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Hepatoprotective study of seeds of *Cassia fistula* Linn.

N.B.Chaudhari*, K.P.Chittam, V.R.Patil

College of Pharmacy, Faizpur, Dist: Jalgaon, Maharastra-425503, (INIDA)

Tel : 09970384456

E-mail : nileshmpfarm@gmail.com

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ABSTRACT

Hepatoprotective activity of the methanolic extract of *Cassia fistula* seeds was investigated by inducing hepatotoxicity with CCl₄ in rats. The extract at a dose of 300 mg/kg body wt. exhibited orally, significant protective effect by lowering the serum levels of transaminases (SGOT and SGPT), bilirubin and alkaline phosphatase (ALP). The effects produced were comparable to that of a standard hepatoprotective agent.

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KEYWORDS

Cassia fistula;
Methanolic extract;
SGOT;
SGPT;
ALP;
Bilirubin;
CCl₄;
Hepatic damage;
Rats.

INTRODUCTION

Cassia fistula Linn. (Leguminosae) is cultivated widely throughout India as an ornamental and deciduous plant. It is also being cultivated in the tropics, including in the West Indies, Ceylon, China, Egypt and many other countries. As a drug it is obtained chiefly from the West Indies, namely Dominica and Martinique^[19]. "Purging cassia" was known in Europe in the thirteenth century and was used by the school of medicine at Salerno^[20]. In Ayurvedic medicine, this plant is used as a treatment for hematemesis, pruritus, leucoderma and diabetes. The powder of the seeds is given for erysipelas and skin diseases^[4]. In Sri Lanka, this plant is used for skeletal fractures (Ekanayake, 1980). The wound-healing^[2] and hypoglycemic activity^[1] of this plant have been reported and The hepatoprotective activity of *Cassia tora* Linn. has been evaluated by using the same method^[3,12]. It has come to our attention that urban people of Northeastern India use pods and leaves of this plant antiallergic and as

hepato- protective agents. Based on ancient practices and traditional uses of this plant as hepatoprotective agent, we undertook the present study using chloroform as the hepatotoxic agent. Results are reported hereunder.

MATERIALS AND METHODS

Plant material

The seeds of *Cassia fistula* Linn. were collected from local area of faizpur, India and identified by Dr. P.G.Diwakar, Botanical Survey of India, Pune, Maharastra. A voucher specimen (NBC-1) has been kept in our laboratory for future references. The seeds were shade dried, powdered and passed through a 40-mesh sieve, and kept in a well-closed container for further extraction.

Preparation of extract and standard use

The powdered drug (500 g) was percolated using 90% methanol (1500 ml). The extract was concentrated under reduced pressure, and the semi-solid mass thus

obtained was used for the experiment (yield 10.01% w/w with respect to dry powdered material). The extract was administered at dose of 300 mg/kg body wt. (p.o.). Popular liver tonic Liv-52 syrup, obtained from The Himalaya Drug Company, Bangalore., India was used as a standard liver tonic for comparison. The liver tonic was administered at a dose of 5 ml/kg body wt. (p.o.) to the respective standard-drug treated groups of animals in each experimental model. The semi-solid extract was suspended in normal saline and administered orally to specific groups of animals.

Phytochemical screening

On phytochemical screening of brown coloured mass indicate the presence of flavonoids^[10], triterpenoids^[13] and anthraquinone^[19].

Animals

White albino rats (Wistar strain) weighing between 180-200 g each of either sex, were maintained at uniform laboratory conditions in standard steel cages and provided with food and water *ad libitum*. The animals were maintained under laboratory condition for an acclimatization period of seven days before performing experiments.

CCl₄ induced hepatotoxicity

Carbon tetrachloride intoxication in rats is an experimental model widely used to study necrosis and steatosis of liver^[16]. Forty albino rats of either sex were divided into four groups of ten animals each. Group I received only normal saline and served as control. Animals of Group II were treated with carbon tetrachloride (CCl₄):liquid paraffin (1:1). For a total period of 8 weeks, 0.1 ml carbon tetrachloride in liquid paraffin to make the volume 0.2 ml, was administered subcutaneously daily, as recommended by Slater^[17]. Group III animals received carbon tetrachloride as mentioned plus oral administration of *C.fistula* seed extract at the dose of 300 mg:kg daily up to 8 weeks. Both the drug and carbon tetrachloride (CCl₄) were started simultaneously. Group IV animals received carbon tetrachloride plus standard liver tonic (Liv-52) for comparative study with hepatoprotective activity of *C.fistula* seed extract. All the animals were observed daily and any dead animals were subjected to post-mortem to find the cause of death.

Assay of serum GOT and GPT activities

All rats were killed under light ether anaesthesia after 36 h of CCl₄ administration and blood withdrawn from the carotid artery was centrifuged at 300 rpm for 10 min (Lin and Lin, 1995) to separate the serum. Serum transaminase activity was measured according to the method of Reitman and Frankel^[15].

Assay of serum bilirubin and serum alkaline phosphatase

Serum bilirubin concentration was estimated following the method of Malloy and Evelyn (1937). Serum alkaline phosphatase was estimated following the method of Kind and Kings method^[9].

Histopathological examination of hepatocytes

Each rat was laprotomized to obtain the liver immediately after collecting blood under ether anaesthesia. Small fragments of the rat liver were fixed in 10% formalin solution, dehydrated with ethanol solution from 50% to 100%, embedded in paraffin and cut into 5 μm thick sections which were stained using haemotoxylineosin dye for photomicroscopic observation^[7] including necrosis, steatosis and fatty change of hepatic cells.

Statistical analysis

The data are expressed as mean + SEM and the statistical significance was evaluated using the student's t-test^[21].

RESULTS

The Brown coloured mass indicates the presence of flavonoids, triterpenoids and anthraquinones.

The results of CCl₄-induced hepatotoxicity are presented in TABLE 1. In rats treated with CCl₄ alone (group II), there was a significant rise in SGOT, SGPT, alkaline phosphatase (ALP) and bilirubin values (TABLE 1). Pretreatment with *C. fistula* seeds extract and standard liver tonic (Liv-52) resulted in significant (p<0.001, p<0.01) protection against the increase of SGOT, SGPT, ALP and bilirubin in rats of groups II and IV, as compared to the CCl₄ control group II (TABLE 1, Figures 2-4). However, the liver tonic exhibited more significant protection. Histologically CCl₄

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treated animals showed central or submassive necrosis (Figure 2) whereas in the *C. fistula* extract and liver tonic treated groups (Figures 3 and 4) necrotic lesions were absent and were comparable with the control (Figure 1).



Figure 1

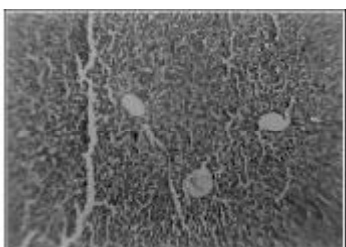


Figure 2

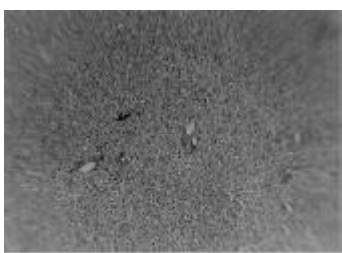


Figure 3



Figure 4

DISSUSSION

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^[5]. Carbon tetrachloride produces an experimental liver damage which histologically resembles viral hepatitis^[8]. SGOT, SGPT, ALP and serum bilirubin are the most sensitive tests which are considered as the index for diagnosis of liver diseases^[11]. In our present investigation rats treated with chronic dose of CCl_4 developed significant hepatic damage which was observed through a substantial increase in the concentration of GOT, GPT, ALP and bilirubin. Treatment of rats with Methanolic extract of *C. fistula* prior to and concomitant 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 levels 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^[6]. In view of this, the extract mediated reduction in levels of GOT, and GPT towards the respective normal values is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl_4 . This effect is in agreement with the commonly accepted view that serum levels of transaminases return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes^[18]. Alkaline phosphatase (ALP) is the prototype of these enzymes that reflect the pathological alteration in biliary flow^[14]. The use of ALP in chemical induced liver dysfunction has been 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 the concurrent depletion of raised bilirubin level suggests the possibility of the extract being

TABLE 1: Effect of *Cassia fistula* leaf extract and liver tonic on serum biochemical parameters during CCl_4 -induced acute liver damage in rats (n = 10)

Groups	SGOT	SGPT	ALP	Bilirubin
Group - I (Control)	62.83 + 3.93	34.18 + 3.81	70.23 + 6.01	0.238 + 0.04
Group - II (CCl_4 Contol, 750 mg/kg)	189.93+ 6.72a	105.56+5.92a	198.72+8.32a	0.714 + 0.02
Group - III (Extract,300 mg/kg + CCl_4 750 mg/kg)	87.33 + 4.55b	56.61+3.24b	91.63+5.91b	0.328 + 0.06b
Group – IV (Liver tonic, 5 ml/kg + CCl_4 750 mg/kg)	67.78 + 4.12a	38.92+3.71a	83.11+5.02b	0.239 + 0.08b

Group II was compared with Group I. Group III and Group IV were compared with Group II. All values were compared using student's *t*-test. *ap* < 0.01, *bp* < 0.001

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able to stabilize biliary dysfunction in rat liver during chronic hepatic injury with CCl_4 .

Thus the present study confirms the liver protective action of the Methanolic extract of *C.fistula* against experimentally induced liver damage in rats, which was comparable to that of a standard hepatoprotective drug, Liv-52. The elevated levels of these parameters were significantly reduced by treatment with *C.fistula* seeds extract as well as liver tonic. This indicates that the extract may be used as an effective hepatoprotective agent.

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