Prevention of Carbon Tetrachloride-Induced Hepatotoxicity by *Urtica urens* in Rats

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Abstract

In this study, the effects of *Urtica urens* L. (dwarf nettle, UU) seed extract on lipid peroxidation, antioxidant and xenobiotic metabolizing enzymes in both control and carbon tetrachloride (CCl_4)-treated rats were investigated. Male Wistar rats were randomly allotted into one of the four experimental groups. A (Control), B (UU-treated), C (CCl_4-only treated) and D (UU+CCl_4-treated), each having 5-24 animals. Some of rats in group A were treated with physiological saline, i.p. daily for 4 days; Group B were treated with UU 200 mg/kg, i.p. daily for 4 consecutive days; Group C were administered with CCl_4 at dose of 10 ml/kg, i.p. for 2 consecutive days; and group D rats were pretreated with UU 200 mg/kg, i.p. daily for 4 consecutive days prior to administration of CCl_4 10 ml/kg, i.p. daily for 2 consecutive days. At the end of the experimental period, rats were sacrificed, and tissues were taken. Results have indicated that treatment of rats with *U. urens* increased hepatic antioxidant enzymes without changing the levels of serum Lactate DeHydrogenase (LDH), ALanine aminoTransferase (ALT) and ASpartate aminoTransferase (AST). Moreover, *U. urens* treatment decreased the CCl_4 dependent elevated lipid peroxidation and serum LDH, ALT and AST activities. Furthermore, *U. urens* protected the inhibitory effect of CCl_4 on CYP2E1 catalyzed aniline 4-hydroxylase activities. As a result, as indicated by these *in vivo* data, *U. urens* seed extract contains constituents protecting liver against hepatotoxic effects of CCl_4.

Key words: Dwarf nettle, Hepatoprotective, Antioxidant, Lipid peroxidation, CYP2E1, Aniline 4-Hydroxylase

INTRODUCTION

In the last ten years, consumption of medicinal herbs or herbal preparations is tremendously increasing in order to identify alternative approaches to improve the quality of life and maintain a good health. In the meantime, extensive studies of the adverse effects of these herbal medicines and establishments of a good correlation between biomarkers and plants are essential for ensuring the efficiency and quality of herbal medicines. We report here such a study on *U. urens*.

Carbon tetrachloride (CCl_4) is a selective hepatotoxic chemical agent. CCl_4-induced reactive free radicals initiate cell damage through two different mechanisms of covalent binding to the membrane proteins and cause lipid peroxidation [1]. Many investigators have utilized this chemical to induce liver cirrhosis in experimental animals [2]. Liver cirrhosis is a major public health problem all over the world. Nowadays, the prevention of liver cirrhosis and fibrosis is the major and vital concern of the therapy in hepatology.

Urtica urens L. (dwarf nettle) is a perennial plant with stinging hairs belonging to the plant family *Urticaceae*. *U. urens* seeds are widely used in folk medicine in many parts of Turkey, particularly in the therapy of advanced cancer patients [3].

The purpose of the present study was to investigate the preventive effects of *U. urens* on lipid peroxidation, antioxidant and xenobiotic metabolizing enzymes in both control and carbon tetrachloride (CCl_4)-treated rats.

MATERIALS AND METHODS

Plant material. The seeds of *U. urens* were purchased from a local herbal store in Denizli, Turkey.

Extraction. The seeds were powdered in a blender and were extracted with hexane using soxhlet extraction apparatus according to the Soxhlet's method. Materials are extracted by repeated percolation which lasts about 6-8 hours with hexane under reflux in a special glassware. At the end of extraction process, the solvent is then evaporated and the remaining mass is measured. The percentage yields were calculated as mg per g seeds.

Animal. Healthy male Wistar rats, weighing 200-250 g, about 12 weeks old were used. The rats were randomly allotted into one of the four experimental groups. A (Control), B (UU-treated), C (CCl_4-only treated) and D (UU+CCl_4-treated), each having 5-24 animals. Some of rats in group A were treated with physiological saline, i.p. daily for 4 days; Group B rats were treated with UU seed extract 200 mg/kg, i.p. daily for 4 consecutive days; Group C rats were administered with CCl_4 to induce hepatotoxicity at dose of 10 ml/kg, i.p. for 2 consecutive days; and group D rats were pretreated with UU seed extract 200 mg/kg, i.p. daily for 4 consecutive days prior to administration of CCl_4 10 ml/kg, i.p. daily for 2 consecutive days. They were housed at University Animal House in standard conditions and fed with standard diet with water ad libitum. All experimental procedures in animals are performed to a high standard under appropriate regimes with Veterinary services and licensed projects.

At the end of experimental period, the rats were anesthetized with alfamine and alfazyne solutions following a 16-hr fasting. Blood samples were taken from aorta to determine the plasma enzymes. The livers, lungs and kidneys were removed and rinsed with cold physiological saline and stored at -80°C until analyzed.

Preparation of tissues subcellular fractions. The tissues were homogenized in 5 parts homogenization solution [1.15% KCl containing 250 mM EDTA, 100 mM phenylmethylsulfonylflouride (PMSF), 100 mM butylated hydroxytoluene (BHT), 0.025% cholate] using a tissue homogenizer with a teflon pestle at 4°C. The subcellular fractions were prepared by a standard differential centrifugation procedure as described previously [4]. Protein concentrations of the samples were determined by the Lowry Method [5] using crystalline bovine serum albumin as standard.

Determination of lipid peroxidation by thiobarbituric acid test. Detection of lipid peroxidation (LPO) depends on quantitation of end product malondialdehyde (MDA) by 2-thiobarbituric acid (TBA) assay spectrophometrically [6]. The reaction mixture contained 0.2 M Tris-HCl (pH 7.4), 1.1 mg/ml microsomal protein and 100 μM $FeSO_4$ in a final volume of 3.0 ml. Tubes were incubated in a 37ºC water bath for 30 min, cooled immediately in an ice bath for 10 min. 1 ml 87% o-phosphoric acid and 1 ml 67.5% TBA was added, mixed and incubated in a water bath for 45 min. MDA products were collected by extraction into 3 ml of n-butanol after centrifugation at 800 x*g* for 15 min. The absorbance of clear supernatant was read at 532 nm and 1.5 $x10^{-5}$ M extinction coefficient was used in order to calculate the amount of MDA which was expressed in terms of nmoles/min/mg protein.

Determination of serum ALT and AST and LDH activities. In order to evaluate hepatocellular damage and antihepatotoxic potential of the *U. urens* seed extract enzymatic leakage of transaminases [ALanine aminoTransferase (ALT) and ASpartate aminoTransferase (AST)] and Lactate DeHydrogenase (LDH) in serum were measured by the method of Reitman and Frankel [7] and Wroblewski and LaDue [8], respectively. Blood was centrifuged at 4000 rpm at 4°C for 10 min to get serum.

Determination of antioxidant enzyme activity. Catalase (CAT) activity was measured using Abei's method [9]. Ten microliters (100-150 μg protein) of mitochondrial fraction was added to the 3 ml cuvette containing 2.80 ml of a 50 mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1 ml of fresh 30 mM H_2O_2 and the decomposition rate of H_2O_2 was measured at 240 nm for 5 min on a spectrophotometer. A molar extinction coefficient of 0.041 mM^{-1}. cm^{-1} was used to measure CAT activity, which was the expressed as nmole H_2O_2 decreased/min/mg protein.

Determination of phase I and phase II enzyme activities. Cytochrome P450 dependent aniline 4-hydroxylase activity (AH) was determined by Imai et al. [10] with some modifications [11]. NADPH-generating system was used as a cofactor. Microsomal protein of 2 mg/ml incubation mixture was used for each assay. The enzymatic reaction was carried out at 25°C for 25 min.

Glutathione S-transferase (GST) activity using CDNB (measured in 0.1 KPi, pH 7.5 containing 1 mM CDNB, 1 mM GSH and 25 μg cytosolic protein) as substrate was determined at room temperature spectrophotometrically by following the change in absorbencies at 340 nm as described by Habig et al. [12]. Enzymatic rates were obtained by subtracting the nonenzymatic rate from the enzymatic rate and enzyme activities towards CDNB were calculated using extinction coefficient of 9.6 mM^{-1}. cm^{-1}.

Statistical analysis. The results are expressed as Mean ± SD of at least three sets of triplicate determinations for each data point. One way Anova, Tukey and Dunnett tests were applied for analyzing the significance of difference between and among different groups.

RESULTS

We found that CCl_4 treatment significantly increased the lipid peroxidation in rats. In addition, serum levels of ALT, AST and LDH, and microsomal AH were significantly elevated with CCl_4 treatment. On the other hand, no significant changes were observed in CAT activity of CCl_4-treated rats (Table 1).

The effects of *U. urens* seed extract on the serum transaminases, serum LDH, cytosolic GST, microsomal AH and lipid peroxidation in control, CCl_4-treated and UU+CCl_4-treated rats are given in Table 1. In this study, treatment of rats with *U. urens* seed extract alone significantly increased both CAT (2.58-fold) and GST (3.47-fold) activities without effecting serum transaminases and microsomal AH. In addition, *U. urens* seed extract treatment alone did not affect the lipid peroxidation in rats.

Pretreatment of rats with *U. urens* (Group D) decreased significantly the CCl_4-caused elevation in transaminases and LDH activities (Table 1). In addition, *U. urens* pretreatment significantly elevated (2.5-fold to that of Group C) the CCl_4-caused inhibition on CYP2E catalyzed AH activities (Table 1).

Table 1. The effects of *Urtica urens* seed extracts on the serum transaminases, LDH, lipid peroxidation, CAT, GST, and AH activities.

GROUPS	**LPO (nmol/min/mg prt)**		**Serum Enzyme Activities (Units)**			**Cytosolic and Microsomal Enzyme Activities (nmol/min/mg prt)**		
	Serum	Liver	LDH	AST	ALT	CAT	GST	AH
A (Control)	48 ± 3	75 ± 2	670 ± 240	167 ± 16	36.5 ± 4.6	300 ± 183	34 ± 10	0.52 ± 0.26
B (UU)	49 ± 3	65 ± 4	837 ± 396*	170 ± 23	26 ± 6	775 ± 158**	118 ± 24**	0.49 ± 0.22
C (CCl_4)	85 ± 3**	130 ± 5**	4,200 ± 840***	528 ± 74***	460 ± 63***	315 ± 130	106 ± 52**	0.028 ± 0.002***
D (UU+CCl_4)	ND	ND	2,153 ± 919***	183 ± 32*	130 ± 62***	486 ± 28**	172 ± 47***	0.07 ± 0.094***

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ ND. Not Determined

DISCUSSION

In this study, *U. urens* seed extract were evaluated for its protective effects on hepatocellular damage in CCl_4-intoxicated rats. CCl_4 is one of the most widely used toxicant for experimental induction of liver fibrosis in laboratory animals [13]. It is a prototype chemical inducing lipid peroxidation in experimental animals within a few minutes of administration and known to cause severe hepatic injury [14]. CCl_4 toxicity is due to reactive free radical (CCl_3), which is generated by its reductive metabolism by hepatic cytochrome P450s. This reactive free radical initiate cell damage through the two major mechanisms of covalent binding to cell membrane lipids and lipid peroxidation [15]. Several experimental studies have investigated the role of antioxidant vitamins, minerals, drugs and plant-derived compounds in the prevention and therapy of liver fibrosis.

Recently, natural bioactive compounds have been used as chemoprotective agents to inhibit carcinogenesis at different sites. In the literature, there seems to be no investigation on the hepatoprotective effects of *U. urens*.

In our study, the therapeutic effects of *U. urens* were studied by examining the prevention of CCl_4 induced hepatotoxicity in rats. Our biochemical results demonstrated that *U. urens* treatment prevented CCl_4-induced hepatotoxicity in rats by strengthening the antioxidant defense system. Therefore, these results demonstrated that the seed of *U. urens* has protective function against CCl_4 toxicity in rat liver when administered *in vivo* at a dose of 200 mg/kg/day i.p., for 4 consecutive days prior to CCl_4 intoxication. Similar results have been reported for some other ethnobotanical fruits and herbs [16, 17].

In addition, the decrease in serum transaminases, ALT and AST, and LDH activities by *U. urens* can be correlated with the induction of detoxification and antioxidant enzymes at higher dose. Additionally, *U. urens* may have biological significance in the elimination of reactive free radicals. The active components of this plant may up regulate free radical and reactive metabolite scavenging systems. Since significant differences were observed not only in CAT and GST but also in AH activities among groups A, B, and D, it may suggest that *U. urens* exert its hepatoprotective function both with and without involving antioxidant system.

We have also studied the effect of *U. urens* on cytochrome P4502E1 which is an important xenobiotic metabolizing enzyme because it is involved in the metabolism of many dietary compounds and is changed under a variety of pathophysiological conditions such as fasting, diabetes, obesity etc. [18, 19]. Overcoming the inhibitory effect of CCl_4 on AH activity with *U. urens* treatment may be another correlative measure for the cyto- and hepato-protective role of *U. urens*.

The results of the present study may have very important implications for the chemopreventive potentials and antioxidant profiles of *U. urens* seed extract as a traditional herbal medicine. In conclusion, this study may suggest new treatments in the prevention of CCl_4 induced hepatotoxicity and reveals the importance of scientific research on miscellaneous plants with various medicinal properties. Further studies are required to evaluate the possible interactions of *U. urens* with therapeutic drugs and/or other dietary components in order to clarify its possible use as traditional medicinal herb.

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