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Hepatoprotective activity of *Enicostemma littorale* in CCl_4-induced liver damage

S. L. Vishwakarma, R. K. Goyal *

Department of Pharmacology, L. M. College of Pharmacy, Ahmedabad- 380 009, India.

Abstract

Objective: To study the effect of *E. littorale* against experimental hepatotoxicity. Methods: The hepatoprotective action of *E. littorale* was studied in carbon tetrachloride (CCl_4) induced acute liver damage in mice. Results: Concomitant treatment with aqueous extract at 250, 500 and 1000 mg/kg showed marked reduction in the ALT and AST levels as compared to CCl_4 treated group. The reduction produced by aqueous extract on ALT and AST was maximum at 500 mg/kg. Serum alkaline phosphatase (ALP) and bilirubin level was significantly raised by CCl_4 treatment. Concomitant treatment with aqueous extract at different doses showed marked reduction in the ALP and bilirubin levels as compared to CCl_4 treated group. The prolonged pentobarbitone sleeping time was significantly reduced in treatment group. The hepatic damage in animal pretreated with aqueous extract was minimal with distinct preservation of structures and architectural frame of the hepatic cells. Conclusions: Our data suggest that treatment with *E. littorale* extract enhances the recovery from CCl_4 -induced hepatotoxicity and the activity of the extract could be attributed to preservation of structural integrity of cell membrane of hepatocyte and maintaining normal function of the liver.

Key words: *E. littorale*, Hepatoprotective activity, CCl_4.

1. Introduction

A number of plants have been shown to possess hepatoprotective property *viz. Phyllanthus niruri, Tinospora cordifolia, Ricinus communis* [1] and *Swertia chirata* from the gentianaceae family [2]. *Enicostemma littorale* Blume (Gentianaceae) is a glabrous perennial herb belonging to the family Gentianaceae. It is called Chota-kirayata or Chota chirayata in Hindi, Mamejavo in Gujarati, Nagajivha in Bengal and Vellarugu or Vallari in Tamil [3]. *E. littorale* plant has been used as a folk medicine for the treatment of diabetes mellitus in Western and Southern India [4]. Ethnomedical studies of North Gujarat (India) reveal the use of hot aqueous extract of *E. littorale* by the tribal inhabitants for the treatment of diabetes, fever, stomach ache, dyspepsia and malaria in interior part of Gujarat. Studies from our laboraory have

*Corresponding author
Email: goyalrk@hotmail.com

shown that aqueous extract of *E. littorale* decreases AST and ALT levels in diabetic treated rats (unpublised data).

Iridoids, a widely distrubuted class of natural product have shown encouranging biological activities including hepatoprotective [5]. Swertiamarin, a secoiridoid glycoside is one of the major compounds in *E. littorale.* There are no pharmacological data available to substantiate the therapeutic value of *E. littorale* in liver disorders. Therefore, in the present study, the hepatoprotective effect of the aqueous extract was evaluated on CCl_4-induced acute liver damage in the mice.

2. Material and methods

2.1 Plant material

Whole plant material of *E. littorale* was collected from Gujarat (India) in August–September (2000) at the end of flowering season.The plant was identified by comparing it morphologically and microscopically as mentioned in different standard texts and floras. Professor O. P. Saxena, Head of the Botany Department, Gujarat University, Ahmedabad, India authenticated its identity. House specimen was deposited at Botany Department, Gujarat University, Ahmedabad, India.The plant was cleaned and dried in shade and powdered to 40 # and the powdered was stored at 25°C.

2.2 Preparation of aqueous extract

One kg of shade-dried herb contaning all vegitative and reproductive parts of *E. littorale* was powderd and boiled with 4 lit water for 8 h then filtered. The filtrate was then concentrated under reduced pressure obtaining yield of 85.23g.

2.3 Preliminary phytochemical screening

The aqueous extract of *E. littorale* was subjected to various preliminary phytochemical tests [6] for the presence or absence of various classes of compounds.

2.4 Experimental animals

Swiss albino mice (20-25 g) of either sex were selected for the study. Animals were maintained under a 12 h light / dark cycle in a temperature and humidity controlled room, with free access to food and water. They were initially acclimatized for the study and the study protocol was approved by the Institutional Animal Ethics Committee of our college as per the requirements of Committee for the Purpose of Control and Supervision on animals (CPCSEA), New Delhi.

2.5 CCl_4- induced liver injury and treatment protocol

Carbon tetrachloride was administered at a dose of 0.8 ml/kg i.p. (30% solution in liquid paraffin) given for 7 days. Silymarin (Ranbaxy) (100 mg/kg p.o.) was used as a standard. The dose of silymarin was selected as reported by Navaro *et al.* [7]. Swiss albino mice (20-25 g) of either sex were selected for the study. A total of 36 animals were equally divided into 6 groups (n = 6 in each group).

Group I, which served as normal control, received distilled water intraperitoneally. Group II received CCl_4 0.8 ml/kg, i.p. once daily for 7 days. Group III received CCl_4 0.8 ml/kg, i.p. and silymarin 100 mg/kg, p.o. simultaneously for 7 days. Group IV received CCl_4 0.8 ml/kg, i.p. and aqueous extract 250 mg/kg p.o. simultaneously for 7 days. Group V received CCl_4 0.8 ml/kg, i.p. and aqueous extract 500 mg/kg p.o. simultaneously for 7 days. Group VI received CCl_4 0.8 ml/kg, i.p. and aqueous extract 1000 mg/kg p.o. simultaneously for 7 days.

At the end of the treatment, blood samples were collected after 6 h after the last treatment from tail vein and the serum was separated for the assay of marker enzymes *viz.*, aspartate aminotransferase (AST), alanine amino-transferase (ALT), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, total protein (TP) and albumin (Alb).

Table 1.
Effect of CCl_4 and aqueous extract of *E. littorale* on various parameters

Parameters	Normal control	CCl_4 treated	CCl_4 treated with			
			silymarin	aqueous extract (250 mg/kg)	aqueous extract (500 mg/kg)	aqueous extract (1000 mg/kg)
Alkaline phosphatase (KA units)	9.8 ± 1.6	29.7 ± 5.9*	13.3 ± 0.6**	10.4 ± 0.9**	10.0 ± 0.7**	18.7 ± 0.8**
Total bilirubin (mg %)	0.3 ± 0.08	21.7 ± 0.5 *	0.7 ± 0.2**	0.6 ± 0.2**	0.5 ± 0.1**	0.8 ± 0.2**
Direct bilirubin (mg %)	0.09 ± 0.07	0.6 ± 0.2 *	0.2 ± 0.2**	0.2 ± 0.08**	0.1 ± 0.09**	0.4 ± 0 .02**
Total protein (mg %)	5.1 ± 0.08	4.0 ± 0.05	4.6 ± 0.2	4.6 ± 0.06	4.6 ± 0.1	4.8 ± 0.09
Albumin (mg %)	3.2 0 ±.1	3.6 ± 0.04	2.9 ± 0.1	2.7 ± 0.1	2.3 ± 0.2	2.9 ± 0.05
Pentobarbitone sleeping time (min)	40.0 ± 17.9	438.0 ± 54.9*	177.0 ± 34.2**	157.5 ± 54.2**	122.6 ± 34.2**	201.6 ± 23.1**

Values are mean±S.E.M, n = 6-8; * Significantly different from normal control $P < 0.05$; ** Significantly different from CCl_4 control $P < 0.05$.

2.6 Enzyme assays

The activities of serum hepatic marker enzymes namely AST, ALT and ALP were estimated by enzymatic method using their respective kits (AST, ALT, ALP kits respectively obtained from Span Diagnostics Limited, Surat. The results were expressed as units/ liter (IU/L).

2.7 Protein and Bilirubin estimation

The levels of total protein (TP) and albumin (Alb) were estimated in serum of animals by modified biuret method and Dumas method. Bilirubin was determined spectrophotometrically by the modified method of Malloy and Evelyn. Standard kits (Span Diagnostics Limited, Surat) were used for these estimations.

2.8 Pentobarbitone-induced sleeping time

In a group of mice, the sleeping time was measured using sodium pentobarbitone (35 mg/kg, i.p.). The sleeping time was calculated as the interval lapsing between the loss and recovery of the righting reflex.

2.9 Histological observation

Animals were sacrificed on the day of withdrawal of blood and liver was removed, sliced and washed in saline. Liver sections were fixed in 10 % formalin solution. After dehydration, the tissue was embedded in paraffin, cut into 3-5 µm sections, stained with the haematoxylin-eosin dye and finally, observed under a photomicroscope and morphological changes such as cell necrosis, ballooning degeneration, fatty changes or inflammation of lymphocytes were observed.

2.10 Statistical analysis

Result were analyzed statistically using analysis of variance (ANOVA) followed by Tukey's test. Values of $P< 0.05$ were considered significant.

3. Results

Preliminary phytochemical screening of aqueous extract fraction showed the presence of triterpeniods, flavanoids, alkaloids and

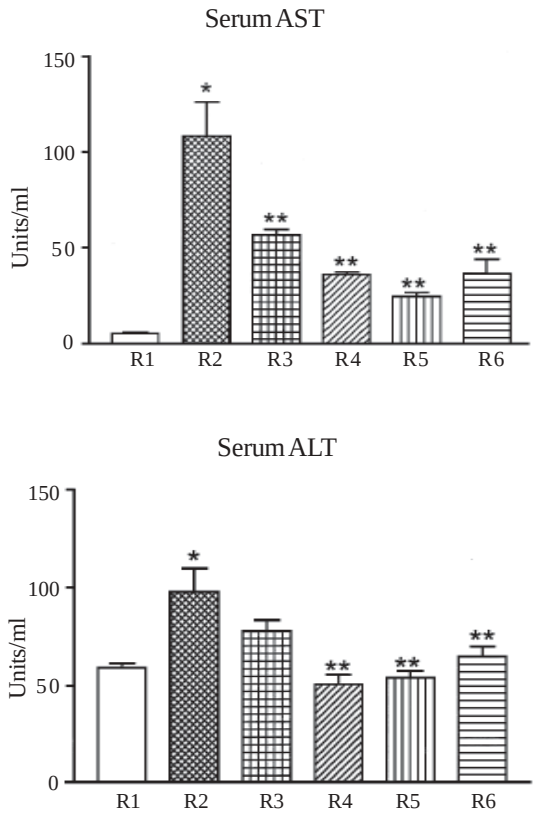

Fig. 1. Effect of aqueous extract treatment on serum AST and ALT in CCL_4 induced liver toxicity.
Each bar represents Mean ± S.E.M. number of animals in each group = 6. R1 = normal control, R2 = CCl_4 treated, R3 = CCl_4 + silymarin 100 mg / kg, R4 = CCl_4 + aqueous extract 250 mg / kg, R5 = CCl_4 + aqueous extract 500 mg/kg, R6 = CCl_4 + aqueous extract 1000 mg/kg.
*Significantly different from normal control,
**Significantly different from CCl_4 control $p<0.05$.

coumarins while saponins, anthraquinone, tannins and phenols were absent. ALT and AST levels were significantly increased in CCl_4 treated group when compared with the normal control group. Concomitant treatment with aqueous extract at 250, 500 and 1000 mg/kg showed marked reduction in the ALT and AST levels as compared to CCl_4 treated group (Fig. 1).

The reduction produced by aqueous extract on ALT and AST was the maximum at 500 mg/kg. Silymarin treated group showed decrease in ALT and AST levels (Fig.1). Serum ALP level was significantly raised by CCl_4 treatment as shown in (Table 1). Concomitant treatment with aqueous extract at different doses showed marked reduction in the ALP levels as compared to CCl_4 treated group. Similar results were observed in Silymarin treated group (Table 1).

There was a significant increase in the total bilirubin content in CCl_4 treated group when compared with the control one. The increased serum bilirubin level was significantly declined in animals receiving aqueous extract.

These results were comparable to that of silymarin (Table 1). No significant change was observed in total protein and albumin levels after CCl_4 alone and aqueous extract plus CCl_4 treated animals, when compared with the control values (Table 1). Pentobarbitone-induced sleeping time in mice was prolonged by CCl_4 treatment. There was significant reduction in the pentobarbitone-induced sleeping time by aqueous extract in CCl_4 treated group.

These results were comparable with the result of silymarin treated animals (Table 1). Histopathological studies of liver of animal in group II showed zonal necrosis around central vein, inflammatory changes, lymphocyte infiltration, vacuolation of hepatocyte (Fig.2b). The hepatic damage in animal pretreated with aqueous extract or silymarin was minimal with distinct preservation of structures and architectural frame of the hepatic cells (Fig. 2c & 2d).

4. Discussion

We found that aqueous extract of *E. littorale* produced significant reduction in CCl_4 induced increase in AST, ALT also ALP levels. It was also found to preserve the structural integrity

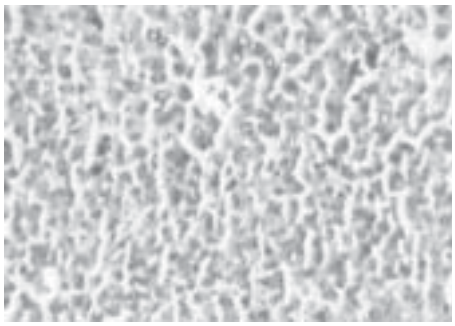

Fig. 2a. Normal rat liver

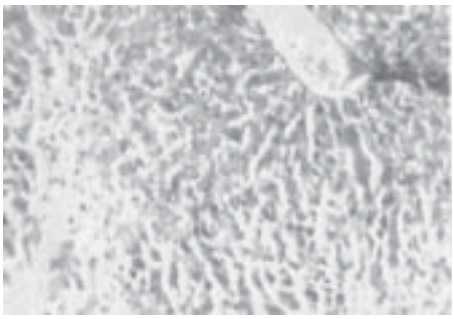

Fig. 2b. Liver of rat treated with CCl_4

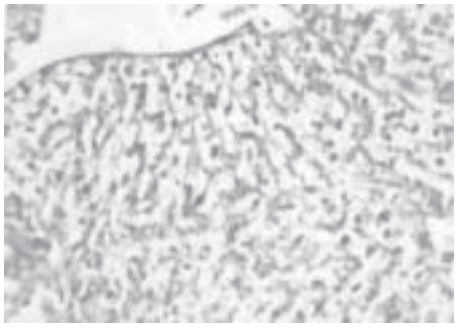

Fig. 2c. Liver of rat treated with aqueous extract of *E. littorale* (500 mg/kg)

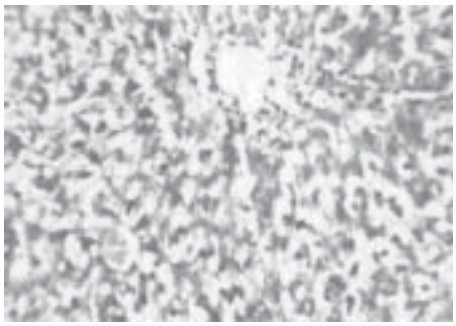

Fig. 2d. Liver of rat treated with silymarin (100 mg/kg).

of the hepatocellular membrane. All these results suggests that *E. littorale* exerts a protective action against CCl_4-induced hepatic damage. The action was prominent at the dose of 500 mg/kg of aqueous extract of *E. littorale*. The marked reduction by aqueous extract of *E. littorale* in CCl_4 induced elevated levels of bilirubin in mice further substantiates the hepatoprotective action of *E. littorale.*

It is known that CCl_4 toxicity is dependent on one of its highly reactive product the trichloromethyl radical (CCl_3^-). This radical binds covalently to neighbouring proteins and lipids, and initiates lipid peroxidation that causes severe membrane alterations this in turn causes leaking of transaminases through damaged membrane and there by resulting in the elevation of transaminases in plasma/serum [8].

Many compounds exhibit hepatoprotective activity against CCl_4 either by decreasing the production of CCl_3 free radical or by impairment of CCl_4 induced lipid peroxidation [9]. The rise in serum levels of AST, ALT and ALP following CCl_4 administration could also be attributed to the damaged structural integrity of the liver cell membrane [10] causing leakage of the cellular enzymes into the blood. Inhibition of CCl_4 bioactivation could reduce this toxic effect of CCl_4.

It is possible that aqueous extract of *E. littorale* produces reduction in the levels of AST, ALT and ALP by preserving the structural integrity of the liver cell membrane. We found that aqueous extract of *E. littorale* not only reduces the levels of various marker enzymes of liver but also preserves the structural integrity of the

hepatocellular membrane as revealed from histological studies.

There was no change in serum protein levels in control, CCl_4 alone and drug treated animals. This observation is in accordance with the observation that proteins in general and albumin level in particular remain unchanged in acute liver damage [11].

It has been established that since barbiturates are metabolized exclusively in the liver, the sleeping time after a given dose is a measure of hepatic metabolism. If there is any pre-existing liver damage, in this case by CCl_4-toxicity, the sleeping time after a given dose of the barbiturate will be prolonged because the amount of the hypnotic broken down per unit time will be less [12].

We found that aqueous extract of *E. littorale* reduces the CCl_4-induced prolongation of the pentobarbitone sleeping time in mice. This further supports the antihepatotoxic potential of the aqueous extract of *E. littorale.*

Phytochemical studies of this plant showed that it contains triterpeniods, flavonoids, alkaloids and coumarins. Some flavonoids have been reported to inhibit drug metabolism. It is also possible that the active component in the aqueous extract of *E. littorale* may owe its antihepatotoxic effect to the inhibition of the biotransformation of CCl_4 into the active free radical CCl_3. Flavonoids are also known to scavenge free radicals [13].

Based on the results of the present study, it can be suggested that the aqueous extract of *E. littorale* prevents changes in plasma enzyme concentration and other metabolic concentrations as well as diminish the destruction of liver cell architecture initiated by administration of CCl_4. Further studies with isolated active principles of the plant may throw more light on the use of *E. littorale* for hepatoprotective activity.

Based upon the results of the present study, it can be concluded that the aqueous extract of *E. littorale* prevent changes in plasma enzyme concentration and other metabolic concentrations as well as diminish the destruction of liver cell architecture initiated by administration of CCl_4. Further studies with isolated active principles of the plant may throw more light on its exact use.

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