Original Article

Effect of dill tablet (*Anethum graveolens* L) on antioxidant status and biochemical factors on carbon tetrachloride-induced liver damage on rat

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Abstract

Background: Liver damage induced by carbon tetrachloride (CCl_4) has been presented as an experimental model for research in hepatoprotective effects of natural product. A commercial medicine prepared from *Anethum graveolens* L (dill) is being used as dill tablet (DT) as a hypolipidemic agent. This experiment aimed to investigate the protective effect of DT against hepatic damage. **Materials and Methods:** Male Wistar rats were randomly divided into four groups (n = 6) as following for a 10 days experiments. (1) Normal animals; (2) normal animals +CCl_4 1 ml/kg (1:1 of CCl_4 in olive oil, by gastric tube); (3) CCl_4 treated animals +100 mg DT/kg; (4) CCl_4 treated animals +300 mg DT/kg. After 10 days of treatment, biochemical factors were measured; also antioxidant tests such as thiol group, malondialdehyde (MDA), total antioxidant capacity (TAC), and catalase (CAT) activity in the liver samples were carried out. **Results:** In dill treated animals, a significant decrease in liver enzymes lactate dehydrogenase, alkaline phosphatase, aspartate transaminase, alanine transaminase, γ-glutamyl transferase, total bilirubin, direct bilirubin, as well as triglyceride, total cholesterol ($P < 0.05$) were observed. Total protein and albumin concentrations were significantly increased in dill treated groups ($P < 0.05$). Furthermore, treatment with dill declined liver cholesterol, triglyceride, MDA, and increased TAC and CAT activity compared with untreated group ($P < 0.05$). **Conclusion:** Dill displayed a potential hepatoprotective effect against CCl_4-induced liver damage based on both biochemical markers and antioxidant status.

Key words: *Anethum graveolens*, carbon tetrachloride, catalase, dill, hepatoprotective

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Introduction

Free radicals and other reactive oxygen species are involved in many disorders in humans, including cardiovascular disease, arthritis, diabetes, and cancer.[1] Various studies reported that carbon tetrachloride (CCl_4)-induced liver damage is related to high levels of free radicals that cause oxidative stress. In this state, CCl_4 provoked destruction of hepatocyte structure and leads to release liver enzyme to blood circulation.[2]

Many people currently like to use herbal medicine for treatment of different disease. Interestingly, WHO has

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stated the use of herbal medicine for treatment of health problems.[3]

Anethum graveolens is a traditional herb which has been used for many medicinal purposes. This plant is administrated for many health matters such as digestive disorders, bad breath, lactation problems, hypercholesterolemia, cancer, infection, diabetes, gastric problems, and inflammation.[4] *A. graveolens L* (dill) is the member of *Apiaceae* family and is cultivated in many parts of the world. Dill is known as a rich source of flavonoids, phenolic, saponin, tannins, and terpene; these substances are attributed in therapeutic effects of this plant.[4] A commercial medicinal form of this plant is available as dill tablet (DT) that contains *A. graveolens* (68%), *Cichorium intybus* (5%), *Fumaria parviflora* (5%), and *Citrus aurantifolia* sp. (4%), and nowadays is used as hypolipidemic agent.[1]

As a matter of the fact that dill has high levels of antioxidant polyphenols and may decrease hepatoprotective damage; consequently, this study planned to evaluate the impact of dill on CCl_4-induced liver damage in rat.

Materials and Methods

Male Wister rats weighing 200–220 g were used in this experiment; they were on standard diet and water. DT was purchased from Iran Darouk Company (Iran). Following 5 days of acclimatization in animal cage, rats were divided into four groups (n = 6): (1) normal animals; (2) normal animals +CCl_4 1 ml/kg (1:1 of CCl_4 in olive oil, by gastric tube); (3) CCl_4 treated animals + 100 mg DT/kg; (4) CCl_4 treated animals + 300 mg DT/kg. The treatment of rats was performed according to Bhandarkar *et al*. method.[5] CCl_4 in olive oil was administered every 72 h and treatment was done for a period of 10 days. After 10 days of treatment, animals were anesthetized, and blood was collected and serum was separated for biochemical tests. Furthermore, liver was removed and washed immediately with ice cold phosphate buffer saline buffer and homogenized in lysis buffer (Sigma). After centrifugation, the supernatant was used for antioxidant tests.

The biochemical factors in serum including lactate dehydrogenase (LDH), alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), γ-glutamyl transferase (γ-GT), total bilirubin, direct bilirubin, triglycerides, total cholesterol, total protein, and albumin were determined using commercial kits on an autoanalyzer. Body weight determined every day and liver weight was measured on the final day.[6]

The prepared liver tissue supernatant was used for determination of catalase (CAT) activity, malondialdehyde (MDA), total antioxidant capacity (TAC), and thiol group content.[7] For determination of antioxidant activity, 10 μL of butylated hydroxytoluene (0.5 M in acetonitrile) was added to tissue sample for prevention of homogenate from oxidation and then stored at −70°C until analysis.[7]

Liver lipid was extracted according to the prevously published report.[8] Briefly, one gram of liver was homogenized with chloroform: Methanol solution (2:1). After dispersal, tissue mixture was agitated for 20 min in shaker at room temperature. After centrifugation, liquid phase was washed with a 200 μL of 0.9% NaCl. After vortexing and centrifugation, the chloroform phase was evaporated, and lower phase was used for lipid analysis. Cholesterol and triglyceride assay kits (Pars Azmoon, Iran) were used to measurement of liver cholesterol and triglyceride contents.[8]

The collected data are stated as mean ± standard error of mean and analyzed by SPSS (version 16,Chicago, SPSS Inc.). Differences were tested by one-way analysis of variance. The $P < 0.05$ was considered statically significant.

Results

In DT-treated rats, body and liver weights were significantly decreased ($P < 0.05$) compared to CCl_4 group. The serum levels of LDH, ALP, AST, ALT, γ-GT, total bilirubin, and direct bilirubin were significantly increased, whereas total protein and albumin were significantly reduced in CCl_4 group. These values normalized in the animals that pretreated with dill ($P < 0.05$ for all factors, Table 1). The activity of CAT and TAC significantly increased, whereas MDA reduced in DT group ($P < 0.001$) compared to CCl_4 group [Figures 1-3]. Thiol groups level was significantly reduced in CCl_4 group, while it was markedly increased in DT-treated animal [Figure 4]. The levels of liver total cholesterol and triglycerides are shown in Figure 5. In dill treated group, liver cholesterol and triglycerides concentrations significantly reduced compared to CCl_4 group. This reduction was more in the 300 mg/kg DT-treated rats.

Discussion

Membrane damage or necrosis leads to release of liver enzyme into blood circulation; consequently, they can be determined in serum. Serum activities of ALT, ALP, AST, γ-GT, total bilirubin, direct bilirubin, total protein, and albumin are used as a complete marker of liver damage.[9] CCl_4 treatment in animals produces an experimental liver damage model. In this condition, toxic metabolite CCl_3 radical is generated in the liver and reacts with oxygen to produce trichloromethyl peroxy radicals. This radical is able to bind to lipid and causes lipid peroxidative membrane of the liver.[10] The reduction of

Table 1: Comparison of biochemical factors among different studied rats' group

Biochemical factors	CCl_4-treated	Normal group	Dill tablet (100 mg/kg) + CCl_4	Dill tablet (300 mg/kg) + CCl_4
LDH (U/l)	196.50±2.29	103.00±5.5*	111.24±6.00*	102.10±4.5*
ALP (U/l)	230.17±6.17	154.00±0.54*	170.68±4.58*	130.60±5.20*
AST (U/l)	273.83±8.47	98.17±3.79*	192.03±4.20*	110.10±5.25*
ALT (U/l)	239.00±5.31	54.33±2.69*	112.37±6.27*	89.15±3.30*
γ-GT (U/l)	5.45±0.611	1.32±0.12*	3.00±0.50$^{\neq}$	2.28±0.11$^{\neq}$
Total bilirubin (mg/dl)	3.01±0.14	0.85±0.04*	1.16±0.20*	1.30±0.09*
Direct bilirubin (mg/dl)	1.01±0.08	0.30±0.03*	0.85±0.18$^{¥}$	0.50±0.06$^{\neq}$
Total protein (mg/dl)	5.47±0.30	6.46±0.08$^{\neq}$	6.11±0.24	6.45±0.15$^{\neq}$
Albumin (mg/dl)	2.96±0.14	3.52±0.07$^{\neq}$	3.34±0.11	3.55±0.09$^{\neq}$
Triglycerides (mg/dl)	121.83±5.26	84.16±1.83*	109.49±5.55	80.35±4.12$^{¥}$
Total cholesterol (mg/dl)	110.83±2.78	75.16±7.56$^{\neq}$	90.12±4.49	69.87±4.10$^{\neq}$
Body weight (g)	195.67±4.54	223.33±1.34	226.20±5.00*	218.40±1.33*
Liver weight (g)	4.17±0.11	3.34±0.18$^{\neq}$	3.23±0.18$^{\neq}$	3.0±0.20$^{\neq}$

Data represented as mean±SEM (n=6). $^{¥}P$<0.05; $^{\neq}P$<0.01; and *P<0.001 compared with CCl_4 - intoxicated rats. SEM: Standard error of mean; LDH: Lactate dehydrogenase; ALP: Alkaline phosphatase; AST: Aspartate transaminase; ALT: Alanine transaminase; γ-GT: γ-glutamyl transferase

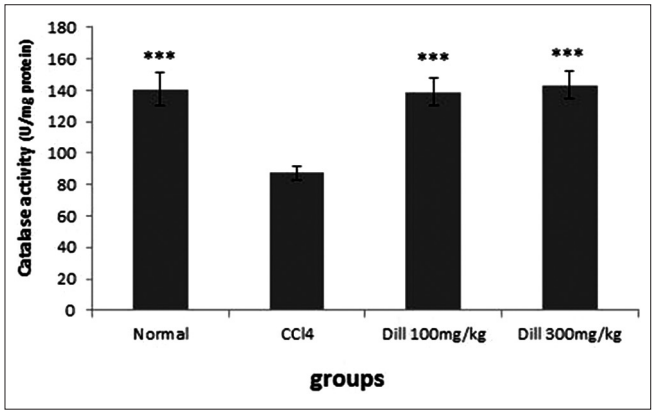

Figure 1: Effect of dill treatment on liver catalase activity in the studied groups. Results are presented as the mean ± standard error of mean. (n = 6). ***P < 0.001 compared with CCl_4 group

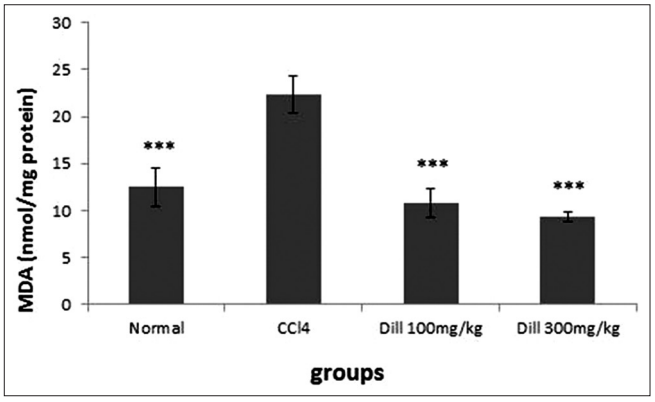

Figure 2: Effect of dill treatment on liver MDA in the studied groups. Results are presented as the mean ± standard error of mean. (n = 6). ***P < 0.001 compared with CCl_4 group. MDA: Malondialdehyde

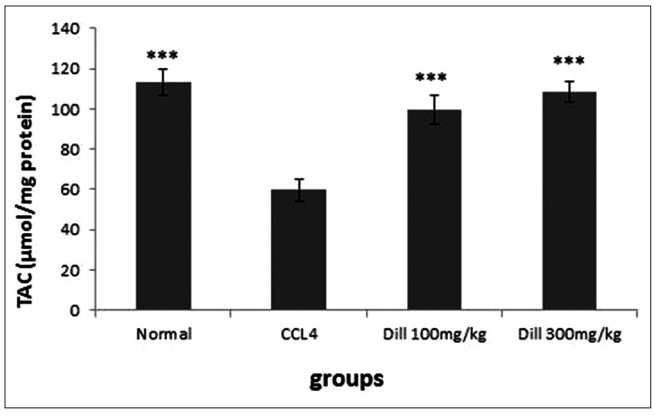

Figure 3: Effect of dill treatment on total antioxidant capacity in the liver of the studied groups. Results are presented as the mean ± standard error of mean. (n = 6). ***P < 0.001 compared with CCl_4 group. TAC: Total antioxidant capacity

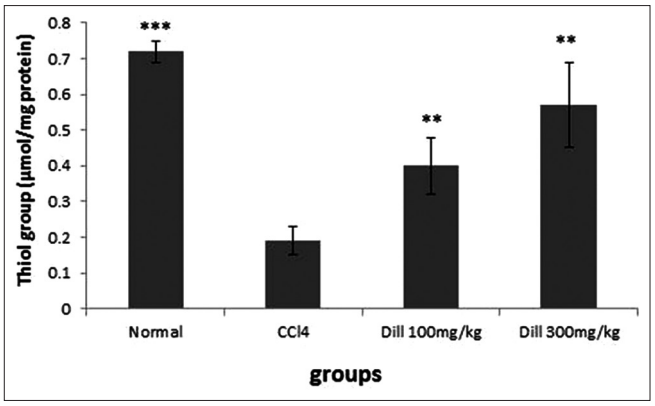

Figure 4: Effect of dill treatment on thiol groups in the liver of CCl_4-intoxicated animals. Results are presented as the mean ± standard. (n = 6). **P < 0.01 and ***P < 0.001compared with untreated rats

liver enzyme activities by DT is a sign of repair of CCl_4-indced liver damage. The administration of DT to animal model potentially prevented the liver from damage which induced by CCl_4 as manifest by improvement of above markers. This is in agreement with Thuppia *et al*., who reported that administration of ethanolic extract of dill in paracetamol-induced hepatic damage in rats has hepatoprotective properties.[11] Rabeh *et al*. also showed that dill or fennel oil and their mixtures have hepatoprotective activities.[2] Tamilarasi *et al*. who found

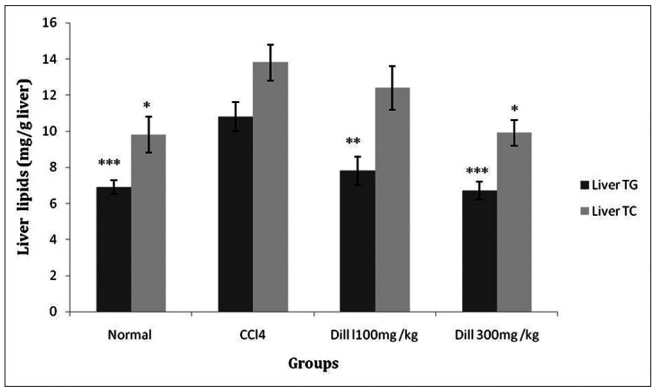

Figure 5: Effect of dill treatment on liver triglycerides and total cholesterol in the studied groups. Results are presented as the mean ± standard error of mean. ($n = 6$). ***$P < 0.001$, **$P < 0.001$, and *$P < 0.05$ compared with CCl_4 group

that crude powder of dill has potential hepatoprotective effect on CCl_4-induced liver damage.[12] Thus, administration of DT revealed hepatoprotective properties against the CCl_4 effect.

Previous studies demonstrated that MDA levels significantly increased, while CAT reduced in CCl_4-induced liver damage.[10] Our experiment on MDA levels and CAT activity are in line with this finding. In DT-treated animals, MDA levels markedly reduced, whereas CAT activity increased in comparison with untreated rats. Previous studies reported that TAC was markedly declined in CCl_4-induced liver damage.[10] This study shows similar results and demonstrates a significant decline in liver TAC value in this animal. Treatment of rats with DT at the dose of 100 and 300 mg/kg markedly normalized the rat antioxidant power.

Thiol groups are important in maintaining the reduced glutathione level that has a main role in the protection of tissue structures from ROS damage.[13] Its role includes detoxification of free radicals, xenobiotics, and also control of immune function. Thiol group level was significantly increased in DT-treated animal.

The result of this study showed that DT has significant hepatoprotective and antioxidant activity at doses of 100 and 300 mg/kg. These findings suggest that DT administration can be useful in the treatment of some hepatic disorders.

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Conflicts of interest

There are no conflicts of interest.

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