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Evaluation of Hepatoprotective Potential of *Cassia tora* Leaf Extract

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Abstract – Methanolic extract of the leaves of *Cassia tora* was evaluated for its hepatoprotective potentials in rats by inducing hepatotoxicity with carbon tetrachloride. The extract at a dose of 400 mg/kg orally showed significant protective effect by lowering the serum levels of transaminase (SGOT and SGPT), bilirubin and alkaline phosphatase (ALP). The effects produced were comparable to that of a standard hepatoprotective agent.

Key words – *Cassia tora*, methanolic extract, SGOT, SGPT, ALP, Bilirubin, CCl_4, hepatic damage.

Introduction

Cassia tora Linn. (Family-Leguminosae) is a well known plant, widely distributed in India and other tropical Asian countries. It is an annual undershrub and grows well in wasteland. It is commonly known as sicklepod. Various parts of the plants are reputed for their medicinal value (Nadkarni *et al.*, 1954). The seeds of *Cassia tora* have been used in Chinese medicine as aperient, antiasthenic, diuretic agents, to improve the visual activity and constitute a valuable remedy in skin diseases, chiefly for ringworm and itch (Kirtikar and Basu, 1975; Chatterjee and Pakrashi, 1992). The leaves are useful against eczema (Asolkar *et al.*, 1992) and have also been found to possess significant antifungal potential (Mukherjee *et al.*, 1997). The leaves of *Cassia tora* contain several anthraquinone glycosides which are well known for their medicinal value. The extract of the *Cassia tora* leaves showed purgative action (Pal *et al.*, 1984). A significant hepatoprotective activity was observed in carbon tetrachloride induced rats, when the drug was administered orally at a dose of 400 mg/kg body weight.

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Materials and Methods

Plant materials – The fresh leaves of *Cassia tora* were collected in the month of August-September. It was identified by Botanical Survey of India, Shibpur, Howrah. A voucher specimen (C-02) has been kept in our laboratory for future references. The leaves were air dried, powdered and passed through 40 mesh sieve and kept in a well closed container for further extraction.

Preparation of extract – The powdered drug (500 G) was percolated with 90% methanol (1500 ml). The extract was concentrated under vacuum. Yield of the extract was 25 G. which is brownish black in color. The con-

centrated extract was used further to study the hepatoprotective activity.

On phytochemical screening the extract showed the presence of an anthraquinone glycoside, characterization of which is under processes.

Carbon tetrachloride induced hepatotoxicity – Carbon tetrachloride intoxication in rats is an experimental model widely used to study necrosis and steatosis of liver (Rubin *et al.*, 1963; Recnagel *et al.*, 1983). Forty albino rats of either sex and weighing between 120 and 150 g were divided into four groups of 10 animals each. Group-I received only normal saline and served as control. Group-II animals were given carbon tetrachloride (CCl_4): liquid paraffin (1 : 1) 0.1 ml in liquid paraffin to make the volume 0.2 ml, subcutaneously daily for a total of 8 weeks, as recommended by Slater (Slater *et al.*, 1978). Group-III animals received carbon tetrachloride as mentioned plus oral administration of *Cassia tora* leaf extract at a dose of 400 mg/kg daily upto 8 weeks. Both the drug and carbon tetrachloride (CCl_4) were started simultaneously. Group-IV animals received carbon tetrachloride plus standard liver tonic (*Neutrasec* containing methionine, choline and vitamins compounds) of Tablets India Ltd., Madras, India) for comparative study with hepatoprotective activity of *Cassia tora* leaf extract.

All the animals were observed daily and any dead animals were subjeced to postmortem to find the cause of death.

Assay of serum GOT and GPT activities – All rats were killed and blood withdrawn from the carotid artery was centrifuged at 300 g for 10 minutes (Chun-Ching *et al.*, 1995) to separate the serum. Serum transaminase activity were measured according to the method of Rietman & Frankel (1975).

Assay of serum bilirubin and serum alkaline phosphatase – Serum bilirubin was estimated following Malloy and Evelyn method (1937). Serum alkaline phosphatase was estimated following Kind and Kings method (1954).

Results

Rats subjected to CCl_4: liquid paraffin (1:1) regimen alone developed significant hepatocellular damage as evident from a significant elevation in serum activities of GOT, GPT, ALP, and bilirubin concentration as compared to their normal counter part, which have been used as reliable markers of hepatotoxicity (Table 1). Oral administration of *Cassia tora* leaf extract (400 mg/kg, p.o.) exhibited a significant reduction in liquid paraffin: CCl_4 (1:1) induced increase in the levels of GOT, GPT, ALP and plasma or serum bilirubin concentration. Treatment with Neutrosec (a popular liver tonic) also reversed the hepatotoxicity significantly.

At the end of 8 weeks percentage mortality was observed in CCl_4 group due to chronic CCl_4 toxicity as evidenced in their autopsy

Table 1. The effect *Cassia tora* leaf extract on serum levels of transaminases, alkaline phosphatase and bilirubin in rats subjected to (carbon tetrachloride induced hepatic damages (n=10)

Treatment	SGOT (μ/ml)	SGPT (μ/ml)	Alkaline Phosphatase (KA units)	Bilirubin (mg/dl)
Control	52.2 ±0.496	58.42±0.68	80.52±0.35	1.12±0.08
CCl_4:Liquid Paraffin (1:1)	123.18±6.32	112±7.63	159.76±6.39	3.64±0.31
Leaf extract (400 mg/kg, p.o.)	62.48±7.36*	65.1 ±5.44*	123.12±5.31*	1.74±0.29*
Liver tonic (5 ml/kg, p.o.)	55.92±7.24*	61.24±5.31*	90.28±5.29*	1.36±0.35*

p-value was calculated by comparing with control by student's 't' test. *p<0.001.

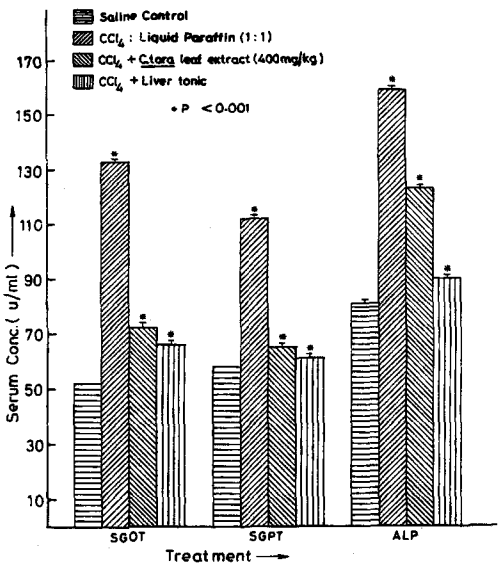

Fig. 1. Hepatoprotective activity of *C. tora* leaf extract on CCl_4 treated liver damage in rats. (A) SGOT, (B) SGPT, (C) Alkaline phosphatase.

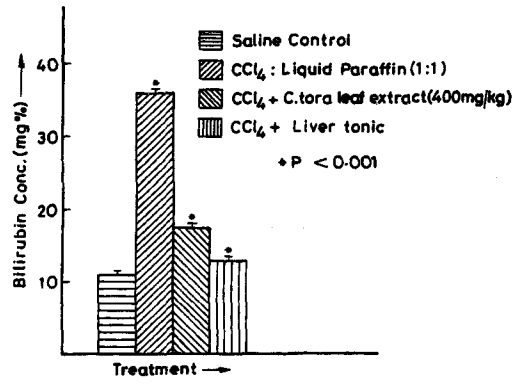

Fig. 2. Effect of *C. tora* leaf extract on bilirubin conc. in CCl_4 induced liver damage in rats.

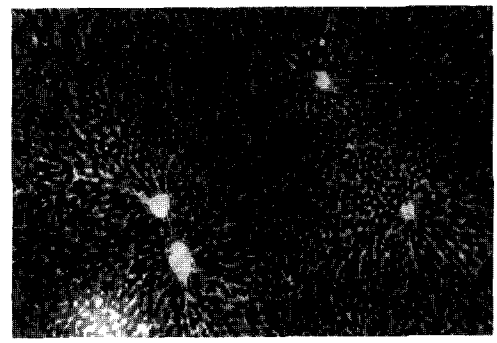

Fig. 3. Normal control rat. Section of liver showing normal hepatic cells with brought out nuclei, cytoplasm, central vein and portal triad (H & E×65).

which showed congested and enlarged liver, sometimes associated with intestinal bleeding and inflammation. However no mortality was observed with either control or *C. tora* leaf extract treated group. Continuous administration of methanol extract of *C. tora* leaves prior to and concurrent with CCl_4 treatment reversed in varying degrees the alteration induced by CCl_4. The extract at 400 mg/kg dose exhibited significant protection against CCl_4 induced hepatotoxicity through its ability to reduce the elevated serum activity of the hapato specific enzymes (Table 1, Fig. 1). Serum levels of bilirubin was also restored towards normal following the extract treatment (Table 1, Fig. 2). The activity of this plant extract was compared with that of a standard hepatoprotective drug, Neutrosec. From Table 1, Fig.s 1 and 2, it is evident that the effect of extract under study and Neutrosec was comparble in all the parameter tested. However, Neutrosec provided better hepatoprotection in terms of the inhibition of elevated serum activities of these enzymes as well as serum bilirubin concentration by CCl_4.

Histopathological profiles of the liver from liquid paraffin: CCl_4 (1:1) treated rats revealed intense centrilobular necrosis, steatosis and often swelling of hepatic cytoplasm (Fig. 4). The protective effect of *C. tora* leaf extract (400 mg/kg, p.o.) was confirmed by histopathological examination of the liver section of control, liquid paraffin: CCl_4 (1:1) and the extract treated groups of rats (Fig. 3-5). Administration of extract to the experimental animals at a dose of 400 mg/kg, p.o. exhibited a significant improvement of hepatocellular architecture over liquid paraffin: CCl_4 (1:1) treated control group as evident from considerable reduction in necrosis and fatty changes. (Fig. 5).

Liver section of rats treated with Neutrosec (Fig. 6) showed significant signs of amelioration of liquid paraffin: CCl_4 (1:1) evoked

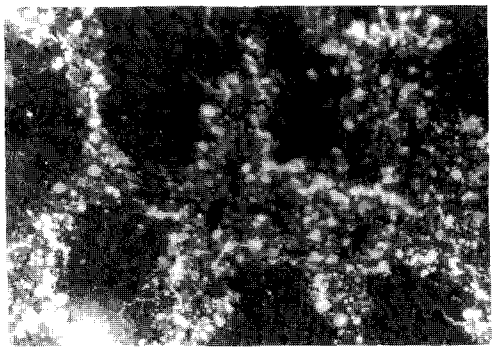

Fig. 4. Liquid Paraffin-CCl_4 treated rat. Section of liver showing marked nacrosis, severe fatty degeneration and extensive vacuolization with disappearance of nuclei (H & E × 65).

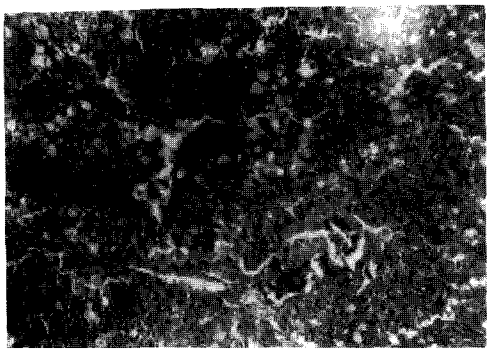

Fig. 5. Representative photmicrograph of liver section from rat guin *C. tora* leaf extract at 400 mg/kg (Group III) showing moderate improvement over liquid paraffin-CCl_4 control (Group II)(H & E × 65).

liver injury which was evident from the presence of normal hepatic pods, absence of necrosis and steatosis (Fig. 6). This study showed that the extract at 400 mg/kg and liver tonic showed significant protective effect against liver injury which is evident from their histopathological examination (Figs. 5 and 6).

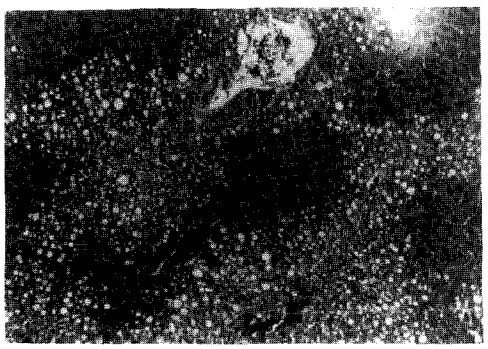

Fig. 6. Liquid paraffin-CCl_4 treated (1:1) and liver tonic (neutrosec) treated rats. Section of liver showing normalcy of hepatic cells, central vein and portal triad (H & E × 65).

Discussion

Hepatoprotective activity has been established on *Cassia tora* seeds but the same activity has not been studied early on the leaves of *Cassia tora*. Hence the leaves has been selected to study its hepatoprotective potential.

Preventive action in liver damage induced by CCl_4 has widely been used as an indicator of the liver protective activity of drugs in general (Clauson, 1989). CCl_4 produces an experimental liver damage which is histologically resembles viral hepatitis (James *et al.*, 1976). SGOT, SGPT, ALP and serum bilirubin are the most sensitive test which are considered as the index for diagnosis of liver diseases (Mehendale *et al.*, 1985).

In our present investigation rats treated with chronic dose of CCl_4 developed a significant hepatic damage which was observed from a substantial increase in the concentration of GOT, GPT, ALP and bilirubin. Treatment of rats with methanol extract of *C. tora* prior to and concomittant with the challenge of CCl_4 produced an alleviation of the hepatic injury to a considerable extent which was reflected by the ability of the extract to lower the elevated serum enzymes activities resulting from the administration of CCl_4 alone. The increased levels of GOT and GPT in serum are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotmann and Lawhorn, 1978). In view of this the extract mediated reduction in levels of GOT, GPT towards the respective normal value is an indication of stabilization of plasma membrane as well as repairement of hepatic tissue damages caused by CCl_4. These effect is an agreement

with the commonly accepted view that serum levels of transaminases return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew *et al.*, 1987).

Alkaline phosphatase (ALP) is the prototype of these enzymes that reflect the pathological alteration in biliary flow (Ploa and Hewitt, 1989). The use of ALP in chemical induced liver dysfunction has been fairly investigated in our study. CCl_4 induced elevation of this enzymatic activity in serum is in line with high level of serum bilirubin content. The extract mediated suppression of the increased ALP activity with a concurrent depletion of raised bilirubin level suggests the possibility of the extract being able to stabilize biliary dysfunction in rat liver during chronic hepatic injury with CCl_4.

Thus the present study confirm the liver protective action of the methanol extract of *C. tora* against experimentally induced liver damage in rats, which was comparable to that of a standard hepatoprotective drug Neutrosec. Further studies relating to the separation of the active component actually responsible for this activity as well as further confirmation of its hepatoprotective effect and related mechanism of action is under way in our laboratory.

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