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Research Article

EFFECT OF ENTADA PURSAETHA DC. AGAINST EXPERIMENTALLY INDUCED HEPATOTOXICITY IN WISTAR RATS

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ABSTRACT

This study was conducted to investigate the protective activity of alcoholic extract prepared from the stem of *Entada pursaetha* (PSE) against carbon tetrachloride (CCl_4)-induced hepatotoxicity in male wistar rats. The animals were divided into six groups (groups I to VI) with six animals in each and different treatments were given for seven days. Groups I and II were naïve and vehicle control groups and were given NSS and 2% polysorbate 80 aq. solution, respectively. In group III, hepatoprotective drug, silymarin, was given orally @ 50 mg/kg bwt. as standard control. In groups IV to VI, hepatoprotective effect of PSE was determined at three different oral doses of 30, 100 and 300 mg/kg bwt., respectively. On 7th day, three hour after the last administration, CCl_4 intoxication (@2ml/kg b.wt., 1:1 v/v in olive oil,i.p.) was used to induce acute hepatotoxicity in all groups except group I. Animals of all groups were sacrificed 24 hour after the CCl_4 administration. Liver weight, relative liver weight, alkaline phosphatase (ALP) and creatinine levels were determined in different groups. The PSE @ 100 and 300 mg/kg b. wt. resulted in a significant dose dependent hepatoprotective effect comparable to standard control, by preventing increase in liver weight and relative liver weight. In addition, serum ALP level in rats given PSE at all the three doses was significantly lower than vehicle control rats. There was no significant effect of PSE on serum creatinine at any of the three dose levels in comparison to vehicle control group. The present study reveals that alcoholic extract of *E. pursaetha* has significant hepatoprotective activity similar to standard drug, silymarin, in CCl_4^- induced acute hepatotoxicity in rats.

Key words: *Entada pursaetha*, silymarin, rats, carbon tetrachloride, hepatoprotective effect

Liver diseases are mainly caused by toxic chemicals, excessive drug therapy, excessive consumption of alcohol, infections and autoimmune disorders. Inspite of advances in modern medicine, there are not much effective drugs available that stimulate liver function, offer protection to the liver damage or help to regenerate hepatic cells (Chattopadhyay, 2003). Herbal drugs offer an alternative approach to drug discovery systems with their multifactorial and multitarget actions. A number of medicinal preparations in ayurveda are recommended for the treatment of liver disorders and their usage is in vogue since centuries and quite often claimed to offer significant relief (Chatterjee, 2003). *Entada pursaetha* DC (Mimosae) is an endangered woody liana commonly found in forests from Northern Luzon (Cagayan) to Mindanao and Palawan and widely distributed in tropical Africa, India, China, Philippines, Guam and northern Australia. Seeds and stems of *E. pursaetha* have been used in the treatment of various liver ailments since centuries in Ayurvedic and African systems of medicine. But all this is known by its use in folklore. However, there is no scientific evidence available to support the ethnic/folklore use of *E. pursaetha.*

Studies have revealed different extracts of leaves/ bark of *Entada africana* had free radical scavenging activity (Tibiri *et al.*, 2007) and anti-inflammatory, antiulcer and wound healing properties (Diallo *et al.*, 2001). Similarly, anti-inflammatory activity has been reported for alcoholic extract of *Entada abssyinica* (Olajide and Alada, 2001) and anti-ulcer activity for *Entada phaseoloides* (Ramakrishna *et al.*, 2008). Some triterpene and saponins have been reported to be hepatoprotective (Cioffi *et al.*, 2006). There seems to be no scientific study in the literature regarding hepatoprotective activity of *E. pursaetha.* Therefore, the present study was conducted to evaluate the hepatoprotective potential of alcoholic extract of *E. pursaetha* against carbon tetrachloride (CCl_4)-induced

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liver injury in rats. Also, its hepatoprotective effect was compared to the effect of silymarin, a known hepatoprotective agent against CCl_4 or acetaminophen induced liver damages (Chrungoo *et al.*, 1997).

MATERIALS AND METHODS

Collection of Plant Material: The stems of *E. pursaetha* were obtained from the jungles of Bhawanipatna, district Kalahandi, Odisha (India). The plant specimen was botanically authenticated. A voucher specimen has been maintained in the Department of Pharmacology and Toxicology, Indian Veterinary Research Institute, Izat Nagar Bareilly (India) for ready references.

Extract Preparation: The stems collected from the fully mature plant were shade dried, powdered and then extracted with 85% ethanol under reflux. The ethanolic extract of stem was concentrated to a semi-solid mass under reduced pressure and made free from any solvent. The alcoholic extract of *E. pursaetha* stem was hereafter, referred to as "PSE" and was further used in all the experimental studies. The extract was then suspended in 2% polysorbate 80 and used in different pharmacological studies. The yield of the extract (PSE) by the above described method was 8.4% with reference to dry starting material.

Animals: Colony bred 36 adult male wistar rats of 6-8 weeks of age (weight 180-220g) were obtained from Laboratory Animal Resource Section of the Institute and acclimatized for 7 days to the laboratory environment, prior to the start of the experiment. The animals were kept in polypropylene cages with chopped paddy straw as the bedding material in a temperature-controlled room (22 ± 2^0C) with relative humidity of 30-70%. A 12:12 light:day cycle was followed. The animals were maintained on a balanced ration obtained from the Feed Technology Unit of the Institute. Fresh drinking water was provided to the animals daily ad libitum. All animal experiments were carried out according to CPCSEA guidelines, after getting the approval of the Institute's Animal Ethics Committee.

Carbon Tetrachloride-Induced Hepatotoxicity: Assuming that PSE may act as hepatoprotective agent, chemical model employing CCl_4 (Merck Chemicals) was used to produce hepatotoxicity in rat (Similie *et al.*, 2001). Rats were divided into six groups with six animals in each group. Group I received normal saline solution (NSS) by oral gavage for seven days and served as the naïve control. Group II received 2% polysorbate 80 aqueous solution by oral gavage for seven days and acted as a vehicle control. Proportionate volume of NSS and polysorbate 80 were given in naïve and vehicle control groups, respectively as given in other drug treated groups. Group III served as standard control and received silymarin by oral gavage at 50mg/kg as suspension in 2% polysorbate 80 aqueous solution for seven days. Groups IV, V and VI received PSE by oral gavage at 30, 100 and 300 mg/kg, respectively as suspension in 2% polysorbate 80 aqueous solution, for seven days. The CCl_4@ 2ml/kg bwt. (1:1 v/v in olive oil) was given intraperitonealy for production of hepatotoxicity on 7^{th} day, 3 hour after the last drug administration in all groups except group I (naïve control). Dose and route of CCl_4 were selected on the basis of literature (Raja *et al.*, 2007) and exploratory studies.

Liver Weight and Relative Liver Weight Estimation: At the end of experiment, 24 hour after CCl_4 administration, animals of all groups were sacrificed under ether anesthesia. When animals were under anesthesia, blood samples were collected by cardiac puncture. The blood was allowed to clot for 30 min at room temperature; serum was separated and was kept at-20^0C till further use. Livers were excised rapidly by midline laparotomy, rinsed in normal saline, blot dried and weighed. As all the animals were weighed before being sacrificed, relative liver weight was determined as liver weight per 100 gm of body weight.

Serum Alkaline Phosphatase (ALP) and Creatinine Estimation: The activity of serum ALP was estimated by Kind and King´s method (King and Armstrong, 1934) and the results were expressed in IU/L. Serum level of creatinine was estimated following Jaffe's reaction (Husdan and Rapoport, 1968) and the results were expressed in mg/dl.

Statistical Analysis: Statistical analysis of data was performed using Graph pad prism and Microsoft Excel. Data were analyzed by ANOVA and means of various parameters were compared with Tukey's multiple comparison post-hoc test. A value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Carbon tetrachloride is a potent hepatotoxin producing centrilobular hepatic necrosis and widely used for animal models of hepatotoxicity. It is biotransformed by the NADPH-cytochrome P-450 in the liver endoplasmic reticulum to the highly reactive trichloromethyl (CCl_3O^-) free radical by reductive dehalogenation (Ha *et al.*, 2005). This free radical in turn reacts with oxygen to form a trichloromethylperoxy radical, which may attack lipids on the membrane of endoplasmic reticulum more readily than the trichloromethyl free radical. The trichloromethylperoxy radical leads to elicit lipid peroxidation, the disruption of Ca^{+2} homeostasis, elevation of hepatic enzymes and finally results in cell death (Clawson, 1989). Acute poisoning with CCl_4 becomes manifested as a multisystem disorder, involving the liver, kidneys, brain, lungs, adrenal glands, and myocardium (Gitlin, 1996).

There was a significant ($P<0.05$) increase in liver weight and relative liver weight in the vehicle control group (group II) as compared to naïve control group (group I) (Table 1) indicating inflammation of the hepatocytes, produced by the oxidative and nitrosative stress by the exposure to CCl_4. Pre-treatment with PSE (groups V and VI) and standard drug silymarin (group III) showed significant ($P<0.05$) improvement in liver weight and relative liver weight when compared with the vehicle control group (group II) (Table 1). The improvement in liver weight towards normalcy after pre-treatment with PSE might be due to its antioxidant and anti-inflammatory action on hepatocytes. However, PSE@30 mg/kg (group IV) failed to reverse liver weight and relative liver weight significantly ($P<0.05$) in comparison to vehicle control group (group II).

Our data in this study revealed a significant ($P<0.05$) increase in serum ALP level in group II as compared to group I (Table 1). During liver injury, these enzymes being cytoplasmic in nature, enter in to the circulatory system due to necrosis or altered permeability of membrane (Maiti *et al.*, 2005). Administration of PSE at all three doses (groups IV, V and VI) significantly ($P<0.05$) decreased the increased levels of this biomarker, produced by CCl_4 administration and caused a subsequent recovery towards normalization almost like that of silymarin treatment (group III) (Table 1). This is an indication of the stabilization of permeability of plasma membranes as well as repair of hepatic tissue damage caused by CCl_4 administration. It has been reported that serum levels of transaminases return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes (Maiti *et al.,* 2005).

Administration of CCl_4 induced a significant ($P<0.05$) increase in serum creatinine level in group II as compared to group I (Table 1). Marked increase of serum creatinine indicates a severe damage to kidney tissue membranes due to CCl_4 exposure. This enzyme is cytoplasmic in nature and enters into the circulatory system during kidney injury (Recknagel and Glende, 1983). The CCl_4 induced increased level of serum creatinine was not significantly ($P<0.05$) increased further by any of the doses of PSE (groups IV, V and VI), which indicates that it has no nephrotoxic effect. However, administration of standard drug silymarin

Table 1
Effects of *Entada pursaetha* stem extract on organ weight and some biochemical parameters in CCl_4-induced hepatotoxicity in rats

Treatment	Dose (mg/kg)	Liver weight (g)	Relative liver weight (g/100 g body weight)	Serum ALP (IU/L)	Serum creatinine (mg/dl)
Naive control	-	8.43±0.20	4.34±0.10	170.74±3.24	0.60±0.07
Vehicle control	-	12.27±0.32†††	6.17±0.17†††	276.36±6.05†††	1.90±0.07†††
Silymarin	50	9.12±0.21***	4.59±0.11***	196.29±7.03***	1.53±0.08*
PSE	30	11.56±0.44	5.78±0.21	251.74±5.19*	2.05±0.08
PSE	100	10.38±0.25**	5.11±0.13***	227.90±4.74***	1.84±0.05
PSE	300	9.49±0.37***	4.72±0.19***	203.34±5.10***	1.77±0.10

n=6, †††p<0.001 in comparison to naïve control; * p<0.05; **p<0.01; *** p<0.001 in comparison to vehicle control in Tukey's multiple comparison post hoc test. PSE=*Entada pursaetha* stem extract; ALP=Alkaline phosphatase

(group III) showed significant ($P<0.05$) reduction in serum creatinine level when compared with the vehicle control group (group II).

Phytochemical screening of *E. pursaetha* seed revealed the presence of triterpenes, saponins, tannins, flavanoids alkaloids and oleogenic acid (Larsen *et al.*, 1973). Many triterpenoids and their glycosides have been shown to possess antioxidant activity (Montilla *et al.*, 2003). These antioxidant phytochemicals might contribute to its hepatoprotective effect. In conclusion, this study reveals that *Entada pursaetha* may be a promising candidate herb for the development of a phytomedicine against liver ailments, however, its hepatoprotective mechanism needs to be elucidated further.

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