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Original Research Article

Evaluation of Hepatoprotective Activity of the Fruits of *Coccinia grandis* Linn

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Abstract

Purpose: *To evaluate the hepatoprotective activity of alcoholic extract of the fruits of Coccinia grandis Linn (Curcubitaceae) using carbon tetra chloride (CCl_4)-induced hepatotoxicity in rats.*

Methods: *The levels of serum glutamate oxaloacetate transminase (SGOT), serum glutamate pyruate transminase (SGPT), alkaline phosphatase (ALP), total protein, total and direct bilirubin were evaluated in experimental rats (with or without CCl_4-induced hepatotoxicity) following administration of alcoholic extract of the fruits of C. grandis using standard procedures. The potency of the extract was compared with standard silymarin at a dose of 100 mg/kg p.o. Histopathology of the liver tissues of the animals treated with the extract was also studied.*

Results: *At a dose level of 250 mg/kg, the alcoholic extract significantly ($p<0.05$) decreased the activities of serum enzymes (SGOT, SGPT, and ALP) and bilirubin which were comparable to that of silymarin.*

Conclusion: *Alcoholic extract of the fruits of C. grandis offers protective effect against CCl_4-induced hepato-toxicity in experimental rats.*

Keywords: *CCL_4-induced hepatotoxicity; Coccinia grandis; hepatoprotective effect; silymarin*

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Introduction

The liver is a major detoxifying organ in vertebrate body, which involves intense metabolic activities. Certain toxic chemicals and medicines can cause liver damage, which has been recognized as a toxicological problem. However, herbal medicines are known to play an important role in the treatment of various ailments, including hepatopathy[1]. Many traditional practitioners have claimed that numerous medicinal plants and their formulations can be effectively used for the alleviation of different types of liver diseases[2]. But most claims are anecdotal and very few have received adequate medical and scientific evaluation.

Coccinia grandis Linn (Curcubitaceae) is a climber herb cultivated throughout India. In folklore medicine, the fruit is used to treat leprosy, fever, asthma, infective hepatitis, jaundice, and sore throats. It is also used as expectorant and astrigent[3]. The alcoholic extract of the plant is used as hypoglycemic[4], and anti-oxidant[5] agent. A compound polyprenol isolated from the ethanol extract posseses anti-dyslipidimic activity[6]. However, no work has been reported on the hepatoprotective properties of this plant.

The present study has been undertaken to evaluate the hepatoprotective activity of the alcoholic extract of fruits of *Coccinia grandis* Linn against CCl_4-induced hepatotoxicity in rats.

Materials and Methods

Plant materials

The fruits of *Coccinia grandis* were purchased from the local market in Chennai in March and April, 2007. They were authenticated by Prof P Jayaraman, a Botanist and Director, Plant Anatomy Research Centre (PARC), Chennai and the voucher specimen was deposited in PARC (PARC/22/07). The fruits were air dried, coarsely powdered, and then subjected to successive extraction using petroleum ether and 95% alcohol (Analytical grade, SISCO Research Laboratory, Bombay) in soxhlet apparatus. Solvent elimination, using rotary evaporator under reduced pressure, was adopted producing the hexane extract (2.3% w/w) and alcohol extract (20.5%w/w yield). The alcoholic extract (250 mg/kg) was formulated as suspension in 1% w/v tracaganth gum and used for the study. Silymarin was used as a standard drug.

Animals

Male Wister strain albino rats (24) weighing 150 – 200 g and albino mice weighing 22 – 25 g were used for the study. They were maintained under standard environmental condition (temperature 25 – 28 ^{o}C and 12 hr light/dark cycle) and allowed access to standard laboratory feed and water *ad libitum*. The animals were allowed to acclimatize to the laboratory condition for a week before they were used for the experiment. Ethical approval for the use of the animals was obtained from the institutional committee constituted for the purpose (IAEC 14/2007).

Acute toxicity studies

Acute oral toxicity was performed as per OECD-423 guidelines[7]. The albino mice were fasted overnight, provided only water, after which the alcoholic extract of the fruits of *Coccina grandis* Linn was administered by gastric intubation to the relevant animals orally at the dose of 5 mg/kg body weight. The animals were then observed for 14 days. When mortality was observed in 2 or 3 animals, the dose administered was recorded as a toxic dose. But when mortality was observed in one animal, then the same dose was repeated again for confirmation. However, if mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2,000 mg/kg body weight. Toxic symptoms for which the animals were observed for 72 hr include behavioral changes, locomotion, convulsions and mortality.

Hepatoprotective activity

Hepatotoxicity was evaluated as previously described[8]. A total of 24 albino rats were divide 4 groups of 6 animals each and treated as follows:

Group I (Control): received subcutaneous administration of 1%w/v of gum tracaganth at the dose of 1ml/kg /day per oral for 14 days.
Group II: received subcutaneous administration carbon tetra chloride (CCl_4) at a dose of 0.1 ml/kg/day of body weight i.p for 10 days.
Group III (test): served as test and received alcoholic extract of fruits of *Coccinia grandis* Linn (250 mg/kg p.o) daily for 14 days along with CCl_4 subcutaneous for 10 days.
Group IV (standard): received silymarin (100 mg/kg) per oral for 14 days along with CCl_4 subcutaneously for 10 days.

At the end of the 14th day the blood was collected from all the animals from the rectero-orbital plexus and the serum was separated by centrifugation at 2000 rpm for 10 min. The serum was then assayed for hepatic marker enzymes, serum glutamate oxaloacetate transminase (SGOT), serum glutamate pyruate transminase (SGPT), alkaline phosphatase (ALP), total and direct bilirubin and total protein.

Assessment of biochemical parameters

The estimation of SGOT and SGPT was based on the reference method described in International Federation of Clinical Chemistry[9]**.** The reagent supplied in the kits (Bayer Diagnostic Kits) were reconstituted and mixed with the serum. SGOT and SGPT were measured at 340 nm and expressed as IU/L. Serum ALP was estimated by mixing the reagent (*p*-nitrophenyl phosphate, magnesium, buffers and stabilizers) with the serum and measuring the absorbance at 405 nm. The value obtained was expressed as IU/L. Total protein was measured using Biuret method[10]. In this method, the serum was mixed with Biuret reagent and incubated for 10 min at 37 ^{o}C. The absorbance of the solution was then measured at 555 nm and the estimated total protein was expressed as g/dl. Total and direct bilirubin were estimated by the method of Jandrassik and Grof at 546 nm and expressed as mg/dl[11].

Histopathological examination

Animals were sacrificed by cervical dislocation and the blood was collected from the rectero-orbital plexus. The liver was removed, sliced and washed in saline and the pieces were preserved in 10% formasal (10% formaldehyde diluted using normal saline) for histopathological evaluation. Sections of pieces of the liver (about 4-6 mm in thickness) were processed and embedded in paraffin wax, stained with haematoxylin and eosin, mounted and observed under light microscope for histological changes.

Data analysis

As appropriate, measurements were carried out in triplicates and descriptive statistics (mean and sd) were used in presentation of the results. Data comparison was carried out using one way analysis of variance (ANOVA). At 95% confidence interval, 2-tailed p-values less than 0.05 were considered to be significant.

Results

The marker enzyme levels are provided in the Table. There was significant increase in the levels of the marker enzymes (SGOT, SGPT, ALP) as well as total and direct bilirubin in the animals treated with CCl_4 when compared with the control animals. For the animals given the extract (250 mg/kg), the levels of these enzymes and bilurubin were relatively normal when compared with CCl_4 treated group ($p<0.05$). The serum total protein concentration of the CCl_4 treated group was significantly smaller than the extract treated group.

Table: *Effect of hepatoprotective activity of the fruits of Coccinia grandis against CCl_4-induced hepatotoxicity in rats*

Treatment	SGOT (IU/L)	SGPT (IU/L)	Alkaline Phosphatase (IU/L)	Total Protein (g/dl)	Bilurubin	
					Total (mg/dl)	Direct (mg/dl)
Vehicle control	56.38± 6.22	64.34± 4.08	195.7±15.4	5.76± 0.38	0.62± 0.03	0.32 ±0.05
Carbon tetrachloride treated	132.85±3.29**	155.61±4.01**	315.5±23.7**	3.21 ±0.59*	0.92± 0.06*	1.12 ±0.08**
Alcoholic extract (250 mg/kg)	72.44± 3.64***	91.57± 3.71**	200.4±12.3*	4.78 ±0.19	0.71± 0.07**	0.72 ±0.02**
Silymarin (100 mg/kg)	61.81± 2.13**	71.93± 2.48***	198.1±14.7	5.23 ±0.20*	0.64 ±0.02	0.39± 0.04**

*Values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 as compared to control*

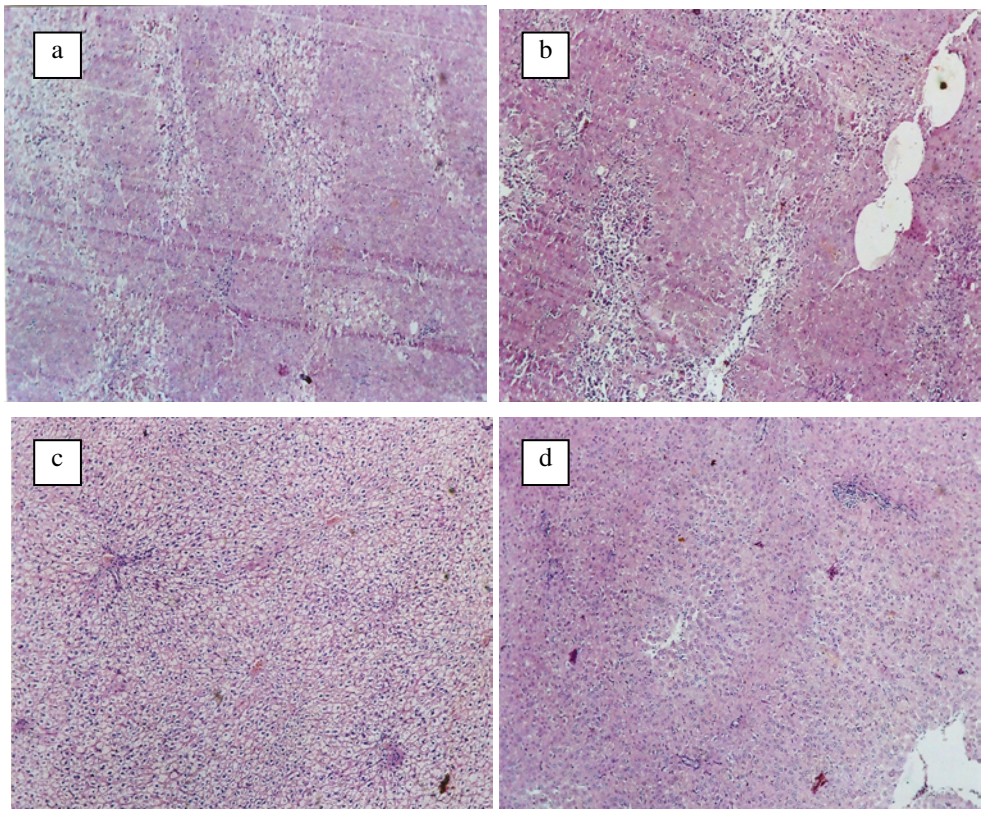

Figure: Photomicrograph of liver cells of control rats (a), and those treated with CCl_4 (b), alcoholic extract (250 mg/kg) of *C. grandis* (c) and silymarin (d)

Histopathological examination of animals in group I showed a normal hepatic architecture (Figure 1a). Animals in group II (CCl_4 treated) demonstrated severe hepatotoxicity as evidenced by profound steatosis, centrilobular necrosis and ballooning degeneration (Figure 1b). In group III and IV, the animal livers exhibited an almost normal architecture barring a little deformation of hepatocytes with pyknosis and clearing of cytoplasm (Figure 1c & 1d).

Discussion

Liver diseases remain as one of the serious health problems. However we do not have satisfactory liver protective drugs in allopathic medical practice for serious liver disorders. Herbal drugs play a role in the management of various liver disorders in addition to other natural healing processes of the liver[12].

Previous studies have demonstrated the use of carbon tetrachloride to successfully induce hepatotoxicity in experimental animals[13]. In experimental hepatopathy, the toxin carbon tetrachloride is biotransformed by cytochrome P-450 to produce the trichloro-methyl free radical, which causes peroxidative degradation in the adipose tissue resulting in fatty infilteration of the hepatocytes. Trichloromethyl free radicals elicit lipid peroxidation of membrane lipids in the presence of oxygen generated by metabolic leakage from mitochondria. All these events culminate in loss of integrity of the cell membranes and damage of hepatic tissue [14].

Histopathological exami-nation of liver section of normal rats showed normal hepatic cells with cytoplasam and nucleus whereas CCl_4 treated group showed that the liver cells are intoxicated with CCl_4 the normal architecture of the liver was completely damaged. The treatment of the rats with ethanolic extract exhibited protection against liver damage by CCl_4, which is confirmed by the results of biochemical studies.

The increase in the levels of transaminase reflects a clear indication of cellular leakage and loss of functional integrity of the cell membrane[15]. Assessment of liver function can be made by estimating the activities of serum GOT and GPT, which are originally present in higher concentrations in cytoplasm. In hepatopathy, these enzymes leak into blood stream in conformity with the extent of liver damage[16]. The elevated levels of marker enzymes (SGOT, SGPT), TP in carbon tetrachloride treated rats in the present study corresponded to the extensive liver damage. Treatment with the 250mg/kg ethanolic extract of the fruit significantly reduced the elevated liver enzymes and bilurubin level, indicating hepatoprotective action. Hepatotoxic effect of carbon tetrachloride is due to oxidative damage by free radical generation and antioxidant property is claimed to be one of the mechanisms of hepatoprotective drugs[17].

In order to provide a better understanding of the possible role of the extract of *Coccinia grandis* fruits in the hepatoprotective effect observed in this study, we carried out a preliminary phytochemical screening of the extract of the fruit and found it to contain flavonoids and glycosides. Earlier report indicated that the flavonoids are phenolic compounds exert multiple biological effects, including antioxidant properties and free radical scavenging abilities[18] Over expression of fibrogenic cytokines as well as increased transcription and synthesis of collagen can be down regulated, at least in experimental models by the use of antioxidants and a study has demonstrated that natural phenolics inhibit stellate cell activation by perturbing signal transduction pathway and cell protein expression. The co-administration of hepatoprotective agents may induce the hepatocytes to resist the toxic effects of carbon tetrachloride. Therefore, the hepatoprotective activity of the extract may be due to its antioxidant property exerted by flavanoids in the fruits.

Conclusion

Extract of the fruits of *Coccinia grandis* offers protective effect against CCl_4-induced hepatotoxicity in experimental rats. The mechanism of action is yet to be investigated but may be due to the antioxidant effects of flavonoids found to be present in the fruits.

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