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Protective effect of leaves of *Cassia alata* Linn. in CCl_4 induced Hepatotoxicity in rats

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ABSRACT

Hepatoprotective activity of ethanolic extract of leaves of *Cassia alata* was studies against CCl_4 induced hepatic injury in albino rats. Pretreatment of ethanolic extract (ECA) reduced the biochemical markers of hepatic injury like serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), bilirubin and gamma glutamate transpeptidase (GGTP). Histopathological observations also revealed that pretreatment with ECA protected the animals from CCl_4 induced liver damage. The results indicate that the leaves of *C. alata* possess hepatoprotective activity. This property may be attributed to the flavonoids present in the leaves of *C. alata*.

Key words: *Cassia alata*, hepatoprotective, carbon tetrachloride, biochemical parameters, histopathological studies.

INTRODUCTION

Liver, largest organ in the body is being evolved to maintain the body's internal milieu and also protect itself from the challenges it faces during its functioning. Since it is involved in the biochemical conversions of various endogenous and exogenously administered substances, there is a possibility of generating various highly reactive species of free radicals. In spite of this free radicals generated by hepatotoxins like CCl4 may overpower the protective mechanism of the liver and cause hepatic damage. Though the modern medicinal system has grown phenomenally, the drug for treating hepatic disease is still a dream. Hence, people are looking at traditional systems of medicines for remedies to hepatic disorders.

Cassia alata Linn. (Leguminoseae) is a shrub found throughout India, which is traditionally used for the treatment of various ailments including asthma, ringworm, skin diseases, liver disorders and rheumatism[1]. Literature review reveals that the plant possesses antiplasmodial, antimicrobial, antinflammatory, larvicidal, antimutagenic, antifungal, analgesic and hypoglycemic activities[2-4]. The plant contains flavonoids, glycosides, tannins, phenolic compounds, sterols and terpenoids[5,6]. However, there are no reports regarding the hepatoprotective activity of the leaves of this plant. Preliminary phytochemical screening of the extract shows the presence of flavonoids. Flavonoids are reported to possess various properties including hepatoprotective property[7]. The present study has been undertaken to screen for hepatoprotective activity of the leaves of Cassia alata and to verify the claim using CCl_4 induced hepatic injury model in rats.

MATERIAL AND METHODS

The leaves of *Cassia alata* were collected from foothills of Yercaud, Salem, Tamilnadu, during the month of April 2006. The plant material was identified and authenticated by the Botanist, Botanical Survey of India, Coimbatore, Tamilnadu.

A voucher specimen was kept in our laboratory for future reference. The plant material was shade dried and pulverized.

Preparation of the extract

The powdered plant material (500 g) was packed in a soxhlet apparatus and subjected to continuous hot percolation for 8h using 450 ml of ethanol (95 %v/v) as solvent. The extract was concentrated to dryness under reduced pressure and controlled temperature and dried in a desiccator (yield, 75 g, 15 %w/w). The extract was suspended in 5 % gum acacia and used for further experiments.

Animals

Swiss albino mice (20-25g) and male Wister rats (150-175 g) were procured from Venkatershwara Enterprises, Bangalore, Karnataka, India, and used throughout the study. They were housed in microlon boxes in a controlled environment (temperature 25±2 °C and 12 h dark/ light cycle) with standard laboratory diet and water *ad libitum*. The study was conducted after obtaining Institutional Animal Ethical Committee clearance.

Acute toxicity studies

Acute oral toxicity (AOT) of ECA was determined using Swiss albino mice. The animals were fasted for 3 h prior to the experiment and were administered with single dose of extracts dissolved in 5 % gum acacia and observed for mortality up to 48 h (short term toxicity). Based on the short-term toxicity, the dose of next animal was determined as per OECD guideline 425. All the animals were also observed for long-term toxicity (14 days). The LD_{50}- of the test extract was calculated using 'AOT 425' software provided by Environmental Protection Agency, USA.

Evaluation of hepatoprotective activity

Four groups of animals containing six each were used for the study. The animals from Group I served as the control and received the vehicle 5% w/v gum acacia at a dose of 1 ml/kg/day, p.o. for 7 days. Groups II – IV received 1.25 ml/kg/day p.o. of CCl_4 (Ranbaxy, Mumbai, India) for 7 days (3). The standard drug Silymarin (Micro Labs, Silyban) was administered to Group III animals in the dose of 100 mg/kg/day, p.o. for 7 days. Group IV was treated with the ECA in the dose of 200 mg/kg/day, p.o. for 7 days, respectively. The CCl_4, Silymarin and the extract were administered regularly to the respective groups of animals. On the 7th day, CCl_4 was given 30 min after the administration of silymarin and ECA. After 36 h of CCl_4 administration all the animals were killed under chloroform anesthesia. The blood samples were collected separately into sterilized dry centrifuge tubes and allowed to coagulate for 30 min and serum was collected. The separated serum was analyzed to assess various biochemical markers like serum glutamic pyruvate transaminase (SGPT)[8], serum glutamic oxaloacetate transaminase (SGOT)[8] alkaline phosphatase (ALP)[9], total bilirubin[10] and gamma glutamate transpeptidase (GGTP)[11] .

Statistical analysis

The mean values ± SEM are calculated for each parameter. For determining the significant intergroup difference each parameter was analyzed separately and one-way ANOVA was carried out. Then individual comparisons of the group mean values were done by using Dunnett's procedure.

Histopathology

After draining the blood, the abdomen of each animal was cut opened and the liver samples were excised, washed with normal saline and processed separately for histopathological observation. The ratio of wet liver weight was calculated. The livers were examined grossly, were fixed in 10% buffered neutral formalin for 48 hour and then with bovine solution for 6 hour. Paraffin sections were taken at 5 µm thickness processed in alcohol-xylene series and was stained with alum hematoxylin and eosin[12].The sections were examined microscopically for histopathological changes.

RESULTS AND DISCUSSION

Hepatoprotective activity of ECA was studied. The results of biochemical parameters revealed the elevation of biochemical markers like SGPT, SGOT, ALP, bilirubin and GGTP in toxicant treated group indicating that CCl_4 induces damage to the liver. Pretreatment with ECA (200 mg/kg) significantly reduced ($P<0.001$) the elevated levels of all the above mentioned biochemical indicators. The enzyme levels were almost restored to the normal (Table 1).

It was observed that the size of the liver was enlarged in CCl_4 intoxicated rats but it was normal in ECA treated group. A significance ($P<0.001$) in liver weight supports the findings (Table-2). Histopathological examination of the liver section of the rats treated with CCl_4 showed an intense centrilobular necrosis and vacuolization. The rats treated with silymarin and ECA showed a good sign of protection against the toxicant to considerable extent as it was evident from the formation of normal hepatic cords and absence of necrosis and vacuoles.

CCl_4 induced hepatic damage is due to its Cytochrome P-450 enzyme system catalyzed hepatic conversion into highly reactive trichloromethyl radical (CCl_3*), which upon reaction with oxygen radical gives trichloromethyl peroxide radical ($OOCCl_3$*). This radical forms covalent bond with sulphydryl group of several membrane molecules like glutathione, which is considered as the initial step in the chain of events leading to lipid peroxidation and hepatic tissue destruction[13-16]. This is evidenced by an elevation in the serum marker enzymes namely SGPT, SGOT, ALP, total bilirubin and GGTP.

The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effects or restoring the normal hepatic physiology, which has been distributed by hepatotoxins. The silymarin and the ethanolic extract of *Cassia alata* significantly decreased the CCl_4 induced elevated levels of the enzymes in the treatment group, indicating the enhancement of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the extract. Decrease in the bilirubin after treatment with ECA indicated the effectiveness of the extract in the normal functional status of the liver.

Histopathological analyses were good in agreement with the biochemical changes. The chemical constituents of *Cassia alata* responsible for their hepatoprotective activity are not known. Preliminary phytochemical studies and literature review revealed the presence of flavonoids in ECA. Flavonoids are reported to possess antioxidant and hepatoprotective properties[7,17]. The hepatoprotective activity of the leaves of *C. alata*

Table 1: Effect of ECA on biochemical markers of CCl_4 induced hepatic injury

Design of treatment	SGPTU/l	SGOTU/l	ALPU/l	Total bilirubinmg/dl	GGTPU/l
Control (Vehicle 1 ml/kg/day, p.o.)	81 ± 0.76	121 ± 0.97	168 ± 0.01	0.6 ± 0.04	129 ± 0.89
CCl_4 (1.25 ml/kg/day, p.o.)	348 ± 0.85	352 ± 0.76	408 ± 1.17	1.2 ± 0.052	253 ± 1.29
Silymarin(100 mg/kg/day, p.o.)	86 ± 0.67*	140 ± 0.97*	180 ± 1.1*	0.7 ± 0.02*	136 ± 1.33*
ECA200 mg/kg/day, p.o.)	90 ± 0.52*	151 ± 0.33*	188 ± 0.76*	0.8 ± 0.33*	140 ± 0.41*

N=6
P<0.001 Vs CCl_4 group.
Data were analyzed by One way ANOVA followed by Dunnett's *t* test.

Table 2: Effect of ECA on average liver weight of treated animals

Design of treatment	Liver weight/ 100 g of body weight
Control (Vehicle 1 ml/kg/day, p.o.)	3.8 ± 0.044
CCl_4 (1.25 ml/kg/day, p.o.)	6.03 ± 0.07
Silymarin (100 mg/kg/day, p.o.)	4.2 ± 0.062*
ECA (200 mg/kg/day, p.o.)	4.6 ± 0.05*

N=6 P<0.001 Vs CCl_4 group.
Data were analyzed by One way ANOVA followed by Dunnett's'*t* test.

may be assigned to flavonoids. However, further studies are needed for confirmation.

In conclusion, the present study demonstrated that the leaves of *Cassia alata* possess hepatoprotective activity. In addition, the hepatoprotective property may be attributed to the active principles of the plant namely, flavonoids, tannins and other polyphenolic compounds. Further study is warranted to isolate, characterize and screen the active principles from the leaves of Cassia alata that possess hepatoprotective activity.

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