Hepatoprotective Effect of Dill (Anethum graveolens L.) and Fennel (Foeniculum vulgare) Oil on Hepatotoxic Rats

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Abstract: The present study was carried out to determine the hepatoprotective effect of some herbal oils as Dill (Anethum graveolens L.) and Fennel (Foeniculum vulgare) oil seeds against carbon tetrachloride (CCL₄) that caused hepatotoxicity in rats. The experiment was performed on 30 adult rat that classified into two main groups, the first main group (6 rats) was kept as control (-ve) group while the second main groups (24 rat) were administered a dose of (2 mL CCL₄/kg b.wt) twice a week for two weeks to induce chronic damage in the liver then classified into four subgroups (six rats each) as follow, one of them (6 rats) was fed on the basal diet and used as a positive control group (+ve), however, the other three subgroups were fed on basal diets and obtained orally dill oil (1 mL/kg), Fennel oil (1 mL/kg), mixture of (0.5 dill and 0.5 mL/kg fennel) oil, respectively for 4 weeks. The hepatotoxicity produced by CCL₄ administration was found to be inhibited by either Dill (Anethum graveolens L.) or Fennel (Foeniculum vulgare) oil or by the mixture of both Dill and Fennel oil with evidence of significant (p<0.05) decrease levels of serum AST and ALT and significantly (p<0.05) increase the level of serum total protein and albumin. Moreover, Dill and Fennel oil supplementation induced suppression of the increased ALP activity with the concurrent depletion of raised bilirubins suggests the possibility of these oils to have ability to stabilize biliary dysfunction in rat liver during hepatic injury by CCL₄. On the other hand, the increase in MDA level and the decrease activity of SOD enzymes in liver induced by CCL₄ suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Treatment with either Dill or Fennel oil and their mixture significantly (p<0.05) reverses these changes. Also, the studied oils have hypolipidemic effects. Hence it is likely that the mechanism of hepatoprotection of either Dill or Fennel oil is due to its antioxidant effect. Dill or Fennel oil and their mixtures have a potent hepatoprotective action against CCL₄ induced liver toxicity in rats. So that, the use of Dill and Fennel oil in food formulations may be beneficial to patients who suffer from liver diseases associated with oxidative stress.

Key words: Dill oil, Fennel oil, hepatoprotective, antioxidant, lipid peroxidation, carbon tetrachloride, rats

INTRODUCTION

The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. The liver helps to purify the blood by changing potentially harmful chemicals into harmless ones (MC Quid, 2003; Ozbek et al., 2006).

Hepatotoxicity implies chemical-driven liver damage. Chemicals that cause liver injury are called hepatotoxins. CCL₄ is a volatile organic alkyl halogen that is present in the environment largely because of release from mammmade sources. High exposure to CCL₄ can cause liver, kidney damages, headaches, dizziness, sleeping, nausea and vomiting (Beartram and Recnagel, 2005).

The liver disorders are one of the world problems. Despite its frequent occurrence, high morbidity and high mortality, its medical management is currently inadequate, so far not yet any therapy has successfully prevented the progression of hepatic disease, even though newly developed drugs have been used to treat chronic liver disorders, these drugs have often side effects (Bruck et al., 1996). Therefore much attention has been focused on natural antioxidants. These antioxidants occur in all higher plants and in all parts of the plant (wood, bark, stems, pods, leaves, fruits, roots, flowers and seeds).

The use of medicinal plants for health started from thousands of years and still a part of the medical practice in Egypt and other developed countries.

Dill "Anethum graveolens L." has been recognized in different system of traditional medicines for the treatment of different diseases and ailments of human beings. Preliminary phytochemical screening of this plant revealed the presence of flavonoids, essential oil, phenolic compounds. It has been reported as antibacterial, antispasmodic, antiulcer activity, antioxidant, hypolipidemic, genotoxicity and diuretic effect (Heamalatha et al., 2011).

Dill is an annual herb which is native to Mediterranean countries and southeastern Europe, used primarily as a
condiment. Dill seed and leaves are used as flavouring in sauces, vinegars, pastries and soups. Dill has medicinal value as a stimulant and a carminative. The dill seeds have essential oil as an active substance, while carvone and limonene are the main constituents of essential oil (Kruger and Hammer, 1996; Bailly et al., 2001; Singh et al., 2005; Callan et al., 2007). The essential oil extracted from both leaves and seeds of dill, could be used in chewing gums, candies and pickles (Zohary and Hopf, 2000) and moreover provide good antioxidant activities (Singh et al., 2005). Carvone (38.8%), apioi (30.8%), limonene (15.9%) and trans (+)-dihydrocarvone (10.99%) were the main components of the essential oil of *Anethum graveolens* L. seeds (Babri et al., 2012).

Fennel “Foeniculum vulgare” is a medicinal plant belonging to the family Apiaceae (Umbelliferae). The leaves, stalks and seeds of the plant are edible. Essential oil of fennel is used as flavoring agents in food products such as beverages, bread, pickles, pastries and cheese. It is also used as a constituent of cosmetic and pharmaceutical products (Piccaglia and Marotti, 2001; Boskabady et al., 2004; Modaress and Asadipour, 2006). Herbal drugs and essential oils of fennel have hepatoprotective effects (Ozbek et al., 2003). They are also known for their diuretic, anti-inflammatory, analgesic and antioxidant activities (Choi and Hwang, 2004; Miguel et al., 2010).

Many phytochemical studies have been conducted to investigate the chemical composition of the essential oil of fennel from different origins and have shown that the major components are phenylpropanoid derivatives and monoterprenoids (Renjie et al., 2010). Ethnobotanical data currently available on wild useful plants in Egypt highlight the importance of fennel's culinary and medicinal uses (Singh and Kale, 2008). Moreover, fennel (essential oil) has been used for centuries in the Mediterranean area as an aromatic herb and also in folk medicine, due to its pharmacological properties. Major components of fennel seed oil samples by chromatographic analysis contain transanethole, fenchone, methylchavicol, limonene, α-pinene, camphene, β-pinene, β-myrcene, α-phellandrene, 3-carene, camphor and cis-anethole (Ozcan et al., 2001; Simandi et al., 1999).

Conventional drugs used in the treatment of liver diseases are generally inadequate and have some serious adverse effects. It is, therefore, necessary to search for alternative drugs for the treatment of liver diseases. So we aimed to examine the protective effect of Dill (*Anethum graveolens* L.) and Fennel (*Foeniculum vulgare*) oil on hepatotoxic rats.

**MATERIALS AND METHODS**

**Rats:** Thirty adult male albino rats of Sprague Dawley strain each weighing 200±5 g b.wt. were used in the study. The rats were obtained from the Laboratory Animal Colony, Helwan, Egypt. The animals were housed under hygienic conditions in plastic cages which contain wood shavings. The animals were kept at a room temperature of 25 ± 2°C with relative humidity of 50-60% and on 12 h light/12 h dark cycle. The rats were provided free access of basal diet and water. The rats were allowed to acclimatize to the laboratory environment for 7 days before start of the experiment.

**Chemicals:** Dill (*Anethum graveolens* L.) and Fennel (*foeniculum vulgare*) oil were obtained from Haraz Market for Herbs and Medicinal Plants, Cairo, Egypt. Kits for biochemical analysis were purchased from The Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

**Preparation of basal diet:** Basal diet was prepared according to Reeves et al. (1993). It is consisted of 14% protein (casein), 10% carbohydrate (sucrose), 4.7% fat (corn oil), 0.25% choline chloride, 0.18% L-Cystine, 0.8% Butyl hydroquinone, 1% vitamin mixture, 3.5% salt mixture and 5% fibers (cellulose). The remainder was corn starch up to 100%.

**Experiment and grouping of rats:** After one week of adaptation, rats (n = 30) were divided into two main groups. The first main group (6 rats) was used as a negative control group (-ve) and fed on the basal diet only. The second main group (24 rats) were subcutaneously injected with CCl4 in paraffin oil (50% V/V 2 mL/kg b.wt.) twice a week for two weeks to induce chronic damage in the liver (Jayasekhar et al., 1997). The injected rats were divided into four subgroups (six rats each). One of them (6 rats) was fed on the basal diet used as a positive control group (+ve). However, the other subgroups (3 subgroups) were fed on basal diets and obtained orally dill oil (1 mL/kg), fennel oil (1 mL/kg) and mixture of (0.5 dill and 0.5 mL/kg fennel) oil respectively for 4 weeks.

**Blood sampling:** At the end of the experimental period (30 day), diets were withheld from experimental rats for 12 h and then rats were sacrificed. Blood samples were collected from the portal vein into dry clean centrifuge tubes. For serum separation, blood samples were left at room temperature to get clot and then centrifuged for 15 min at 3000 rpm. Serum was carefully aspirated using a needle and transfers into dry clean test tubes and kept frozen at -10°C until chemical analysis. Liver was removed for antioxidant analysis.

**Biochemical analyses:** Biochemical parameters i.e., aspartate amino transferase (AST) and alanine amino transferase (ALT) (Bergmeyer et al., 1978), alkaline phosphatase (ALP) (Roy, 1970), serum total cholesterol
(Richmond, 1973), triglycerides (TG) (Wahlfeld, 1974), high density lipoprotein (HDL-c) (Albers et al., 1983), were analyzed according to the reported methods. Meanwhile low density lipoprotein (LDL-c) was calculated according to (Friedwald et al., 1972) using the following equation:

\[ LDL-c = TC - (HDLC + VLDL-c) \]

where, also very low density lipoprotein (VLDL-c) was estimated according to the equation of (Friedwald et al., 1972), as follow:

\[ VLDL-c = TG/5 \]

where, serum total protein and albumin were determined according to Kingsley (1939), while serum bilirubin by was determined according to (Magos, 1960).

Preparation of liver homogenate: One gram of each frozen liver tissue was washed in ice-cold 0.9% NaCl and homogenized in ice-cold 1.15% solution of potassium chloride in 50 mM potassium phosphate buffer solution (pH 7.4) to yield a 10% (W/V) homogenate. Homogenization was performed using Sonicator, 4710 Ultrasonic Homogenizer (Cole-Parmer Instrument Co., USA). The homogenate was centrifuged at 3000 rpm for 10 min. at 4°C. The supernatant was collected and used as liver homogenate sample.

Analytical procedures: The prepared liver homogenates were used for measurement of tissue malondialdehyde (MDA) according to (Rui-Larea et al., 1994) and determine the activity of antioxidant enzyme Superoxide Dismutase (SOD) according to the method of (Kakkar et al., 1984).

Statistical analysis: Results were expressed as mean±SE. All data from the experiment were examined statistically by one-way analysis of variance with computerized SPSS package program (SPSS 16.00 software for Windows) by ANOVA test to test the variations among groups and Post Hoc test (Duncan’s test) was used to compare group means. A p<0.05 was considered statistically significant.

RESULTS
The obtained results in Table 1 showed that subcutaneous administration of CCL4 to rats significantly (p<0.05) increased levels of serum AST, ALT and ALP as compared to (-ve) group, indicating liver damage. CCL4 is reported to produce free radicals, which affect the cellular permeability of hepatocytes leading to elevated levels of serum biochemical parameters like ALT, AST and ALP. Administration with dill or fennel oil or their mixture significantly (p<0.05) decreased the elevated serum levels of liver enzymes in CCL4-intoxicated rats as compared to (+ve) group. Also, there is no significant differences in the level of liver enzymes between the group fed on mixture of (dill and fennel oil) and (-ve) group.

The effect of dill and fennel oil on serum levels of Total Protein and Albumin is presented in Table 2. The data revealed that CCL4 treatment caused significant (p<0.05) decreased in the levels of Total Protein and Albumin and significantly (p<0.05) increased the serum level of Bilirubin as compared to (-ve) group.

The administration of rats with the dill and fennel oil and their mixture significantly (p<0.05) increased the lowered levels of total protein, while there is no significant differences in serum Albumin for the tested groups as compared to +ve group as shown in Table 2. On the other hand, the group fed on mixture of (dill and fennel oil) had no significant differences in the level of Total Protein, Albumin and Bilirubin as compared to (-ve) group. Also, the best results were found in the group fed on mixture of dill and fennel oil, while was brought to near normal. The alkaline phosphatase is the prototype of enzymes that reflects the pathological alteration in biliary flow. The CCL4 induced elevation of this enzymatic activity in the serum is in line with high level of serum bilirubins content. The ethanol extract of Anethum graveolens Linn CCL4 induced suppression of the increased ALP activity with the concurrent depletion of raised bilirubins suggests the possibility of the extract to have ability to stabilize biliary dysfunction in rat liver during hepatic injury by CCL4. The results illustrated in the Table 1 and 2 exhibit the significant hepatoprotective effects of the dill and fennel oil and their mixtures.

It is clear from Table 3 that, CCL4 treatment caused significant (p<0.05) increased in the content of MDA and significantly (p<0.05) decreased the activity of SOD in the homogenates liver of the rats as compared to (-ve) group.
Liver is one of the vital organs in the body and it is responsible for detoxification of toxic chemicals and drugs. Thus it is the target organ for all toxic chemicals. Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver disease (Parola et al., 1992). It is widely used to induce liver damage because it is metabolized in hepatocytes by cytochrome P450, generating a highly reactive carbon centered trichloromethoxy radical (Johnson and Kroening, 1986). These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides. This lipid peroxidative degradation of biomembranes is one of the principle causes of hepatotoxicity of CCl4 (Poli, 1993). This is evidenced by an elevation in the serum marker enzymes namely AST, ALT, ALP, total bilirubin and decrease in total protein and albumin.

Naturally occurring antioxidants can be used to protect human beings from oxidative stress damage (Scalbert et al., 2005). Fennel was known as excellent sources of nature antioxidants and contributed to the daily antioxidant diet (Shahat et al., 2011). The volatile oil of fennel showed strong antioxidant activity in comparison with butyraldehyde in comparison with butyraldehyde and butyraldehyde and hydroxylozone (Singh et al., 2008).

Volatile components of fennel seed extracts contain β-myrcene (Ozbek et al., 2004) that elevates the levels of apoproteins CYP2B1 and CYP2B2, which are subtypes of the P450 enzyme system (De-oliveira, 1997). The cytochrome P450 (CYP) enzyme system consists of a superfamily of hemoproteins that catalyse the oxidative metabolism of a wide variety of exogenous chemicals including drugs, carcinogens, toxins and endogenous compounds such as steroids, fatty acids and prostaglandins (Shimada et al., 1994).

The mechanism of the hepatoprotective action of the fennel oil is uncertain but may be related to the ability of the fennel oil to inhibit lipid peroxidation in the liver and decreasing levels of serum AST, ALT, ALP and bilirubin. The CCl4 induced hepatotoxicity produced in rats leading to hepatic injury triggers the generation of toxic radicals which can be masked by using a correct antioxidant in adequate amount. The presence of D-limonene and β-myrcene of the fennel oil explain its role in hepatoprotection by inhibiting the free radicals mediated damage (Ozbek et al., 2003).

The hepatotoxicity produced by chronic carbon tetrachloride administration was found to be inhibited by Foeniculum vulgare essential oil with evidence of decreased levels of serum AST, ALT, ALP and Bilirubin (Hanefi et al., 2004).

The use of fennel oil and their mixtures in our study protects the liver from damage by CCl4 as evident by improved biochemical markers of liver damage. This
is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew and Joice, 1987). The explanation to our results were in agreement with Taher et al. (2007) who mentioned that active ingredients of volatile oil of dill (1000 µL/kg) are D-carvon and D-limonen and may act as an antioxidant or to decrease the production of free radicals, causing stabilization of hepatocyte membrane and decreasing the release of enzymes into the blood. Also our results were agreement with Choi and Hwang, (2004) who reported that fennel essential oil significantly increased the specific activities of SOD and Catalase. Also, the essential oil of fennel exhibited antibacterial and antiviral activities (Ruberto et al., 2000). The increase in MDA level in liver induced by CCl4 suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Treatment with either dill or fennel oil and their mixture significantly reverses these changes. Hence it is likely that the mechanism of hepatoprotection of either dill or fennel oil and their mixture is due to its antioxidant effect. Oral administration of the essential oil of Anethum graveolens seeds, at two different doses, reduced the triacylglyceride levels by almost 42%. The total cholesterol level was not reduced by the same doses of the essential oil (Yazdanparast and Alavi, 2001). Hajhashemi and Abbasi (2008) indicated that, Anethum graveolens essential oil (AGEO) was prepared by hydrodistillation and analyzed using GC/MS. AGEO had a yield of 2% and GC/MS analysis showed that alphaphellandrene (32%), limonene (28%) and carvone (28%) were its major components. Daily oral administration of AGEO to rats at doses of 45, 90 and 180 mg/kg for 2 weeks significantly and in a dose-dependent manner reduced TC, TG and LDL-C. AGEO also increased significantly HDL-C. so that Anethum graveolens has significant lipid lowering effects and is a promising cardio protective agent. These results were in agreement with our study.

Conclusion: The efficacy of any hepatoprotective material is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been disturbed by a hepatotoxin, the results of the present study indicated that under the present experimental conditions, both dill and fennel oil and their mixtures showed hepatoprotective effects against carbon tetrachloride induced liver damage in albino rats.

REFERENCES


