosa* and *Cassia occidentalis.*

From Table 1 it was obvious that the roots selected for the study are fairly good source of non-enzymic antioxidants. The plant samples were analyzed for the presence of phytochemicals such as steriods, terpenes, alkaloids, saponins, flavonoids and tannins and the samples were found to contain the above mentioned phytochemical constituents as given in Table 1.

Assessment of serum marker enzymes : The activity of serum marker enzymes such as alanine aminotransferase

Table 1 : Levels of Non-Enzymic Antioxidants, Tannin and Flavonoids in *Hygrophila spinosa* and *Cassia occidentalis*

Sample	Ascorbic acid (mg/g)	α - Tocopherol (μg/g)	Reduced glutathione (nmol/g)	Total phenols (mg/g)	Total carotenoids (μg/g)	Tannin (mg/g)	Flavonoids (mg/g)
Hygrophila spinosa	0.164	0.83	68.9	0.18	BD	0.57	0.79
Cassia *occidentalis*	0.132	0.62	69.7	0.39	BD	0.89	0.58

BD – Below Detectable level

Table 2 : ALT, AST and GGT Levels in Control and Experimental Groups of Rats (U/L)

Groups	Treatment	Hygrophila spinosa			Cassia occidentalis		
		AST	ALT	GGT	ALT	AST	GGT
I	Normal	43.2	47.10	62.5	42.2	46.3	60.4
II	CCl_4 control	142.3 [a]	106.23 [a]	81.4 [a]	140.4[a]	104.2 [a]	80.2[a]
III	Paraffin oil control	39.2 [a]	43.28 [a]	56.3 [a]	37.4 [a]	41.24 [a]	56.2[a]
IV	Aqueous extract control	44.42 [a]	46.24 [a]	64.2 [a]	43.4 [a]	48.2 [a]	62.4 [a]
V	CCl_4 Aqueous extract	47.6 [a]	48.18 [a]	67.2 [a]	48.3 [a]	44.2 [a]	65.1 [a]
	CD (0.05)	2.0	2.45	1.81	0.63	0.22	0.22
	SED	0.89	1.0	0.81	0.28	0.09	0.09

a – significantly different at 5% level compared to the control

(ALT), aspartate aminotransferase (AST) and g-glutamyl transferase (GGT) were analyzed in serum samples of different groups of rats are shown in Table 2. Serum marker enzymes such as ALT, AST and GGT were analyzed in different groups of rats. In group II there was a significant increase ($P<0.05$) in the serum levels of ALT, AST and GGT. But when the aqueous extract of *Hygrophila spinosa* and *Cassia occidentalis* was given in groups V, there was a significant decrease in the value, which tends to reach the normal value.In group III paraffin oil control and group IV plant extract control the level approached the normal values.

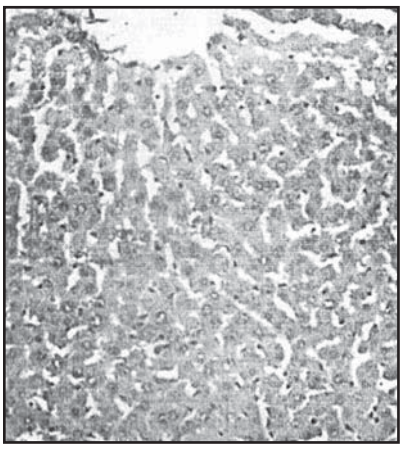

Fig 1 : Normal

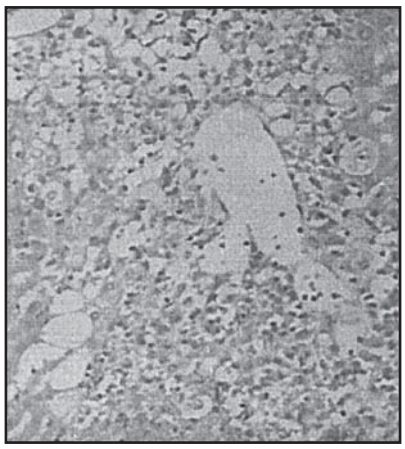

Fig 2 : CCl_4 Control

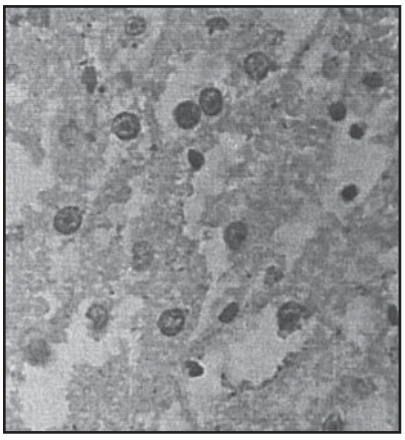

Fig 3 : CCl_4 + Hygrophila spinosa

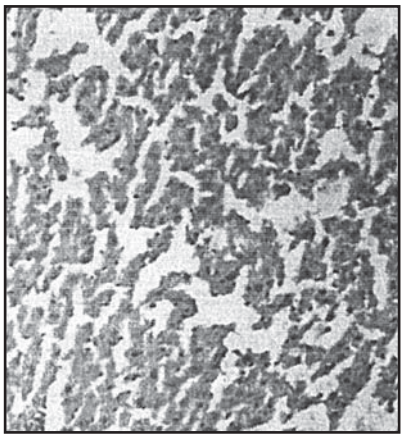

Fig 4 : CCl_4 + Cassia occidentalis

Carbon tetrachloride is reported to produce free radicals, which affect the cellular permeability of hepotocytes leading to elevated levels of serum biochemical parameters like ALT, AST, alkaline phosphatase and bilirubin. It causes massive histopathological changes in the experimental dose like necrosis, congested vessels, fatty changes, nuclear disintegration, kupffer cell hyperplasia etc. The reverse of this phenomenon can be considered as the index of hepatoprotective activity (19).

Histopathological studies in liver : Histopathological studies of sections of liver of control and experimental rats were carried out to test the hepatoprotective effect of the aqueous extract of *Hygrophila spinosa* and *Cassia occidentalis*. The results obtained are shown in Plate I.

In group I (normal control) rats, liver showed normal architecture. The central veins, portal tracts, hepatocytes and sinusoids appear normal. The lobular unit is well identified (Fig 1). Group II (CCl_4 control) shows loss of the normal liver architecture. There are extensive areas of patchy and confluent hepatocyte necrosis and lobular inflammation is intense. Surviving hepatocytes show steatosis and feathery degeneration.

Sinusoidal spaces are flooded with inflammatory cells and RBCs (Fig 2). Group III (Paraffin oil control) shows structure of the normal liver tissue. There are no significant pathological changes. Group IVa (Hygrophila spinosa only) shows structure of the normal liver. The central vein, portal traids and hepatocytes appear normal. In group IVb (*Cassia occidentalis* only) the administration of aqueous extract of Cassia occidentials preserved the histological structure of liver showing normal hepatocytes and a congested central vein. Group Va (CCL_4 + *Hygrophila spinosa*) showed complete reversal of toxic effects. No necrosis seen. The central vein and portal triads appear normal. Some of the hepatocytes show binucleation suggesting regenerative activity (Fig 3). Group Vb (CCl_4 + *Cassia occidentalis*) CCl_4 damaged and treated with the extract showed normal architecture with feathery degeneration of hepatocytes (Fig 4).

The improved histology of the liver as seen in histopathological observations on animals treated with the plant material as compared to that seen in animals administered only CCl_4 indicated the possibility of the plant material being able to induce accelerated regeneration of the liver.

To conclude the antioxidant content of *Hygrophila spinosa* and *Cassia occidentalis* might play a major role in controlling the tissue damage caused by reactive oxygen species. The biochemical and histopathological studies confirmed the protective effect of the aqueous extract of *Hygrophila spinosa* and *Cassia occidentalis* against CCl_4 induced liver damage in rats. However, the exact nature of the hepatoprotection exhibited by the plant sample has to be studied for further details.

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