EFFECT OF ETHANOL EXTRACT OF FLOWERS OF *VITEX TRIFOLIA* LINN. ON CCl_4 INDUCED HEPATIC INJURY IN RATS

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ABSTRACT

Hepatoprotective activity of ethanolic extract of flowers of *Vitex trifolia* (Verbenaceae) was studied against CCl_4 induced hepatic injury in albino rats. The plant extract (EVT) at the dose of 200 mg/kg, p.o. showed a remarkable hepatoprotective activity. CCl_4 induced a significant rise in serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), total bilirubin and gamma glutamate transpeptidase (GGTP). Treatment of rats with EVT significantly ($P<0.001$) altered serum biomarker enzyme levels to near normal against CCl_4 treated rats. The activity of the extract was comparable to the standard drug, silymarin (100 mg/kg, p.o.). Histopathological observations also revealed that treatment with EVT protected the animals from CCl_4 induced liver damage. The results indicate that the flowers of *V. trifolia* possess hepatoprotective activity on CCl_4 induced hepatic injury in rats.

Keywords: *Vitex trifolia*, 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 CCl_4 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.

Vitex trifolia Linn. (Verbenaceae) is a stout, aromatic shrub or a small tree found wild in several parts of India, which is traditionally used by the tribes and native medical practitioners for the treatment of various ailments including liver disorders, tumours, rheumatic pains, inflammation, sprains, fever and used in the treatment of tuberculosis (Anonymous, 2003). Literature review reveals that the plant *Vitex trifolia* possess larvicidal, wound healing, anti HIV, anticancer, trypanocidal, antibacterial and antipyretic activities (Kannathasan *et al*., 2007; Manjunatha *et al*., 2007; Li *et al*., 2005; Woradulayapinij *et al*., 2005; Kiuchi *et al*., 2004; Hossain *et al*., 2001; Hernandez *et al*., 1999; Ikaram *et al*., 1987). Vitexicarpin, a flavonoid was isolated from *Vitex trifolia* Linn, which induces apoptosis in K562 cells via mitochondria – controlled apoptotic pathway (Wang *et al*., 2005). Four new halimane type diterpenes, vitetrifolins D – G, were isolated from the fruits of *Vitex trifolia* (Ono *et al*., 2001). Preliminary phytochemical screening of the extract and literature survey shows the presence of flavonoids. Flavonoids have been shown to inhibit lipid peroxidation, iron induced hepatotoxicity, and acetaminophen induced lipid peroxidation and liver damage. Their antioxidant activity has been linked to all of these hepatoprotective effects (Allan and Miller, 1996). However, there are no scientific evidences regarding the hepatoprotective activity of the flowers of this plant. The present study has been undertaken to screen for hepatoprotective activity of the flowers of *Vitex trifolia* and to verify the claim using CCl_4 induced hepatic injury model in rats.

MATERIALS AND METHODS

Plant material

The flowers of *Vitex trifolia* 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 (V/03/2006). 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

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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, 90 g, 18 %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 (Protocol No. PCol/02/2006 dated 28.01.2006).

Acute toxicity studies

Acute oral toxicity (AOT) of EVT 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 (doses ranges from 500 – 2000 mg/kg at various dose levels) 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. 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 EVT 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 7^{th} day, CCl_4 was given 30 min after the administration of silymarin and EVT. 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) (Reitman and Frankel, 1957), serum glutamic oxaloacetate transaminase (SGOT) (Reitman and Frankel, 1957), alkaline phosphatase (ALP) (Kind and King, 1954), total bilirubin (Mallay and Evelyn, 1937) and gamma glutamate transpeptidase (GGTP) (Szaszi, 1969).

Statistical analysis

All values were expressed as mean ± SEM. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Dunnett's '*t*' test. *P* values < 0.05 were considered to be statistically significant when compared to CCl_4 group.

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 (Galigher and Kozloff, 1971). The sections were examined microscopically for histopathological changes.

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

Design of treatment	SGPT U/l	SGOT U/l	ALP U/l	Total bilirubin mg/dl	GGTP U/l
Control (Vehicle 1 ml/kg/day, p.o.)	81 ± 1.22	126 ± 0.96	160 ± 0.73	0.7 ± 0.025	123 ± 0.66
CCl_4 (1.25 ml/kg/day, p.o.)	342 ± 0.58	358 ± 1.11	416 ± 0.82	1.2 ± 0.05	251 ± 0.82
Silymarin (100 mg/kg/day, p.o.)	84 ± 0.93*	132 ± 0.58*	174 ± 0.82*	0.8 ± 0.03*	130 ± 1.06*
EVT 200 mg/kg/day, p.o.)	88 ± 1.15*	136 ± 0.58*	180 ± 0.82*	0.8 ± 0.06*	136 ± 1.05*

N=6; Values were expressed as mean ± SEM; P<0.001 Vs CCl_4 group.
Data were analyzed by One way ANOVA followed by Dunnett's '*t*' test.

Table 2: Effect of EVT 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.)	3.2 ± 0.12
CCl_4 (1.25 ml/kg/day, p.o.)	6.2 ± 0.058
Silymarin (100 mg/kg/day, p.o.)	3.8 ± 0.12*
EVT (200 mg/kg/day, p.o.)	4 ± 0.12*

RESULTS

Hepatoprotective activity of EVT was studied. For the acute oral toxicity studies, the extract treated animals were observed for mortality up to 48 h (short term toxicity) and for long-term toxicity (14 days). Based on the results the extract did not produce any mortality up to 2000 mg/kg body weight.

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 EVT (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 EVT treated group. A significance ($P<0.001$) in liver weight variation 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 EVT 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.

DISCUSSION

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 (Brattin *et al.*, 1985; Ahmed *et al.*, 2000; Kyung Jin Lee *et al.*, 2004; Be-Jen Wang *et al.*, 2004). 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 *Vitex trifolia* 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 EVT indicated the effectiveness of the extract in the normal functional status of the liver. Histopathological analyses were good in agreement with the biochemical changes.

Preliminary phytochemical studies and literature review revealed the presence of flavonoids, phenolic compounds and tannins in EVT. Flavonoid derivatives silymarin, apigenin, quercetin and naringenin are active against microcystin LR induced hepatotoxicity. The flavonoid rutin and venoruton showed regenerative and hepatoprotective effects in experimental cirrhosis. Their activities are due to strong free radical scavenging property (Allan and Miller, 1996; Wegener and Fintelmann, 1999, Tapas *et al.*, 2008). Therefore there is a possibility that the flowers of *Vitex trifolia* may possess hepatoprotective activity.

In conclusion, the present study demonstrated that the flowers of *Vitex trifolia* 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 flowers of *Vitex trifolia* that possess hepatoprotective activity.

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