Volatile compounds and antioxidant activity of the aromatic herb *Anethum graveolens*

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Background/Aim

Anethum graveolens L. (dill) (Apiaceae) is one of the most popular culinary herbs in the world. Dill has been cultivated since ancient times, and the use of this plant for medicinal and consumption purposes has been recorded dating back to the Greek and Egyptian civilizations. Dill is widely used to give flavor to food. The aim of this work was to assess the chemical composition of the volatile compounds in dill as well as their hepatoprotective and nephroprotective activity against free radicals generated by paracetamol.

Materials and methods

The chemical composition of the volatile compounds of dill was assessed by gas chromatography and gas chromatography-mass spectrometry. Four groups of rats were used (the normal control group, the *A. graveolens*-supplemented group, the paracetamol-intoxicated group, and the *A. graveolens*-protected group) for studying the effect of plant infusion on panadol (paracetamol)-induced free radicals and hepatotoxicity. Plasma total antioxidant capacity, plasma catalase, cellular glutathione peroxidase, plasma total protein, albumin, alanine amino transferase, aspartate amino transferase, alkaline phosphate, γ-glutamyl transferase, total bilirubin, and direct bilirubin levels were determined. In addition, kidney functions (plasma urea and creatinine) and histopathological and histochemical changes in the liver were investigated.

Results

The phytochemical results identified volatile components 7-α-hydroxy manool (24.43%), l-carvone (14.28%), limonene (13.9%), epi-α-bisabolol (6.81%), α-terpinene (5.44%), and α-phellandrene (4.63%) as the main constituents. *p*-Cymene (2.13%), sabinene (1.98), and α-pinene (1.43%) were determined as the minor constituents. The biochemical results showed that a mega dose of paracetamol induced the production of free radicals, which caused damage to hepatocytes and nephrocytes in rats. The aqueous extract of dill revealed high antioxidant properties and acted as an extracellular neutralizer of free radicals. Histopathological and histochemical observations showed severe damage in the liver. Supplementation with dill in paracetamol-intoxicated rats attenuated the damage to the liver.

Conclusion

The present study revealed that *A. graveolens* has antihepatotoxic properties that could minimize the deleterious effects generated by hepatotoxin paracetamol, and therefore it can be used as a potent antihepatotoxic agent.

Keywords:

Anethum graveolens, antioxidants, hepatoprotective, nephroprotective, rats, volatile compounds

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Introduction

Anethum graveolens is a short-lived perennial herb. The *A. graveolens* fruit is oval, compressed, winged, and about a tenth of an inch wide. The genus name *Anethum* is derived from a Greek word meaning strong smelling. The *Anethum* spp. fruit is used as a spice and the fresh and dried leaves of the plant called dill are used as a condiment and in tea. The use of this plant for medicinal and consumption purposes has been recorded dating back to the Greek and Egyptian civilizations. The Egyptians used dill in the production of cosmetics and perfume. This aromatic herb was commonly used for flavoring and seasoning of various foods such as pickles, salads, sauces, and soups [1,2]. Dill fruits have a strong aromatic smell and taste but they lose these properties on cooking because of the loss of essential oils. Dill oil is extracted from the seeds, leaves, and stems of the plant, and is an essential oil used as flavoring in the food industry [1,3]. Further, chewing the seeds improves bad breath. *Anethum* spp. stimulates milk flow in lactating mothers and cures urinary complaints, piles, and mental disorders [4]. It has been reported that the aqueous extract of *A. graveolens* has a broad-spectrum antibacterial activity. The essential oil and extract of dill have been reported to possess various degrees of antimycobacterial activity and to exhibit strong antifungal activity [5,6]. Antioxidants are compounds that can delay, inhibit, or prevent the oxidation of oxidizable matters by scavenging free radicals and diminishing oxidative stress [7].

Herbs contain a wide variety of antioxidant phytochemicals or bioactive molecules that can neutralize the free radicals and thus retard the progress of many chronic diseases associated with oxidative stress and reactive oxygen species [8,9]. *A. graveolens* has been reported to contain flavonoids, phenolic, and essential oil [5]. The increased consumption of vegetables and herbs containing high levels of phytochemicals has been recommended to prevent or reduce oxidative stress in the human body [8–10]. Endogenous antioxidant defense mechanisms may be insufficient and hence dietary intake of antioxidant compounds is essential [11]. The intake of natural antioxidants has been associated with reduced risk for cancer, cardiovascular disease, diabetes, and diseases associated with aging [12]. The liver is associated with many important life functions; it has great capacity to detoxicate toxic substances and synthesize useful principles [13]. The extract of *A. graveolens* could protect the liver against high-fat-diet-induced oxidative damage in rats [14].

The crude extract of *A. graveolens* has been reported to have antihypercholesterolemia and antihyperlipidemic properties. The effect of *A. graveolens* extract on serum lipoproteins in hypercholesterolemic rats and the possible mechanism of action of a crude extract on liver enzymes activity have been studied [15]. The present study was designed to specifically investigate the antioxidant efficacy of the aqueous extract of *A. graveolens* against paracetamol drug-induced oxidative stress in albino rats.

Materials and methods

Isolation and characterization of *Anethum graveolens* volatile compounds

Two hundred grams of dried fruit of *A. graveolens* were ground to a fine powder using an electric grinder. The essential oil was obtained by steam distillation in 3000 ml H_2O for 3 h using a Clevenger apparatus. The oil was dried over anhydrous sodium sulfate and filtered. Extraction was carried out in duplicate and the results were averaged. Analysis of the volatile compounds of the plant infusion was carried out using chromatographic techniques such as gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

Gas chromatography

The volatile oil of *A. graveolens* was thermally desorbed using a modified injector port directly on the front of a (DB5) (60 m × 0.32 mm internal diameter) fused-silica capillary column, in the oven of a Hewlett-packard HP 5890 gas chromatograph, Agilent technologies company, USA and the temperature was increased from 45 to 240°C at a rate of 4°C/min. Kovat's indices were determined by coinjection of the sample with a solution containing homologous series of *n*-hydrocarbons (C_6–C_{26}) under the same conditions as described above. The separated components were identified by matching with NIST mass-spectral library data, and by comparison of Kovat's indices with those of authentic samples and with published data [16]. Quantitative determination was carried out on the basis of peak area integration. Retention indices of each compound were calculated from the standard alkane retention time and the peak retention time.

Gas chromatography-mass spectrometry

Analyses were performed on an HP model 6890 GC, Thermoscientific company, USA interfaced to an HP 5791A mass selective detector (GC-MS), Thermoscientific company, USA which is used for mass spectral identification of GC components at (MS) an ionization voltage of 70 eV. A 30 m × 0.25 mm internal diameter (DF = 0.25 lm) DB-WAX bonded-phase fused-silica capillary column, Thermoscientific company, USA was used for GC. The linear velocity of the helium carrier gas was 30 cm/s. The injector and the detector temperatures were 250°C. The oven temperature was programmed from 40 to 240°C at 4°C/min and held for 50 min.

Preparation of plant infusion

Dry fruits of *A. graveolens* were purchased from Omdurman market in Khartoum State, Sudan. The dry fruits under study were ground separately and a quantity of 3 g was infused with 100 ml of freshly boiled water for 5 min, followed by filtration.

The infusion filtrate of *A. graveolens* was subjected to the following tests:

(1) Quantitative determination of the total phenolic content (TPC) was performed using the Folin–Ciocalteu method [17].
(2) Quantitative determination of antioxidant activity was performed according to the β-carotene bleaching method [18] and diphenyl picryl hydrazyl (DPPH) free radical scavenging assay [19].

Biochemical study

Experimental animals

Adult male Swiss albino rats with initial weights ranging from 120 to 150 g were used as experimental animals for biochemical and histological studies. All animals were from the Breeding Unit of the National Research Centre (Cairo, Egypt). The animals were housed individually in stainless steel wire mesh cages and acclimatized for 1 week. Commercial standard pellets and tap water were supplied *ad libitum*.

Experimental design

Twenty-eight adult male albino rats were used for studying the effect of plant infusion on panadol (paracetamol)-induced free radicals and hepatotoxicity [20]. The rats were divided equally into four groups (seven rats in each group): group 1 (the normal control group) rats received tap water. Group 2 (the *A. graveolens* group) rats were given a supplement of freshly prepared aqueous extract of *A. graveolens* (3 g/100 ml water) for 30 days to examine the beneficial effects of the plant. Group 3 (the paracetamol group) rats received a single dose of paracetamol orally (2 g/kg of rat body weight) after 4 weeks (28 days) [20]. Group 4 (the protected group) rats received a supplement of freshly prepared *A. graveolens* (3 g/100 ml) for 28 days, and then received paracetamol (2 g/kg of rat body weight). The experiment duration was continued for 30 days. The rats were killed 2 days after the single oral dose of paracetamol (panadol).

Blood and tissue sampling

At the end of the experimental period, blood samples were drawn into heparinized tubes. Plasma was used for determination of liver and kidneys function and for the presence of antioxidant biomarkers. The RBCs were washed several times with cold saline solution. The packed RBCs were stored at –20°C for determination of glutathione peroxidase (GPx). The liver was excised and rinsed with cold saline, dried on filter paper, and weighed. A portion of the liver tissue of each rat was kept in 10% formalin for histological and histochemical examinations.

Biochemical assays

In this study, assay kits were purchased from Elitek Diagnostic (Spain), Boehringer-Mannheim (Germany), Lincer Chemicals (Italy), Stanbio (Spain), Sigma Diagnostic (USA), and RANDOX (USA).

Plasma total antioxidant capacity (TAC), plasma catalase (CAT), and cellular GPx levels were determined using the methods of Koracevic *et al.* [21], Aebi [22], and Paglia and Valentine [23], respectively.

Plasma total protein, albumin, alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), total bilirubin, and direct bilirubin activities were determined according to the methods described by Gornall *et al.* [24], Doumas *et al.* [25], Reitman and Frankel [26], Belfield and Goldberg [27], Persijn and Van der Slik [28], and Walters and Gerarde [29], respectively. Kidney functions (plasma creatinine and urea) were also assessed according to the method of Bartles *et al.* [30] and Fawcett and Soctt [31].

Histological study

To examine the extent of cellular damage caused by paracetamol, the liver samples of experimental and control rats were fixed in 10% formalin saline for 24 h. Following a rinse with water, the tissues were dehydrated in graded series of alcohol, cleaned in xylol, and embedded in paraffin wax (58–60°C). Using a rotary microtome, 6-μm-thick sections were obtained. The sections were deparaffinized in xylene and hydrated in graded series of alcohol ranging from 100 to 90, 70, 50, and 30% and then in distilled water. Thereafter, the sections were stained with hematoxylin and counterstained with aqueous eosin for microscopic investigations [32]. The stained sections were mounted in DPX.

Histochemical study

Total proteins

The mercury bromophenol blue method was used for the histochemical demonstration of total proteins [33].

The polysaccharide inclusions

The periodic acid Schiff (PAS) method was applied for visualization of polysaccharide materials [34].

Statistical study

The data presented in the study were statistically evaluated as mean ± SE for each group. Statistical evaluation of the difference between the group mean values was carried out by analysis of variance (ANOVA) analysis. *P* values less than 0.05 were considered significant [35].

Results

Phytochemical analysis

Composition of the essential oil

Composition of the essential oil of *A. graveolens* fruits was established by GC and GC-MS. Figure 1 and Table 1 showed 40 identified compounds and their percentages were obtained by GC-MS as well as by the retention time listed in order of their elution from the capillary column used. Chemical identification of the volatile oil constituents was made on the basis of their retention indices and mass spectral data and authentic samples. According to GC-MS data, monoterpenes were the main constituents of the essential oil. The main volatile components were 7-α-hydroxy manool (24.43%), l-carvone (14.28%), limonene (13.9%), epi-α-bisabolol (6.81%), α-terpinene (5.44%), and α-phellandrene (4.63%). *p*-Cymene (2.13%), sabinene (1.98), and α-pinene (1.43%) were the minor constituents.

Total phenolic content and antioxidant activity

The TPC in the *A. graveolens* aqueous extract was determined by Folin–Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents (GAE); the result is presented as mg GAE/l. The TPC value of the dill aqueous extract was 2040 ± 14.7 mg/l (Table 2). The radical scavenging activity of the dill aqueous extract on β-carotene/linoleic acid and DPPH free radicals increased with increasing concentration of aqueous extract from 50 to 400 μg/ml. Table 2 also illustrates the percentage inhibition of β-carotene-linoleate and DPPH free radicals exhibited by the dill aqueous extract. The dill extract showed excellent radical scavenging activity, with percentage inhibition at the highest concentration of 400 μg/ml being 87.5 ± 2.7 and 89.7 ± 2.4% according to the β-carotene-linoleate method and DPPH free radicals method, respectively.

Biochemical results

Table 3 shows the effect of the aqueous extract of *A. graveolens* fruits on some antioxidant biomarkers (plasma TAC, plasma CAT activity, and erythrocyte GPx) in paracetamol-intoxicated rats. ANOVA analysis indicated that rats supplemented with the aqueous extract had a significant increase in all tested antioxidant biomarkers compared with control. Administration of paracetamol only recorded a significant decrease in TAC, CAT, and GPx compared with normal control rats. Rats treated with the aqueous extract before paracetamol administration showed a significant increase in all tested antioxidant biomarkers compared with paracetamol-intoxicated rats. In contrast, compared with normal control, the values of the aqueous extract-protected group returned to be comparable with control values.

Table 4 illustrates the effect of the aqueous extract of *A. graveolens* fruits on liver and kidneys functions in panadol-intoxicated rats. Plasma activities of ALT, AST, ALP, and GGT, and plasma levels of total proteins, albumin, total bilirubin, direct bilirubin, and indirect bilirubin of all studied groups were evaluated. The results of liver function tests revealed that activities of AST, ALT, ALP, and GGT as well as levels of plasma

Table 1 Volatile compounds of *Anethum graveolens* analyzed by GC-MS

Peak number	KI	Area (%)	Identified compound	Peak number	KI	Area (%)	Identified compound
1	933	1.43	α-Pinene	21	1480	0.45	Gremacene
2	1002	1.95	δ-2-Carene	22	1496	0.3	(Z)-Dihydroapofarnesol
3	1014	4.63	α-Phellandrene	23	1512	0.37	γ-Cadinene
4	1017	5.44	α-Terpinene	24	1527	0.25	Vetivene
5	1040	0.74	Trans β-ocimene	25	1540	0.25	α-Calacorene
6	1067	1.98	Sabinene	26	1553	0.25	Elemicin
7	1080	2.13	*p*-Cymene	27	1566	0.26	Caryophyllene alcohol
8	1110	0.97	Camphenol	28	1577	0.28	Spathulenol
9	1122	0.85	Cis-pinene hydrate	29	1648	0.69	β-Eudesmol
10	1143	1.07	Camphor	30	1675	0.14	Androencocalinol
11	1160	13.9	Limonene	31	1684	6.81	Epi-α-bisabolol
12	1205	0.83	Para-cymen-9-ol	32	1758	2.25	(E)-Nuciferol
13	1307	0.93	Iso-vebranol acetate	33	1760	2.5	β-Bisabolen-12-ol
14	1315	0.29	Terpinyl acetate	34	1773	1.57	(E)-α-atlantone
15	1320	0.51	Dihydro citronellal acetate	35	1797	14.28	l-Carvone
16	1356	0.73	Eugenol	36	1808	2.12	Cryptomeridiol
17	1389	0.58	Cubebene	37	1905	1.63	2-Phenylethyl phenyl acetate
18	1418	0.47	(E)-Caryophyllene	38	1990	0.28	4-Methyl stilbene
19	1442	0.56	γ-Patchoulene	39	2136	0.51	Osthol
20	1462	0.38	Allo-aromadendrene	40	2234	24.43	7-α-hydroxy manool

Compound listed in the order of elution from a DB_5 column; retention indices relative to C_7–C_{20} *n*-alkanes on the DB-5MS column; identification based on retention index; and comparison of mass spectra; GC-MS, gas chromatography-mass spectrometry; KI, Kovats index.

Table 2 Total phenolic content and in-vitro antioxidant activity of *Anethum graveolens* extract

		Inhibition at different concentrations (%)							
		AA at different concentrations by β-carotene method (μg/ml)				AA at different concentrations by DPPH free radical scavenging method (μg/ml)			
	TPC (mg/l)	50	100	200	400	50	100	200	400
Anethum extract	2040 ± 14.7	50.0 ± 1.8	73.5 ± 2.4	82.7 ± 3.0	87.5 ± 2.7	54.7 ± 1.2	71.5 ± 1.8	80.7 ± 2.1	89.7 ± 2.4
TBHQ		75.2 ± 3.1	85.0 ± 2.5	94.0 ± 2.7	99.5 ± 2.7	76.53 ± 2.3	83.7 ± 2.5	95.3 ± 2.6	99.7 ± 2.6

Each value represents the mean ± SE and mean of three replicates; AA, antioxidant activity; TBHQ, tert-butyl hydroquinone, standard synthetic antioxidant; TPC, total phenolic content.

total bilirubin, direct bilirubin, and indirect bilirubin of rats treated with paracetamol only were quite higher than those of the control group. In contrast, protected rats treated with dill extract and paracetamol had significantly lower levels of all previous parameters when compared with the paracetamol-intoxicated group. The content of total proteins and albumin decreased significantly in paracetamol-intoxicated rats and this depletion returned to near control values when rats were protected with the dill aqueous extract. ANOVA analysis indicated that kidney functions deteriorated, and urea and creatinine levels significantly increased in paracetamol-intoxicated rats and this toxic elevation became comparable to control levels when rats were protected with the dill aqueous extract Table 4.

The histopathological results

Histopathological changes were observed in both control and experimental groups. The control rat's liver showed radially placed hepatic cells, with each cell having a large spherical nucleus and granular cytoplasm without any injury (Fig. 2a). The portal lobule is considered the functional unit of the liver tissue. Each portal lobule is a triangular mass of liver tissue, which has the portal area at its center and is outlined by lines that connect the central veins of the three hepatic lobules that surround this portal area (Fig. 2b). Paracetamol-treated rats showed vacuolization, several apoptotic cells, necrotic changes, and lymphocytic infiltration. Loss of liver cell boundaries and portal tracts with dilated and congested veins were observed (Fig. 2c and d). Treatment of animals with *A. graveolens* alone resulted in normal hepatocytes (Fig. 2e). In case of rats pretreated with *A. graveolens* before administration of paracetamol, the liver showed marked reduction in the extent of necrosis and degree of degeneration (Fig. 2f).

Table 3 Effect of paracetamol and *Anethum graveolens* extract on plasma total antioxidant capacity, plasma catalase, and cellular glutathione peroxidase in rats

Groups	Biomarkers		
	TAC (mmol/l)	CAT (U/ml)	GPx (U/ml)
Control	1.15 ± 0.13[a]	386.17 ± 28.02[a]	0.16 ± 0.03[a]
Extract	1.52 ± 0.18[b]	398.38 ± 46.33[b]	0.19 ± 0.2[b]
Paracetamol	0.92 ± 0.06[c]	198.91 ± 19.54[c]	0.12 ± 0.02[c]
Paracetamol+extract	0.99 ± 0.10[a]	364.76 ± 27.84[a]	0.16 ± 0.02[a]

Data are presented as mean ± SD; CAT, catalase; GPx, glutathione peroxidase; TAC, total antioxidant capacity; Values in the same column with the same superscripts are not significant at $p < 0.05$.

Liver total proteins

Examination of liver sections of the control rats showed the proteinic inclusions in the hepatocytes as grayish blue irregular particles of various sizes against weakly to moderately stained ground cytoplasm. The nuclear chromatin and nucleoli were densely stained, indicating their rich content of proteinic constituents (Fig. 3a). In Kupffer cells, the proteinic inclusions are mainly localized at the periphery of the cytoplasm. The nuclei of these cells are moderately stained. In case of treatment with paracetamol, protein inclusions became smaller in size compared with those of control rats (Fig. 3b). Oral administration of *A. graveolens* plant extract showed normal distribution of the protein inclusions in the hepatocytes (Fig. 3c). In contrast, administration of *A. graveolens* plant extract before panadol displayed diffuse staining. A few hepatocytes displayed dense staining compared with others (Fig. 3d). The above results revealed that paracetamol causes a reduction in the content of protein materials in the liver of treated rats and the volume of this reduction is decreased with the treatment of the plant extract.

Liver polysaccharides

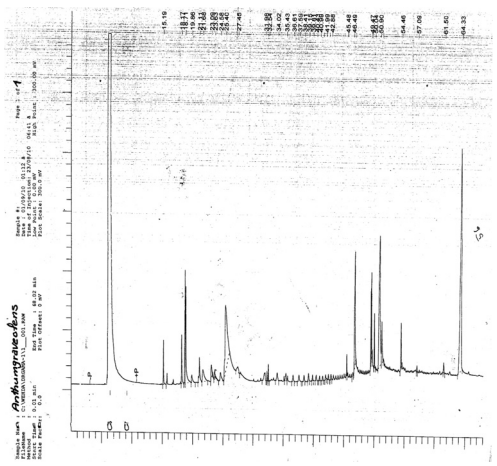
Examination of thin sections of the liver of control rats stained according to the PAS technique showed an abundance of polysaccharide materials (glycogen) in hepatocytes. The nuclei of the hepatocytes gave negative PAS reaction, indicating the absence of polysaccharides (Fig. 4a). Daily treatment with paracetamol induced faint homogenous staining of the polysaccharide inclusions in the hepatocytes (Fig. 4b).

Table 4 Effect of paracetamol and *Anethum graveolens* extract on liver and kidney functions in rats

Groups	Parameters										
	Liver function tests									Kidney function tests (mg/dl)	
	AST (U/l)	ALT (U/l)	ALP (U/l)	GGT (U/l)	Total. proteins (g/dl)	Albumin (g/dl)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Indirect bilirubin (mg/dl)	Urea	Creatinine
Control	61.57[a] ± 4.28	16.43[a] ± 1.54	169.09[a] ± 7.54	23.20[a] ± 1.63	6.74[a] ± 0.19	3.15[a] ± 0.21	0.34[a] ± 0.03	0.14[a] ± 0.01	0.19[a] ± 0.02	37.78[a] ± 1.59	0.70[a] ± 0.04
Extract	59.00[a] ± 3.22	14.50[a] ± 2.81	167.52[a] ± 9.42	23.62[a] ± 1.59	6.90[a] ± 0.44	3.26[a] ± 0.1	0.34[a] ± 0.04	0.15[a] ± 0.01	0.18[a] ± 0.02	36.85[a] ± 3.02	0.67[a] ± 0.02
Paracetamol	85.00[b] ± 5.48	39.57[b] ± 2.64	240.19[b] ± 8.49	34.19[b] ± 1.15	5.97[b] ± 0.18	2.88[b] ± 0.19	0.50[b] ± 0.02	0.24[b] ± 0.02	0.26[b] ± 0.02	43.69[b] ± 1.08	0.79[b] ± 0.03
Paracetamol +extract	74.57[c] ± 3.51	30.17[c] ± 3.10	210.23[c] ± 10.84	30.11[c] ± 0.88	6.29[c] ± 0.12	2.78[c] ± 0.17	0.41[c] ± 0.02	0.20[c] ± 0.01	0.21[c] ± 0.03	38.1[a] ± 1.64	0.69[a] ± 0.03

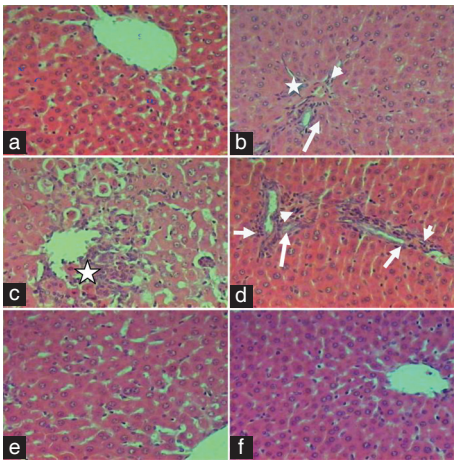
All data are presented as mean ± SE; ALP, alkaline phosphatase; ALT, alanine amino transferase; AST, aspartate amino transferase; GGT, γ-glutamyl transferase; All values in the same column with the same superscripts are not significant at $p < 0.05$.

Figure 1



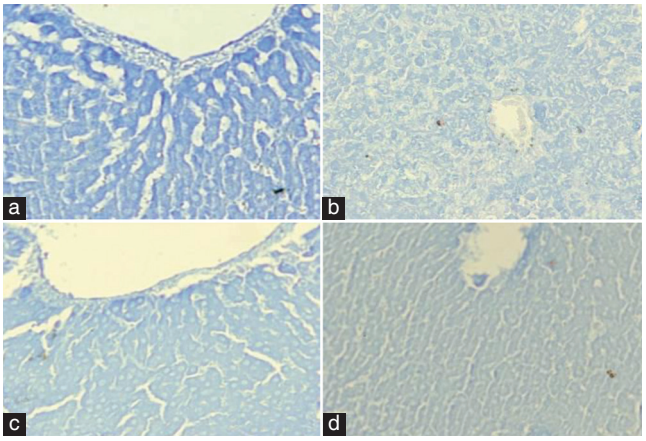
Gas chromatogram of volatiles in the hydrodistilled oil of Sudanese *Anethum graveolens* fruits analyzed by GC-MS. GC-MS, gas chromatography-mass spectrometry.

Figure 2



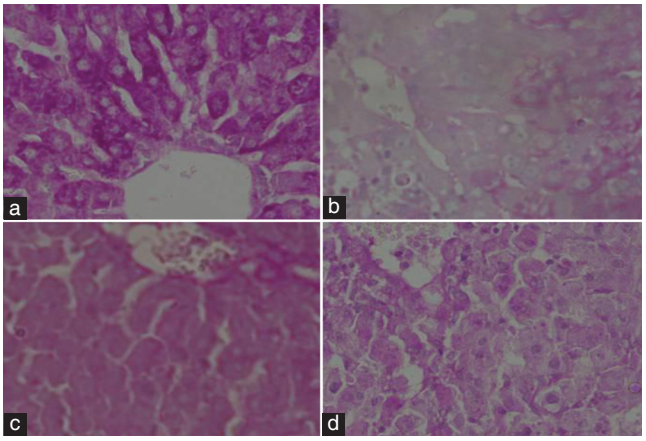
Section of the liver of (a) a control rat showing the normal architecture of a hepatic lobule; (b) control liver showing the normal portal area; (c) several apoptotic cells (arrows) and focal necrosis (arrowheads) associated with lymphocytic infiltration (asterisk) in a rat treated with panadol; (d) a portal tract with a dilated and congested vein (arrow), periportal necrosis of hepatocytes (long arrow), and inflammatory infiltration (arrowhead) in a rat treated with panadol; (e) normal hepatocytes in a rat treated with *Anethum graveolens*; (f) normal structure in a rat treated with *A. graveolens* and panadol (H&E stain, ×300).

Figure 3



Sections of the liver of (a) control rat showing the normal distribution of proteinic contents; (b) proteinic inclusion distribution in a rat treated with panadol. Notice that these inclusions are diminuted and palely stained in the hepatocytes; (c) staining of the proteinic inclusions that is relatively diffused in both the cytoplasm and the nucleus in a rat treated with *Anethum graveolens*; (d) staining of the proteinic inclusions that is relatively diffused in both the cytoplasm and the nucleus in a rat treated with *A. graveolens* and panadol (bromophenol blue reaction, ×400).

Figure 4



Sections of the liver of (a) a control rat showing an abundance of glycogen in the cell of the hepatic lobule; (b) marked depletion of the polysaccharide inclusions in a rat administered paracetamol; (c) normal distribution of polysaccharide inclusions in hepatocytes in a rat treated with *Anethum graveolens*; (d) polysaccharide inclusions in a rat treated with *A. graveolens* and panadol. Notice that such inclusions display diffuse staining. A few hepatocytes display dense staining (PAS/H, ×300).

Oral administration of *A. graveolens* plant extract resulted in normal distribution of the polysaccharide inclusions in the hepatocytes (Fig. 4c).

Oral administration with paracetamol plus *A. graveolens* plant extract before panadol administration led to diffuse staining. A few hepatocytes displayed dense staining compared with others (Fig. 4d). Thus, it was seen that paracetamol causes a reduction in the amount of polysaccharide materials in the liver of treated rats. The degree of this reduction is decreased with the treatment of *A. graveolens* plant extract.

Discussion

The present results indicated that there are 40 volatile compounds in *A. graveolens* (dill) fruits according to their elution order on the DB5 column, with 7-α-hydroxy manool (24.43%), l-carvone (14.28%), limonene (13.9%), epi-α-bisabolol (6.81%), α-terpinene (5.44%), and α-phellandrene (4.63%)

being the main constituents and *p*-cymene (2.13%), sabinene (1.98), and α-pinene (1.43%) being the minor constituents. Terpenes were the most abundant volatiles detected in *A. graveolens*. Dill oil contains carvone and d-limonene, which together represent a high percentage of the compounds identified by GC-MS [5]. The aroma compound of dried dill stems (α-phellandrene) was identified. α-Phellandrene, the main constituent of dill aroma, greatly contributes to the sensory impression of the dill herb. The content of dill ether in dried samples is significantly lower than that in fresh dill. Dried dill stems contain more of *p*-cymene than do fresh ones [36]. The hexane extract of *A. graveolens* contains volatile components such as α-phellandrene, which has been detected as a major compound, as well as β-phellandrene, α-pinene, and β-pinene. Cultivation conditions may have an effect on the bioactivity and phytochemical content of dill samples [37]. The chemical composition of dill volatile oil varies depending on the plant parts. Rădulescu *et al*. [38] reported that the main compound in the essential oil was carvone (75.21%), whereas the content of α-phellandrene was only 0.12% and that of limonene was 21.56%. The essential oil from the dried leaves, flowers, and fruits of *A. graveolens* cultivated in Romania was isolated by hydrodistillation and analyzed with GC-MS. The main components in leaves were α-phellandrene (62.71%), limonene (13.28%), and anethofuran (16.42%). The main components in flowers were α-phellandrene (30.26%), limonene (33.22%), and anethofuran (22%). Cis-carvone and limonene are the major constituents of fruit volatile oil. Monoterpenic hydrocarbons are predominant in fruit oil. Anethofuran (dill ether) is present in leaves and flowers at a proportion of 16.42 and 22%, respectively, but is missing in fruit oil [39]. These data agree with our results with some differences that were observed in dill phytochemical contents. It has been proved that the method of distillation could affect the chemical composition in essential oils. Chemical variations were observed in the essential oil of the same dill sample obtained by hydrodistillation and microdistillation. In addition to the method of distillation, the duration of distillation is another critical factor that may influence the essential oil composition [40]. One of the major classes of natural antioxidants found in the studied plant that could remove free radicals is polyphenol. The presence of phenolic compounds and essential oil in *A. graveolens* has been reported [5]. Polyphenols are able to neutralize free radicals, scavenge singlet and triplet oxygen, and break down peroxides. The TPC in dill aqueous extract was evaluated using the Folin–Ciocalteu method [17]. The extract showed a high level of TPC (2040 ± 14.7 mg/l). The method of determination of the level of total phenolics is not based on absolute measurements of the amount of phenolic compounds but on their chemical reducing capacity relative to gallic acid. It is very important to note that there is a positive relationship between the antioxidant activity potential and the amount of phenolic compounds in the extract. From the phenol antioxidant index a combined measure of the quality and quantity of antioxidants in herbs has been obtained [41]. The excellent antioxidant activity of the aqueous extract of dill showed 87.5 ± 2.7% scavenging activity against β-carotene-linoleate and 89.7 ± 2.4% scavenging activity against DPPH radical when compared with the synthetic antioxidant tert-butyl hydroquinone at the same concentration of 400 μg/ml (Table 1). Our result is compliant with many studies on dill extract of Thia and Turkish origin [5,40,42]. The excellent antioxidant activity of dill aqueous extract is attributed not only to the presence of a high content of polyphenols but also to that of volatile constituents that are present abundantly in dill fruits. These components could change free radicals such as DPPH to nonradical DPPH-H. Monoterpenes are the main chemical constituents of dill essential oil and are found as mixtures of odoriferous components that can be obtained by steam distillation or solvent extraction from a large variety of aromatic plants. Recent works have demonstrated that monoterpenes may present important pharmacological properties, including antioxidant activity. Monoterpenes with oxygen in their structure constitute a wide group of antioxidant molecules, largely because of their functional groups (alcohols) [43,44].

Our data showed for the first time the antioxidant activity of dill aqueous extract on Swiss albino rats treated with a supratherapeutic dose of paracetamol. Rats supplemented with the aqueous extract (group 2) recorded a significant increase in all tested antioxidant biomarkers – TAC, CAT, and GPx – compared with normal control rats. This pointed to its effect as a powerful activator of antioxidant status in normal rats besides its effect as a free radical scavenger and strong antioxidant *in vitro* (Table 2). The major metabolic pathways of paracetamol are glucuronidation and sulfation. Under normal conditions, about 5% of paracetamol is metabolized through the cytochrome P450 system, leading to the formation of the highly reactive intermediate *N*-acetyl-benzoquinone imine (NAPQI) [45]. NAPQI is conjugated with glutathione and eliminated until glutathione and available liver sulfur stores become critically low. If NAPQI is not removed, it binds to critical intracellular molecules and eventually leads to toxicity and cell death. Glutathione, in its reduced (GSH) and oxidized (GSSG) forms, is the main intracellular thiol redox system in erythrocytes [46]. One of its major functions is the detoxification of reactive electrophiles and toxic

oxygen metabolites generated during the metabolism of endogenous and exogenous substances [47]. Intermediates such as hydrogen peroxide and hydroperoxides are reduced by GPx at the expense of GSH. Some studies showed that acute paracetamol overdose can deplete erythrocyte GPx [48]. Moreover, paracetamol overdosing in rats produced significant changes in glutathione, glutathione-dependent enzymes, and antioxidant status [49]. Drugs that lead to the depletion of GSH or influence the activity of any of these GSH-dependent enzymes may result in toxic responses. Glutathione makes a complex with NAPQI in the liver [50] and the depletion of the former increases the risk of hepatotoxicity [51]. CAT is an enzymatic antioxidant widely distributed in all animal tissues. The decreased activity of CAT may be due to these free radicals generated by paracetamol. Treatment with dill aqueous extract significantly ($P < 0.05$) increases the activity of GPx and CAT. Thus, these finding suggest that the aqueous extract contains free radical scavenging activity and is rich in phenolics (2040 ± 14.7 mg GAE/l) besides volatile compounds, mainly monoterpenes hydrocarbones and oxygenated monoterpenes, which could exert a beneficial action against pathophysiological alterations caused by the highly reactive intermediate NAPQI free radicals, indicating the regeneration of damaged liver cells.

The present study indicated that dill aqueous extract is a good source of antioxidants and could be used to reduce biological oxidative stress and prevent cellular damage by scavenging free radical activity of the oxidative stress. As shown in Table 3, paracetamol overdose caused a significant increase ($P<0.05$) in liver biomarkers (AST, ALT, ALP, GGT, total bilirubin, direct bilirubin, and indirect bilirubin), indicating significant liver damage. This finding suggested that mega doses of paracetamol induce the production of free radicals, which cause damage to the hepatocytes of rats. This result correlates with the finding of Yakubu *et al*. [11] who demonstrated that the toxicity of paracetamol occurs when it is taken in high amounts. The elevations of plasma liver enzymes indicated liver damage and this correlates with the report of Sai *et al*. [52]. Increased plasma bilirubin levels in paracetamol-intoxicated rats (group 3) could be looked upon as a compensatory/retaliatory phenomenon in response to cellular peroxidative changes, which cause damage to the biliary gland. This is because bilirubin functions *in vivo* as a powerful antioxidant, antimutagen, and an endogenous tissue protector [53]. The significant ($P < 0.05$) reduction in total protein and albumin levels in paracetamol-intoxicated rats also indicated cellular damage. The damage produced might be due to the functional failure of endoplasmic reticulum, which leads to a decrease in protein synthesis [54]. In contrast, administering albino rats with dill aqueous extract caused a significant decrease ($P \leq 0.05$) in all previous liver function enzymes, and they reached near control values (Table 3). These findings indicated the ability of the extract to protect hepatocytes from oxidative damage caused by paracetamol overdose. Reduction of bilirubin and elevation of total protein and albumin levels in protected rats (group 4) indicated stabilized biliary cell function and endoplasmic reticulum leading to bile acid and protein synthesis [55].

It was noted in the present study that the liver is not the only target organ of paracetamol; it causes free radical generation in other organs such as the kidneys as well. The significant increase in urea and creatinine in paracetamol-intoxicated rats revealed the toxic effect of paracetamol overdose on kidneys. Dill aqueous extract contains powerful antioxidant components that serve as an extracellular neutralizer of free radicals. Rich polyphenols and volatile compounds in dill possess antioxidant capacity and improve the normal renal function as shown in Table 4.

In the present study, the histopathological investigations supported the biochemical findings. The paracetamol-treated rats showed necrosis, vacuoles, space formation, and loss of cell boundaries in the liver. Oral administration of *A. graveolens* before paracetamol administration reverted the above-mentioned changes in the liver to near normal. Coen *et al.* [56] reported marked changes in the liver, such as vacuolated hepatocytes, necrosis, and congested sinusoids, in paracetamol-treated rats. Oliveira *et al.* [57] reported that administration of α-amyrin and β-amyrin to paracetamol-treated rats resulted in a normal histoarchitecture in the liver. Furthermore, Yanpallewar *et al.* [58] and Yadani *et al.* [59] reported that the administration of *Azadirachta indica* to paracetamol-treated rats gave a normal histoarchitectural pattern to the liver.

The present study showed a decrease in the carbohydrate and protein content in the liver of paracetamol-intoxicated rats, as reported by Chen *et al*. [60]. The authors stated that initiation of lipid peroxidation, necrosis, and subsequent impairment in cellular metabolism collectively altered the major cellular components, including protein and glycogen.

Reid and Li [61] found that reactive oxygen species may activate the ubiquitin proteasome pathway. Proteasomes are very large protein complexes located in the nucleus and cytoplasm in all eukaryotes [62]. The main function of the proteasome is to degrade un-needed or damaged proteins by proteolysis.

Proteasomes are part of a major mechanism by which cells regulate the concentration of particular proteins and degrade misfolded proteins.

The presence of phenolic compounds and essential oil in *A. graveolens* has been reported [5]. Polyphenols are able to neutralize free radicals, scavenge singlet and triplet oxygen, and break down peroxides.

Conclusion

The present study revealed that the *A. graveolens* extract has antihepatotoxic properties that may minimize the deleterious effects generated by hepatotoxin paracetamol. Thus, it could be used as a potent antihepatotoxic agent. Also, the extract should be encouraged in diets as it could be used as a functional food to prevent liver and kidneys damage because of its antioxidant properties. Moreover, dill could be recommended for clinical trial as hepatic support.

Acknowledgements

Conflicts of interest
None

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