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• *BRIEF REPORTS* •

Hepatoprotective effects of *Nigella sativa* L and *Urtica dioica* L on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats

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Abstract

AIM: To investigate the effects of *Nigella sativa* L (NS) and *Urtica dioica* L (UD) on lipid peroxidation, antioxidant enzyme systems and liver enzymes in CCl_4-treated rats.

METHODS: Fifty-six healthy male Wistar albino rats were used in this study. The rats were randomly allotted into one of the four experimental groups: A (CCl_4-only treated), B (CCl_4+UD treated), C (CCl_4+NS treated) and D (CCl_4+UD+NS treated), each containing 14 animals. All groups received CCl_4 (0.8 mL/kg of body weight, sc, twice a week for 60 d). In addition, B, C and D groups also received daily i.p. injections of 0.2 mL/kg NS or/and 2 mL/kg UD oils for 60 d. Group A, on the other hand, received only 2 mL/kg normal saline solution for 60 d. Blood samples for the biochemical analysis were taken by cardiac puncture from randomly chosen-seven rats in each treatment group at beginning and on the 60th d of the experiment.

RESULTS: The CCl_4 treatment for 60 d increased the lipid peroxidation and liver enzymes, and also decreased the antioxidant enzyme levels. NS or UD treatment (alone or combination) for 60 d decreased the elevated lipid peroxidation and liver enzyme levels and also increased the reduced antioxidant enzyme levels. The weight of rats decreased in group A, and increased in groups B, C and D.

CONCLUSION: NS and UD decrease the lipid peroxidation and liver enzymes, and increase the antioxidant defense system activity in the CCl_4-treated rats.

Key words: CCl_4; Nigella sativa L.; Urtica dioica L.; Lipid peroxidation; Antioxidant enzymes; Rat

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INTRODUCTION

Carbon tetrachloride (CCl_4) is one of the oldest and most widely used toxins for experimental induction of liver fibrosis in laboratory animals[1]. This model has been used in various studies on examined the deposition of extracellular matrix in the fibrotic and cirrhotic liver[2,3].

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. A number of investigators have utilized this chemical to produce liver cirrhosis in experimental animals[4]. Production of reactive oxygen species and lipid peroxidation induced by iron overload[5], cholestatic injury[6] and intoxication by ethanol[7] and CCl_4[4] is associated with liver fibrosis and cirrhosis. These effects are partially prevented by antioxidant compounds including α-tocopherol[4,8], silymarin[9] and salvianolic acid[10].

The seed of *Nigella sativa* L (NS), an annual *Ranunculaceae* herbaceous plant, has been used traditionally for centuries in the Middle East, Northern Africa, Far East and Asia for the treatment of asthma. NS contains more than 30 of a fixed oil and 0.40-0.45 w/w of a volatile oil. The volatile oil has been shown to contain 18.4-24% thymoquinone and 46% many monoterpenes such as p-cymene, and α-pinene[11]. Recently conducted clinical and experimental researches have shown many therapeutic effects of NS extracts such as immunomodulator[12], anti-inflammatory[13] and anti-tumour agents[14].

Urtica dioica L (UD) is a plant belonging to the plant family *Urticaceae*. Its seeds are widely used in folk medicine in many parts of Turkey, especially in the therapy of advanced cancer patients. Polar extract of the UD contains lignans (+)-neoolivil, (-)-secoisolariciresinol, dehydrodiconiferyl alcohol, isolariciresinol, pinoresinol, and 3,4-divanillyltetrahydrofuran, and has antiinflammatory

effects[15] and stimulates the proliferation of human lymphocytes[16].

The present study aimed to investigate the preventive effects of NS and UD on lipid peroxidation, antioxidant enzyme systems and some liver enzymes in CCl_4-treated rats.

MATERIALS AND METHODS

Plant materials and extraction procedure

The NS and UD seeds were purchased from a local herb store, Zonguldak, Turkey. Voucher specimens were kept at the Department of Biochemistry, Zonguldak Karaelmas University, Zonguldak, Turkey for the future reference. The seeds of NS were powdered in a mixer, placed in a distillation flask and the volatile oil with 0.2 % yield was collected by a steam distillation. The fixed oil of UD was extracted with the help of a rotary evaporator using diethyl ether as solvent.

Treatment of rats

Fifty-six male Wistar albino rats, weighing 150-200 g, averaging 16 wk old, were used in this study. The rats were randomly allotted into one of the four experimental groups: A (CCl_4-only treated), B (CCl_4+UD treated), C (CCl_4+NS treated) and D (CCl_4+UD+NS treated), each containing 14 animals. All groups received CCl_4 (Merck; 153.82 g/moL, 1.59 kg, Germany, 0.8 mL/kg of body weight, sc, twice a week for 60 d). In addition, B, C and D groups also received the daily ip injection of 0.2 mL/kg NS or/and 2 mL/kg UD oils for 60 d. Group A, on the other hand, received only 2 mL/kg normal saline solution for 60 d. The animals were housed in macrolon cages under standard laboratory conditions (light period 7.00 a.m. to 7.00 p.m., 21±1 °C, rat chow and tap water freely available). All animals received human care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health. The experiment lasted for 60 d.

Biochemical analysis

Blood samples for the biochemical analysis were taken from each treatment group at beginning and on the 60th d of the experiment. Rats from which blood samples were taken were excluded from the experiment to eliminate the haemorrhage- and stress-induced complications. At the end of the experiment, rats in all groups were starved overnight, and sacrificed under chloralhydrate (6 mL of 7% chloralhydrate/kg, Sigma, St. Louis, MO, USA) anaesthesia. Blood samples were collected by cardiac puncture using heparinised syringes. Leukocytes and plasma components were seperated by centrifugation of the blood. Erythrocytes were washed three times with 0.9% NaCl solution and packed, and then stored at -70 °C until study.

Blood MDA (mmol/L) was determined by the double heating method of Draper and Hadley[17]. The principle of the method is spectrophotometric measurement of the colour produced during the reaction to thiobarbituric acid (TBA) with MDA. For this purpose, 2.5 mL of 100 g/L trichloroacetic acid solution was added to 0.5 mL erythrocytes in each centrifuge tube and placed in a boiling water bath for 15 min. After cooled in tap water, the mixture was centrifuged at 1 000 r/min for 10 min, and 2 mL of the supernatant was added to 1 mL of 6.7 g/L TBA solution in a test tube and placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance was measured using a Shimadzu UV-1601 (Japan) spectrophotometer at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of MDA-TBA complex 1.56×10^5 /cm, and expressed in μmol/g Hb erythrocytes and μmol/g tissue protein.

Blood GSH concentration was measured by the method described by Beutler *et al*[18]. Briefly, 200 μL of whole blood was added to 1.8 mL of distilled water. Three ml of the precipitating solution was mixed with the hemolysate. The mixture was allowed to stand for approximately 5 min and then filtered. Two milliliters of filtrate were taken and added into another tube, and then, 8 mL of the phosphate solution and 1 mL of the DTNB [5,5'dithiobis-(2-nitrobenzoic acid)] were added. A blank was prepared with 8 mL of the phosphate solution, 2 mL of the diluted precipitating solution (three parts to two parts of the distilled water), and 1 mL of the DTNB reagent. A standard solution of glutathione was prepared (40 mg/100 mL). The optical density was measured at 412 nm with a spectrophotometer.

Serum ceruloplasmin *p*-phenylenediamine (PPD) oxidase activity was measured according to Sunderman and Nomoto[19]. At pH 5.4, ceruloplasmin catalyze the oxidation of PPD to yield a colored product. The rate of formation of the colored oxidation product was proportional to the concentration of serum ceruloplasmin if a correction was made for nonenzymatic oxidation of PPD.

Vitamin E was analyzed colorimetrically with 2,4,6-tripridyl-s-triazin and $FeCl_3$ after the extraction with absolute ethanol and xylene[20]. Serum vitamin C level was determined after derivatisation with 2,4-dinitrophenylhydrazine[21]. The levels of β-carotene at 425 nm and retinol at 325 nm were detected after the reaction of serum : ethanol : hexane at the ratio of 1 : 1 : 3 respectively[22].

Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels were determined by an autoanalyzer (Roche-Hittachi, Japan) using commercial kits (Roche, Basel, Switzerland).

Statistical analysis

The data were expressed as mean±SD and analysed by repeated measures of variance. Tukey test was used to test for differences among means when ANOVA indicated a significant ($P\leqslant0.05$) F ratio.

Table 1 Blood MDA level (nmol/mL erythrocytes) in CCl_4+NaCl-treated (A), CCl_4+UD treated (B), CCl_4+NS treated (C) and CCl_4+UD+NS treated (D) rats (mean±SD)

Groups(d)	A	B	C	D
0	1.23±0.09	1.18±0.14	1.21±0.12	1.25±0.03
60	2.81±0.05	1.35±0.07	1.41±0.09	1.28±0.12

Table 2 Serum antioxidant levels (mg/dL) in CCl_4+NaCl-treated(A), CCl_4+UD treated (B), CCl_4+NS treated (C) and CCl_4+UD+NS treated (D) rats (mean±SD)

Groups(d)	A	B	C	D
GSH				
0	50.76±0.72	47.14±1.02	47.72±1.25	49.36±0.93
60	40.21±2.38	55.44±3.32	50.63±3.41	61.12±8.26
Ceruloplasmin				
0	18.72±0.82	18.47±0.89	17.36±1.13	19.23±0.59
60	12.92±0.49	29.33±0.64	25.90±1.61	30.22±1.23
Vitamin E				
0	0.20±0.12	0.19±0.01	0.18±0.01	0.20±0.13
60	0.11±0.02	0.34±0.03	0.24±0.02	0.45±0.01
Vitamin C				
0	0.53±0.01	0.51±0.01	0.52±0.01	0.54±0.01
60	0.40±0.01	0.61±0.02	0.54±0.02	0.89±0.01
Beta-karoten				
0	26.49±0.85	25.28±0.44	26.41±0.37	25.15±0.83
60	19.28±1.23	25.21±1.55	26.73±1.53	25.25±1.27
Retinol				
0	50.33±0.87	52.72±0.66	51.22±0.39	52.64±0.55
60	44.98±1.18	56.19±0.37	53.52±0.63	61.69±1.69

Table 3 Serum liver enzyme levels (U/L) in CCl4+NaCl-treated (A), CCl4+UD treated (B), CCl4+NS treated (C) and CCl4+UD+NS treated (D) rats (mean±SD)

Groups(d)	A	B	C	D
ALP				
0	957±23.62	961±16.64	987±13.64	1028±36.51
60	1354±32.58	692±24.21	637.82±31.21	772.57±91.45
ALT				
0	89.3±4.86	78.21±17.35	80.14±20.12	83.17±11.21
60	2762±42.45	553±60.29	425±42.24	253±39.27
AST				
0	143.29±6.43	141.28±16.26	143.50±7.85	144.27±20.44
60	2342.25±34.06	554.23±14.83	1031.44±92.68	672.17±58.42

Table 4 Weights (g) of rats in CCl_4+NaCl-treated (A), CCl_4+UD treated (B), CCl_4+NS treated (C) and CCl_4+UD+NS treated (D) rats (mean±SD)

Groups(d)	A	B	C	D
0	189±6.21	181.43±12.21	187.21±4.33	189.63±3.21
60	171.73±3.90	204.02±12.09	214.76±12.72	217.50±11.52

RESULTS

The levels of blood MDA, antioxidants of all groups and the levels of serum liver enzymes are shown in Table 1-3, respectively. The CCl_4 treatment for 60 d significantly ($P<0.05$) increased the MDA and liver enzymes, and also decreased ($P<0.05$) the antioxidant levels. NS or UD treatment (alone or in combination) for 60 d significantly ($P<0.05$) decreased the elevated MDA and liver enzyme levels and also increased ($P<0.05$) the reduced antioxidant levels. The weight of rats decreased ($P<0.05$) in group A, and increased ($P<0.05$) in groups B, C, and D (Table 4).

DISCUSSION

Treatment of animals with CCl_4 is known to cause severe hepatic injury[23]. In our study, we showed that repeated CCl_4 treatment for 60 d increased the lipid peroxidation and liver enzymes, and also decreased the antioxidant enzyme levels. It has been suggested that the lipid peroxidation may be a link between tissue injury and liver fibrosis by modulating collagen gene expression[24]. It was reported that CCl_4 is suitable to induce lipid peroxidation in experimental animals within a few minutes after administration and its long-term use results in liver fibrosis and cirrhosis by lipid peroxidation pathway[25]. It is generally thought that CCl_4 toxicity is due to reactive free radical (CCl_3.), which is generated by its reductive metabolism by hepatic cytochrome P450. The reactive intermediate is believed to cause lipid peroxidation and breakdown of cellular membranes[26].

Recent experimental studies have investigated the role of antioxidative vitamins, minerals, drugs and plant-derived compounds in the prevention and therapy of liver fibrosis. Parola *et al*[4] showed that an increased liver content of vitamin E leads to a significant degree of protection against carbon tetrachloride-induced chronic liver damage and cirrhosis in rats. Ianas *et al*[27] have described the all-round beneficial action of a selenium preparation upon the organism in rats exposed to CCl_4 as well as a strong antioxidative effect, confirming the essential role of selenium in maintaining cellular integrity.

Several plant derived compounds such as colchicine (Colchicum dispert), silymarin (Slybum marianum), polyenylphosphatidyl choline (soy bean), ellagic acid (cruciferous vegetables), Gingko biloba composita and recently Sho-saiko-to (extract of seven herbs in Chinese folk medicine) have been proposed as antioxidants and antifibrotics in the treatment of chronic liver disease[28-30]. The antioxidative and hepatoprotective effects of chitosan against CCl_4-induced liver toxicity in rats have been under investigation by measuring thiobarbituric acid reactive substances (TBARS) and antioxidant enzyme activities[31]. The antioxidant systems such as antioxidant vitamins (A, C, and E), superoxide dismutase (SOD), catalase, glutathione (GSH), ceruloplasmin and glutathione peroxidase (GSH-Px) protect the cells against lipid peroxidation, which is the base of many pathologic processes [32,33].

In our study, we found that NS or UD treatment (alone or combination) for 60 d decreased the elevated MDA and liver enzyme levels and also increased the reduced antioxidant enzyme levels in CCl_4-treated rats. Previously performed clinical and experimental investigations have shown that NS has a protective effect against oxidative damage in isolated rat hepatocytes[34]. It was found that the fixed oil of NS has both antioxidant and anti-eicosanoid effects greater than thymoquinone which is its active

constituent[13]. Furthermore, NS has antioxidant activity by suppressing the chemiluminescence in phagocytes[35].

Recently, Turkdogan *et al*[36] observed that NS has a significant hepatoprotective effect in CCl_4-administrated rabbits, ans that hepatocellular degenerative and necrotic changes are slight without advanced fibrosis and cirrhotic process in NS-treated group. However, Turkdogan *et al*[37] found that NS can prevent liver fibrosis and cirrhosis, suggesting that NS protects liver against fibrosis possibly through immunomodulator and antioxidant activities.

There are no comprehensive studies on the therapeutic effects of UD. Only one study reported that UD extract can inhibit *in vitro* prostate cancer cell proliferation[38]. It has been suggested that the extract of UD is effective in inducing glutathione S-transferase, SOD and catalase activity in the forestomach and SOD and CAT activity in the lung at both dose levels[39]. However, Turkdogan *et al*[36] showed that NS and UD can significantly prevent CCl_4-induced hepatotoxicity in rats. Our biochemical results demonstrated that NS and UD treatment prevented CCl_4-induced hepatotoxicity in rats by decreasing the lipid peroxidation and increasing the antioxidant defense system activity. However, Kanter *et al*[40] also showed the NS and UD increase the antioxidant defense system activity in experimentally CCl_4-treated rats.

In conclusion, NS and UD decrease lipid peroxidation and liver enzymes, and increase antioxidant defense system activity in the CCl_4-treated rats. They also prevent weight lose induced by the CCl_4 treatment. Further studies are required to evaluate the possible hepatoprotective effect of NS and UD which are traditionally used as a medicine for many complaints including liver diseases.

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