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Hepatoprotective Effect of the Ethanolic Extract of *Urtica parviflora* Roxb. in CCl₄ Treated Rats

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Abstract: The ethanolic extract of leaves of *Urtica parviflora* (EEUP) was evaluated for the hepato protective effect in carbon tetrachloride (CCl₄) induced hepatotoxicity in rats to prove its ethnomedicinal claim by the hill people of Sikkim. Hepatotoxicity was induced in Swiss Albino male rats of Sprague Dawley strain by subcutaneous injection of carbon tetrachloride at the dose of 1 mL kg⁻¹ body weight. The hepatoprotective activity was evaluated by the assay of liver function biochemical parameters such as aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), alkaline phosphatase (ALP), total bilirubin, serum protein and by study of histopathology of the livers. The toxic effect of carbon tetrachloride was controlled significantly by the EEUP at 250, 500 and 700 mg kg⁻¹ p.o. (p<0.05) as compared to the CCl₄ treated animals by restoration of the levels of serum bilirubin, proteins and hepato protective enzymes. Histopathological studies revealed that the centrolobular necrosis induced by CCl₄ was recovered to normal state by EEUP in a dose dependent manner. The study confirms the possible hepatoprotective potentiality of the ethanolic extract of leaves of *Urtica parviflora* which had been collected from Sikkim. Studies are under process to isolate and characterize the bioactive component present in the plant as well as to establish the mechanism of action underlying for its hepatoprotective potentiality.

Key words: Hepatoprotective activity, *Urtica parviflora*, carbon tetrachloride

INTRODUCTION

Urtica parviflora Roxb. (Urticaceae) is a perennial shrub used in traditional medicine in Sikkim, Darjeeling and in North Bengal (Gurung, 1999). The roots are employed for the treatment of fractures of bone and dislocations of joints (Ramachandran, 1992). The leaves are used in dysentery, joint pain and liver disorders (Gurung, 1999). The inflorescences are used as cleansing agent after parturition and in the treatment of dermatitis in the alpine region of central and eastern Himalayas (Ramachandran, 1992). Urtication was practiced for the treatment of certain diseases and consisted of beating the skin with a bunch of nettles (White, 1887). The result was erythema and whealing but after the third or fourth successive application, the skin ceased to react under fresh contact (White, 1887). Acetylcholine, histamine and 5-hydroxytryptamine have been implicated in itching from the stinging hairs (Emmelin and Feldberg, 1947; Saxena *et al.*, 1965). The hairs are described by Thurston and Lersten (1969) and Uphof and Hummeh

(1962). Recently, Oxalic acid and tartaric acid were isolated from other species of *Urtica* (*U. thunbergiana*) as major long-lasting pain-inducing toxins (Fu *et al.*, 2006). The roots of stinging nettle (*Urtica dioica*) had been studied for the treatment of Benign Prostatic Hyperplasia (BPH) and associated Lower Urinary Tract Symptoms (LUTS) (Egon, 2001). Liver is an important organ actively involved in many metabolic functions and is the frequent target of number of toxicants (Meyer and Kulkarni, 2001). Therefore, the disorders associated with this organ are numerous and varied (Wolf, 1999). Liver disorders have long been recognized as one of the most important health problems in the developing countries. Hepatitis is one of the most common diseases in the Eastern Himalayan region of Sikkim (Roy Burman, 2003). In absence of a reliable liver protective drug in the modern medicine, there are number of medicinal preparations in Ayurveda, recommended for the treatment of liver disorders (Chatterjee, 2000). In view of this, the present study has been undertaken to investigate the hepatoprotective activity of *U. parviflora* Roxb. leaves against the CCl₄ induced hepatotoxicity in albino rats.

MATERIALS AND METHODS

Plant materials: The leaves of *Urtica parviflora* Roxb was collected from Majhitar, East Sikkim, India in March 2006. The plant was identified by the Botanical Survey of India (BSI), Gangtok, Sikkim. The voucher specimen (HP-124) has been retained in our laboratory for future reference. The collected leaves were air-dried and pulverized in a mechanical grinder.

Preparation of extracts and phytochemical study: The leaves (500 g) were coarsely powered and subjected to successive solvent extraction with petroleum ether (60-80°C), benzene, chloroform, ethanol and water. In the preliminary hepatoprotective studies, ethanolic extract shown to have better protection than the other extracts. Therefore the ethanolic extract was further used for pharmacological screening. The extract was suspended in aqueous Tween 80 solution (0.5%). The chemical constituents of the extracts was identified by qualitative chemical tests and further confirmed by thin layer chromatography for the presence of alkaloids, sterols, tannins, reducing sugars and flavonoids (Trease and Evans, 1996).

Animals: Swiss Albino male rats of Sprague Dawley strain, weighing 150-175 g each, were used. They were housed under standard conditions of temperature (23±1°C) and relative humidity (55±10%); 12/12 h light/dark cycle and fed with standard pellet feed and water *ad libitum*. The Institutional Animal Ethics Committee reviewed the entire animal protocols prior to the experiment (No. HPI/IAEC/PK/08/2006).

Experimental induction of liver damage: Liver damage was induced in rat by administering CCl₄ subcutaneously in the lower abdomen at the dose of 1 mL kg⁻¹ body weight except the animals of first group. CCl₄ was administered on every first and fourth day of the week up to 13 weeks. The rats were divided into 5 groups, 8 animals in each. Group 1 served as control, receiving Tween80 solution (0.5%) orally. Group 2 received only CCl₄. In a pilot study it was observed that the ethanolic extract showed prominent liver protection against CCl₄ induced liver damage. The ethanol extract of *U. parviflora* Roxb leaves (EEUP) was administered orally to groups 3, 4 and 5 at a dose of 250, 500 and 750 mg kg⁻¹ p.o. body weight, respectively. Reference drug Silymarin 100 mg kg⁻¹ p.o. was administered to Group 6 animals in Tween80 solution (0.5%). Every day at 9.00 am, a known quantity of food was replenished. The animals were kept starved over night one day before the last day of the

experiment. On the next day they were sacrificed and blood was collected making an incision on jugular vein.

Enzyme assay: The serum was separated from the blood for biochemical estimation by centrifugation at 2500-3000 rpm. Different parameters like serum alanine aminotransaminase (ALT), aspartate aminotransaminase (AST) and alkaline phosphatase (ALP) activity were measured according to the method of Reitman and Franckel (Reitman and Frankel, 1957).

Estimation of total protein and bilirubin: The level of total protein (TP) was estimated in the serum of the animals by Biuret method (Kingsley and Frankel, 1964). The level of bilirubin was estimated by the method of Mallory with slight modification (Mallory and Evelyn, 1939).

Histopathological examination: The liver lobes of the animals were removed and washed with normal saline (Fig. 1a). Small pieces of liver tissue were preserved in 10% formalin solution for histological analysis. The pieces were dehydrated with 90% ethanol, embedded in paraffin, cut into thin sliced sections (7 µm thick), stained with haematoxylin-eosin dye and observed under a light microscope, for cell necrosis, vascular degenerative changes, inflammation and fibrosis.

Statistical analysis: The data were analysed statistically using one-way analysis of variance followed by Dunnett t-test. The data are expressed as mean±SEM p-values less than 0.05 indicate significance.

RESULTS AND DISCUSSION

The phytochemical studies (chemical test and TLC) revealed that presence of alkaloids, sterols, tannins, flavonoids and reducing sugar (Table 1). The biochemical mechanism of hepatoprotective activity of the EEUP, the levels of ALT, AST, ALP, total protein and bilirubin were shown in Table 2. EEUP has shown dose dependent and significant (p<0.05 compared to CCl₄ group) hepatoprotective effect at the dose levels 250, 500 and 750 mg kg⁻¹ p.o. The increase in the level of hepatic enzymes and serum bilirubin reflected the depth of jaundice, cellular leakage and the loss of cellular integrity of the cell membrane (Sarawat *et al.*, 1993). The ethanol extract at the dose level of 750 mg kg⁻¹ p.o decreased the elevated levels of ALT, AST, ALP and Bilirubin at the levels of 47.9, 58.3, 63.7 and 0.74 IU L⁻¹ (Table 2) after CCl₄ induction. Single dose of CCl₄ caused centrolobular necrosis, extending to midzone with neutrophilic collection inside the lobule (Fig. 1b). The decrease in

Table 1: Thin layer chromatography study of the ethanol leave extract of *U. parviflora*

Chemical group	Solvent system	Spray reagent	R _f values
Alkaloids	Chloroform: Methanol (9:1)	Dragendroff's	28
Sterols	Chloroform: Methanol (1:1)	Liebermann Burchard	65-75
Tannins	Ethyl acetate: Benzene (9:11)	Folin	0-5
Reducing sugars	n-Butanol: Acetic acid: Water (4:1:5)	Aniline hydrogen phthalate	15-22
Flavonoids	Toluene: Ethyl acetate: Acetone (2:4:4)	5% alcoholic AlCl ₃	35

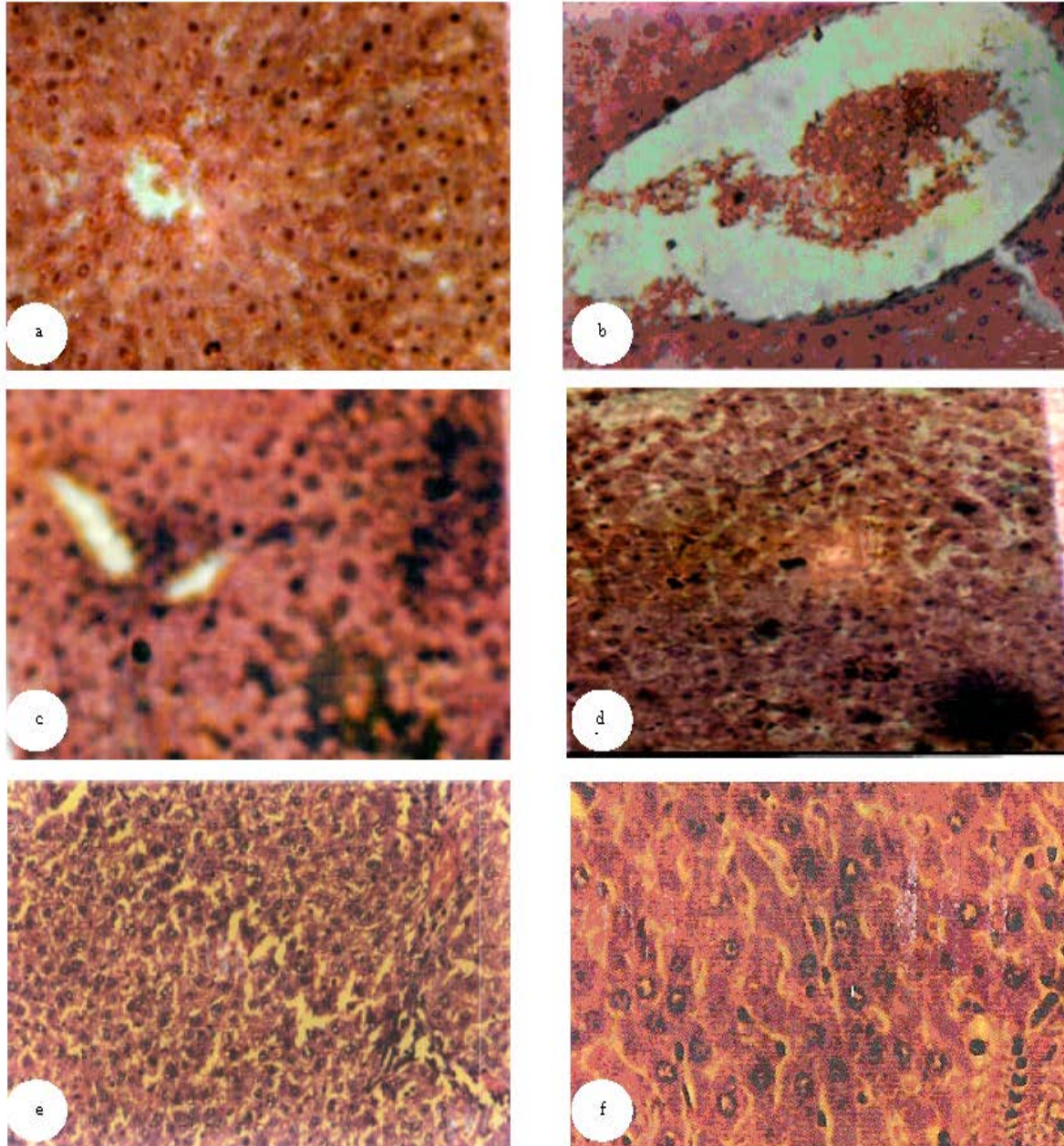


Fig. 1. Photomicrographs of liver sections of rat stained with haematoxylin and eosin (x100). (a) Liver section from normal rat showing normal liver architecture with normal hepatocyte morphology, (b) Liver section from CCl₄-treated rat showing centrilobular necrosis extending to midzone with neutrophilic collection, (c) Liver section recovering from CCl₄ induced toxicity in EEUP 250 mg kg⁻¹ treated rat (d) Liver section depicting the clear bile canaliculi; normal distribution of kupffer cells and sinusoidal cells in EEUP 500 mg kg⁻¹ treated rat and (e, f) Liver architecture almost normal in EEUP 750 mg kg⁻¹ and Silymarin 100 mg kg⁻¹ treated rats

Table 2: Effect of ethanol leaves extract of *U. Parviflora* on CCl₄ induced hepatotoxicity in rats

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
ALT (IU L ⁻¹)	30.63±0.32	120.00±8.81 ^a	118.30±6.44 ^b	96.70±6.43 ^b	47.90±4.98 ^b	30.23±1.82 ^b
AST (IU L ⁻¹)	60.12±0.05	65.00±6.40 ^a	148.90±7.76 ^b	81.20±5.18 ^b	58.30±7.41 ^b	58.10±4.56 ^b
ALP (IU L ⁻¹)	58.29±0.12	108.20±3.39 ^a	89.70±1.73 ^b	71.40±4.81 ^b	63.70±3.67 ^b	57.90±1.89 ^b
Bilirubin (IU L ⁻¹)	0.49±0.05	2.67±0.13 ^a	1.15±0.12 ^b	0.86±0.09 ^b	0.74±0.04 ^b	0.56±0.02 ^b
Total protein (g dL ⁻¹)	8.15±0.27	5.21±0.13 ^a	6.17±0.27 ^b	7.56±3.32 ^b	9.54±2.13 ^b	9.23±1.13 ^b

Values are expressed as mean±SEM from 6 rats in each observation, ^a: p<0.05 compared to control group, ^b: p<0.05 compared to CCl₄ treated group

hepatic transaminase enzymes after the EEUP treatment is an indication of the stabilization of plasma membranes as well as repair of hepatic tissue damage caused by CCl₄. This effect is in agreement with the commonly accepted view that serum levels of transaminases return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes (Raja *et al.*, 2007). In CCl₄ treated rats liver section, the cells of centrolobular region showed vacuolated cytoplasm, the vacuolar size showed variations from spherical to large droplet structures. In most of the necrotic cells centrally placed nuclei were suspended in small amount of cytoplasm, which continued by cytoplasmic strands that traverse through the vacuoles connected to the periphery of cytoplasm. Kupffer cells and sinusoidal cells showed arrest in distribution. The administration of ethanol extract at 250 mg kg⁻¹ p.o. body weight protected the liver partially (Fig. 1c). The extent of the necrotic region was reduced significantly. Numbers of necrotic cells located in this region were considerably reduced and were retained in immediate vicinity of the vein. The centrolobular region of rat liver treated with EEUP at 500 mg kg⁻¹ p.o. body weight along with CCl₄ showed normal cellular architecture without any necrotic cells (Fig. 1d). The histology of the livers was found normal when treated with EEUP and standard drug Silymarin at 750 mg kg⁻¹ and 100 mg kg⁻¹ p.o. body weight, respectively (Fig. 1e, f). Histopathological studies revealed the significant protection of liver by EEUP after CCl₄ induction, the study reports are similar to the literature (Shyamal, 2006), but only the dose was higher (750 mg kg⁻¹) in this study and revealing at higher dose of EEUP can be employed as hepatoprotective medicine. The sub-chronic and chronic toxicity studies to be performed on this plant to assure the safety of *Urtica parviflora*. Further detailed investigations by the researchers are in progress to isolate the phytoconstituents of the plants responsible for the hepatoprotective activity.

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