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Protection Against Polycyclic Aromatic Hydrocarbon Induced Lipid Peroxidation in Mice by Some Essential Oils

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The composition of the essential oils of aerial parts of peppermint (*Mentha piperita* L.), pennyroyal (*Mentha pulegium* L.) and basil (*Ocimum basilicum*) were analyzed by GC/MS. The peppermint and pennyroyal oils were consisted mainly of ketonic compounds (54.9-71.7%), while basil oil was rich in phenolic compounds (60%). Pulegone (65.1%), estragole (55.5%) and menthane (21.1%) were identified as the dominant components in essential oils of pennyroyal, basil and peppermint, respectively. The efficacy of their essential oils to inhibition the peroxidation process caused by 7,12-dimethylbenzo(a)anthracene (DMBA) in female mice was evaluated by estimating some possible hepatic and renal antioxidant markers. Enhanced of the lipid peroxidation in liver and kidney tissues of the mice treated with DMBA was accompanied by significant ($P<0.05$) increase in the enzyme activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and glutathione S-transferase (GST) and reduced glutathione (GSH) level when comparing with untreated mice (control group). However, their values were decreased in the liver with 1.16, 1.10, 1.23, 1.20 and 0.37 times respectively of the control. Administration of essential oils to DMBA-mice caused significantly ($P<0.05$) decreased in hepatic and renal malonaldhyde (MAD) levels and increased the levels of glutathione (GSH) and glutathione dependent enzyme (GST) as well as enhanced the levels of antioxidant defense enzyme. In the liver, the basil essential oil caused significant decrease of MAD level 79% of the control and increase the GSH, GST, SOD, CAT and POD levels was 1.7, 3.2, 2.86, 2.90 and 1.91 times respectively than that in the control group (G1).

Key words: 7,12- Dimethylbenz[a]anthrene, antioxidant, essential oils, superoxide dismutase, catalase, glutathione-S-transferase

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Introduction

A number of naturally occurring compounds from edible plants have shown antagonize the noxious effect of carcinogens in animal modals (Lam and Zheng, 1991; Arivazhagan *et al.*, 2000). Essential oils occurs widely in herbs and plants and traditionally used to extend the shelf life of foods and in folk medicine (Farag *et al.*, 1989; Sivropoulou *et al.* 1995; El-Baroty, 1997). Essential oil derived from many plants are known to possess biological activity against microorganisms (Farag *et al.*, 1989; Daw *et al.*, 1995; El-Baroty 1997; El-Baroty and Abdel-lattif, 1997). Also, a great deal of attention has been given to the used of aromatic plants as potential chemopreventive agents that can be useful in reducing the incidence of cancer in humans (Loub *et al.*, 1975; Zheng *et al.*, 1992; Arivazhagan *et al.*, 2000). Studies indicated that the essential oils are potential source of natural inhibitors of anticarcinogen (Zheng *et al.*, 1993). In this respect, sulfur-containing compounds from onion and garlic oils, d-carvone (present in caraway and dill oil), limonene (citrus oil) were found to possess anticarcinogenic properties (Zheng *et al.*, 1993; Agrawal, 1996; Arivazhanag *et al.*, 1999 and 2000). However, these compounds inhibit chemical carcinogenesis (such as benzo (a) pyrene and nitrosamine) have been found to increased the activities of the number of detoxifying enzymes including GST (Wattenberg *et al.*, 1986; Zheng *et al.*, 1992 and 1993; Abd El-Baky *et al.*, 2002)

Recently, carcinogen-induced cellular oxidative damage and its role in the cytotoxicity and carcinogenesis have attracted much attention (Shen *et al.*, 1997; Abd El-Baky *et al.*, 2002). The biological oxidative damage usually refers to impairment of the function of the cellular components, e.g., enzymes, membranes, protein and nucleic acid by reactive oxygen species: peroxides, singlet oxygen and free radicals such as superoxide, hydroxyl and peroxyl radicals (Ozturk-Urek *et al.*, 2001; Farber *et al.*, 1990). A major form of cellular pathways involved in oxidation damages is lipid peroxidation (LPO) which was found to play an important role in genotoxicity and chemical carcinogenesis (Ames *et al.*, 1993). However, the cellular damage causes through lipid peroxidation is controlled *in vivo* by a wide spectrum of antioxidative defense mechanisms such as glutathione and antioxidant enzymes (Guemouri *et al.*, 1991; Abd-El-Baky *et al.*, 2002). These defenses provide the protection against peroxidation of lipid and destruction of biological molecules in the cell.

In this study, the lipid peroxidation was induced by single oral dose of DMBA (most potent carcinogenic polycyclic aromatic hydrocarbon) in mice was characterized. The effects of some essential oils on antioxidant and detoxifying enzymes and level of lipid peroxidation and glutathione were investigated in liver and kidney tissues of DMBA-mice.

Materials and Methods

Chemicals: Dimethylbenz(a)anthracene (DMBA), glutathione and 1-chloro-2,4-dinitrobenzene were obtained from Sigma Chemical Co. (St. Louis, MA). Chemicals were of reagent grade, purchased from Aldrich Chemical.

Plant materials: Arial parts of three plants: Peppermint (*Mentha piperrita*), pennyroyal (*Menth pulegium*) and basil (*Ocimum basilicum*) were used in the study and collected during spring season 2001 from the Pharmacy Farm, Cairo University.

Extraction of essential oils: The fresh leaves of plants were pulverized and the essential oils was isolated after hydrodistillation for 2 h (Anonymous, 1975).

Gas chromatography–Mass spectrometry (GC/MS) of oil: The samples of essential oils were analyzed by GC/MS, using Hewlett Packard Capillary GC-quadrupole MS system (Model 5970) fitted with a fused silica column (50 m x 0.32 mm, i.d) coated with carbowax (20 m X 0.32 mm). GC programmed is as follows: 60°C (1 min), 60-180°C (4°C/min) and hold at 180 for 15 min. GC-MS analyses were made in splitless mode with helium as carrier gas at a flow rate of 1 m min^{-1}, the mass spectrometer was operated at 70 eV. The identification of the compounds were based on a comparison of retention time and mass spectra with those of authentic samples and with literature data (El-Baroty, 1997).

Animals: Forty female Swiss mice (weighing 23–27 g), six weeks age were obtained from Ophthalmology Research Institute (ORI, Giza, Egypt). Animals were acclimated for one week, they were fed semipurified diet until the end of experiments (in experimental animal house, ORI). Water was given ad libitum.

Treatments: One week after the start, fed of the semipurified diet, the animals were divided into eight groups (each of 6 mice). Group 1 received 50 μl of corn oil by gavage and served as the control. Group 2 treated with 25 mg 7,12- dimethylbenz(a)anthrene/100g in corn oil and served as the DMBA control (negative control). In groups 3-5, animals were treated with DMBA, received total seven doses from essential oils of mint, pennyroyal and basil by gavage once every two days for 2 weeks. Each of dosages consisted of 20 mg dissolved in 50 μl of corn oil.

Twenty four hours after last administration of the dose, mice were killed by cervical dislocation and the liver and kidney were removed and quickly frozen by liquid nitrogen and stored at –40°C for enzymes preparation.

Preparation of cytosolic fraction: The liver and kidney of mice were homogenized in ice cold 1.15% KCl solution using Teflon glass homogenizer. A portion of aliquot of homogenate was kept for determination of glutathione (GSH) and lipid peroxidation levels (as MDA). The cytosol, after 105,000 xg centrifugation for one hour, was obtained and frozen at –40°C until used. Each sample represents one tissue from each individual animal (Zheng *et al.*, 1993).

Enzymes assays: The activity of cytosolic GST (EC 2.5.1.18) was determined according to the method of Habig *et al.* (1974). The activity of SOD (EC 1.15.1.1) was determined by photochemical method (Ginnopolitis and Ries, 1977). The peroxidase activity (EC 1.11.1.7) was assay by spectrophotometrically as described by Chance and Maehly (1955). The catalase enzyme (EC 1.11.1.6) activity was measured spectrophotometrically, measuring the ultraviolet absorption at 240 nm of a 10.5 mM of H_2O_2 solution in present of 50 mM phosphate buffer, pH 7.0 (Aebi, 1987).

Determination of lipid peroxide level: For determination of lipid peroxide level, tissues homogenate was mixed with sodium dodecyl sulfate, acetate buffer (pH 3.3) and an aqueous solution of TBA (0.67%). After heating at 100°C for 30 min, the red pigment produced which was extracted with butanol–pyridine mixture (1:1, v/v) and estimated by the absorbance at 532 nm (Haraguchi *et al.*, 1997). The lipid peroxide level was expressed as micro moles of malondialdhyde (MDA).

Determination of glutathione (GSH): The GSH content was estimated by the acid–soluble sulfhydryl (SH) level in the tissue homogenates as described by Vecchia *et al.* (1992). Aliquots (100 μl) of tissue homogenates were precipitated with equal volumes of 5-sulfosalicylic acid (4%). The precipitate was removed by centrifugation. The supernatants (100 μl) were assayed for the presence of free SH groups by the addition of 0.9 ml of Ellmans reagent [0.1 mM 5,5`-dithiobis (2-nitrobenzoic acid)] in 0.1 M sodium phosphate buffer (pH 8.0). The absorbance was recorded at 412 nm to calculate the GSH concentration. Complete assay mixture without the supernatants was used as control.

Determination of protein: Protein concentration of cytosol was determined spectrophotometrically at 595 nm, using comassein blue G 250 as a protein binding dye (Bradford, 1979). Bovine serum albumin (BSA) was used as a protein standard.

Statistical analysis: Data were analyzed statistically by using the student's t test (Little and Hills, 1978).

Results and Discussion

The essential oils isolated from pepperment (*Mentha piperrita*), pennyroyal (*Menth pulegium*) and basil (*Ocimum basilicum*) were obtained in a yield of 1.2, 1.7 and 1.4% (Table 1). Qualitative and quantitative analysis of the essential oils of mint species showed that their bulk (54.9–80%, of the total oils) consisted of ketoneic compounds while basil oil was rich in phenolic compound (60%). Seventeen compounds, amounting to 95.9% of the essential oil components of the peppermint were identified, of which, menthane (21.1%), menthyl acetate (12.3%), linalool (10.2%) and limonene (11.3%) were identified as major constituent (>10%). The major components of pennyroyal oil were pulegone (50.1%), isomenthone (13.0%) and piperitone (12.3%). The basil essential oil was mainly characterized by high concentration of estragole (methyl chavicol) (55.5%), 1,8 cineole (13.3%) and champhor (11.2%). The composition of essential oil of two mint species and basil were in a good agreement with Masada (1976), Sivropoulou *et al.* (1995) and Faber *et al.* (1994). Also, it was markedly different from the results of Lachowicz *et al.* (1996). The reason for the variability of their chemical composition of essential oil are due to many environmental conditions (i.e, climate, soil type, etc.), stage of plant maturity, geographical origins and degree of freshness (Daouk *et al.*, 1995; Lachowicz *et al.*, 1996; El-Baroty, 1997).

Effect of plant essential oils on lipid peroxidation: The MDA concentration was significantly ($P < 0.05$) increase in liver (340 μM) and kidney (77.5 μM) tissues of mice treated with DMBA (G2), compared with control group (G1), which their values were 60.2 and 20.3 μM, respectively (Table 2). The levels of MDA in the liver and kidney tissues of DMBA-mice administrated with the oil of peppermint (79 and 69%), pennyroyal (75 and 62%) and basil (70 and 56%) were markedly decreased compared with DMBA control group (G2). Limonene group (positive control, G6), had the lowest decrease of MDA levels 71.52 and 21.5 μM in liver and kidney respectively, but no significant variation was observed when compared with the basil oil group (G5).

Lipid peroxidation is a complex process, oxidation of polyunsaturated fatty acids (PUFA) of lipid membrane lead to membrane damage and cell death of toxic MDA and like substances which are accumulated in the cells is generally metabolized by cytosolic enzymatic and non-enzymatic defense systems to prevent any damage to the biological membrane (REF). In this study, MDA levels in hepatic and renal tissues of the essential oils groups were markedly decreased. The significant decrease of MDA in liver varied from 75% for basil group to 79% for the peppermint group in comparison of the DMBA group. This indicates that the essential oil of peppermint, pennyroyal and basil play protective roles against lipid peroxidation.

Effect of plant essential oils on GST: The activity of GST in liver and kidney of DMBA group (G2) showed no significant differences when compared with untreated group (G1). The GST activity in the liver of the peppermint, pennyroyal, basil and limonene groups were significantly increased ($P<0.05$) by 4.65, 3.43 and 3.75 times higher than the normal control group (G1), respectively (Table 3). This means that the increase in GST activity varied according to the kind of oils and results of exposure of animals to DMBA carcinogens. However, the enhancement of GST activity in liver and kidney tissues of peppermint group (G3) was superior to that of other essential oils (G4 and G5) and limonene group (G6). In general, the increase of GST enzyme activity due to administration of essential oils were in the following order: peppermint > limonene > basil >pennyroyal. These sequences indicate that the administration of peppermint oil was much better than the other oils to increase the GST activity.

The epidemiological and plethora studies in various animals models have established unequivocally that some natural substances can influence the incidence of diseases such as cancer by modulating of detoxifying enzyme system (GST). Which, their enzyme is responsible for the metabolic activation and deactivation of chemical carcinogens (Zheng *et al.*, 1993; Bu-Abbas *et al.*, 1995; Burczynski and Penning, 2000; Arivazhagan *et al.*, 2000). The oils under study are very rich in monoterpenes compounds such as limonenen, which has been shown to inhibit a variety of organ-specific cancers in rodent models including mammary, stomach lung, skin and liver cancer (Crowell and Gould, 1994). Also, Watenberg and Lam (1984) shown that limonine was co-administrated the carcinogen benzo(rst) pentaphene or DMBA, developing tumors was reduced.

An enhancement of the activity of GST and other detoxifying enzyme with increasing GSH concentration suggest an increase in the host's ability to detoxify xenobiotics, including carcinogen. GST catalyzes the reaction of GSH with electrophiles to form less toxic conjugates for excretion. However, the positive correlation has been established between several natural compounds of inhibitors of polycyclic aromatic hydrocarbon (such as B(a)P and DMBA) and induction of carcinogenesis in different animal models and their enzyme activity (Zheng *et al.*, 1993). Thus, any compounds that induce an increase in the activity of this detoxifying enzyme system may be considered as potential inhibitors of carcinogenesis (Zheng *et al.*, 1993; Coles *et al.*, 1999; Hu *et al.*,1999; Abd El-Baky *et al.*, 2002).

Effect of essential oils on non-enzymatic defense system: The administrated of DMBA in mice (G2) was significantly ($P < 0.05$) decreased the concentration of GSH in the liver (2.1 μmol g^{-1}) and kidney (1.8 μmol g^{-1}) when compared with untreated mice (G1), which their level were 5.6 and 2.7 μmol g^{-1}, respectively (Table 4). The GSH concentration in liver and kidney tissues of DMBA-mice administrated with the oils of peppermint, pennyroyal, basil and limonine were 8.2 and 6.4, 7.8 and 5.9, 9.4 and 6.8 and 7.9 and 6.0 μmol g^{-1}, respectively. The basil oil was significantly increase ($P<0.05$) the GSH level in liver and renal over than the other groups. It is interesting to note the essential oils was modify the level of reduced glutathione (GSH) which is a limiting factor in several enzymatic activity (Lawlor and O'Brien, 1997). However, it was reported that the toxicity of chemical carcinogens might increase as a result of GSH depletion (Gopalan *et al.*, 1994; Abd El-Baky, 2002). Also, Yang *et al.* (2000) noted that the significant depletion of GSH concentration with increased of reactive oxygen species (ROS) level were found in the tissues of animals exposure to environmental carcinogens (aflatoxins, B(a)P and DMBA). On the other hand, the carcinogen induced GSH depletion may not be due to the reactive oxygen species mediated oxidation of GSH, but rather to the conjugation of carcinogen with GSH (Toshida *et al.*, 1997; Yang *et al.*, 2000). It is agreement with this study, DMBA reduced GSH levels in liver and kidney tissue of mice. This means that the increase of GSH level might be an adaptive mechanism of the liver and kidney to enhance the GSH conjugation capacity of accumulation of DMBA in order to reduce their toxicity. Vecchia *et al.* (1992) reported that the induction of the phase II detoxifying enzyme, and the level of mandatory substrate GSH are considered favorable for the detoxification of carcinogens such as PAH_s. However, Singh *et al.* (1999) reported that the peppermint was significantly elevated the sulfhydryl (-SH) and GST level in the liver of murine mice.

Limonine (G6) was noted to significantly ($P<0.05$) elevated the intracellular GSH level in mice tissues expose to DMBA-carcinogens when compared with negative control (G2). Limonene be able to increase GSH concentration both in *in vivo* and *in vitro* studies (Kodam *et al.*, 1996; Padmaja *et al.*, 1996; Shen *et al.*, 1997). The increase of intracellular GSH level due to administration of essential oils may provide more conjugative reaction and thus lead to detoxification of the carcinogen. Presently, the mechanism how essential oils increase intracellular GSH concentration is seems possible that it may work through the following pathway: (I) increasing GSH synthesis (ii) preventing GSH efflux (iii) increasing the utilization of GST (Yang *et al.*, 1997).

Table 1: Quantitative composition (percent) of some plants of Labiatae family

Components	*Mentha piperita* (Peppermint %)	*Mentha pulegulm* (Pennyroyal %)	*Ocimum basilicum* (Basil %)
Octanol	1.6	1.0	1.2
Limonene	11.3	3.2	4.3
1,8 Cineole	4.2	2.1	13.3
Linalool	10.2	tr	4.5
Champher	3.2	tr	11.2
Pulegone	5.6	50.1	tr
Estragole (Methyl chavical)	5.5	tr	55.5
Menthane	21.1	3.1	tr
Isomemthane	tr	13.0	tr
Menthol	5.3	5.3	tr
Piperitone	6.2	12.3	tr
Carvane	2.1	1.1	tr
Anethole	tr		tr
Methyl acetate	12.3	2.6	tr
Geraniol	tr	tr	1.8
Eugenol	1.0	tr	1.8
Methyl eugenol	1.3	tr	1.1
Caryophellene oxide	1.0	tr	tr
Valencene	1.0	tr	tr
Tau-cadinol	1.7	tr	tr
Essential oil yield (ml/100 g of dry wt. of plant tissue)	1.2	1.7	1.4

tr : Trace amount

Table 2: Effect of volatile oils of some plants on inhibition of microsomes lipid peroxidation in target tissues of mice treated with DMBA

	MDA μM[a]			
Groups	Liver	Inhibition (%)[b]	Kidney	Inhibition (%)[b]
G1: Not treated control	60.23		20.30	
G2: Treated control	340.00	100%	77.50	100.00
G3: Peppermint (*Mentha piperrita*)	75.23	79.00	24.15	69.00
G4: Pennyroyal (*Menth pulegium*)	88.12	75.00	29.65	62.00
G5: Basil (*Ocimum basilicum*)	100.90	70.30	34.25	56.00
G6: Limonene	71.52	79.00	21.50	72.20

a: Thiobarbturic acid reactive substance b: Inhibition % was expressed as the reduction of TBARS formation from sample compared to negative control

Table 3: Effect of volatile oils of some plants on glutathione s-transferase (GST) in target tissues of female mice treated with DMBA

	Organs					
	Liver			Kidney		
Groups	Sp. activity	Ratio a	Ratio b	Sp. activity	Ratio a	Ratio b
G1: Not treated control	1.15	1.0		0.65 $\pm$ 0.02	1.0	
G2: TREATED control	1.35 $\pm$ 0.17	1.2	1.0	0.82 $\pm$ 0.17	1.26	1.0
G3: PEPPERMINT (*Mentha piperrita*)	*5.35 $\pm$ 0.08	4.65	3.96	*2.61 $\pm$ 0.08	4.0	3.2
G4: PENNYROYAL (*Menth pulegium*)	*3.95 $\pm$ 0.4	3.43	2.92	*2.49 $\pm$ 0.04	3.8	3.0
G5: BASIL (*Ocimum basilicum*)	*4.32 $\pm$ 0.15	3.75	3.2	*1.91 $\pm$ 0.21	2.93	2.3
G6: LIMONENE	*4.72 $\pm$ 0.08	4.1	3.5	*2.52 $\pm$ 0.15	3.9	3.1

Specific activity of GST: mmol/min/mg protein Ratio a: Test/control Ratio b: Test/treated control

Table 4: Effect of volatile oils of some plants on acid-soluble sulfhydryl (SH) level in target tissues of mice treated with DMBA

	Acid-soluble sulfhydryl level μmol/g tissue			
Groups	Liver	Ratio a	Kidney	Ratio a
G1: Not treated control	5.60 $\pm$ 1.0	1.0	2.70 $\pm$ 0.6	1.0
G2: TREATED control	*2.1 $\pm$ 1.2	0.37	*1.8 $\pm$ 1.4	0.66
G3: PEPPERMINT (*Mentha piperrita*)	*8.2 $\pm$ 1.1	1.46	*6.4 $\pm$ 0.9	2.37
G4: PENNYROYAL (*Menth pulegium*)	*7.8 $\pm$ 0.85	1.39	*5.9 $\pm$ 1.1	2.18
G5: BASIL (*Ocimum basilicum*)	*9.4 $\pm$ 1.4	1.70	*6.8 $\pm$ 1.5	2.5
G6: LIMONENE	*7.9 $\pm$ 0.9	1.4	*6.01 $\pm$ 0.85	

Ratio a: Test/control * : $P < 0.05$

Effect of essential oils on antioxidant defense system: Results (Table 5) demonstrated that there was a variation in catalase activity due to administration of essential oils to DMBA–mice. The essential oils caused significant ($P < 0.05$) increase in catalase (CAT) activity in both liver and kidney tissues as compared with both control groups (G1 and G2). The basil, pennyroyal, peppermint and limonene were increased CAT activity in liver of DMBA-mice by 2.72, 3.36, 3.85 and 2.85 times over than that DMBA treated mice, respectively. In kidney tissues, their oils were increased CAT activity 2.65, 3.24, 3.32 and 2.94 times that of the DMBA-control group (G2). However, the peppermint oil had significantly higher CAT activity in the liver and kidney than that of the other groups. The CAT activity was of following order peppermint > pennyroyal > limonene > basil.

The administration of essential oils was enhancement of POD activity (Table 6) in the tissues of liver and kidney of DMBA-mice ranged from 1.91 to 2.73 and from 1.34 to 2.3 times of the DMBA group (G2). The oil of peppermint was significantly ($P < 0.05$) increased peroxidase activity in liver and kidney of DMBA-mice over than the limonene group (G6).

Table 5: Effect of volatile oils of some plants on catalase (CAT) in target tissues of female mice treated with DMBA

	Organs					
	Liver			Kidney		
Groups	Sp. activity	Ratio a	Ratio b	Sp. activity	Ratio a	Ratio b
G1: Not treated control	1.94±0.05	1.0		1.32±0.09	1.0	
G2: TREATED control	2.05±0.31	1.06	1.0	1.53±0.16	1.16	1.0
G3: PEPPERMINT (*Mentha piperrita*)	*7.9±0.91	4.07	3.85	*5.08±0.72	3.85	3.32
G4: PENNYROYAL (*Menth pulegium*)	*6.9±0.98	3.56	3.36	*4.96±0.45	3.76	3.24
G5: BASIL (*Ocimum basilicum*)	*5.58±0.99	2.9	2.72	*4.06±0.55	3.1	2.65
G6: LIMONENE	*5.85±0.55	3.01	2.85	*4.51±0.15	3.41	2.94

Table 6: Effect of volatile oils of some plants on peroxidase in target tissues of female mice treated with DMBA

	Organs					
	Liver			Kidney		
Groups	Sp. activity	Ratio a	Ratio b	Sp. activity	Ratio a	Ratio b
G1: Not treated control	1.64±0.05	1.0		1.01±0.09	1.0	
G2: TREATED control	2.11±0.21	1.28	1.0	1.56±0.16	1.57	1.0
G3: PEPPERMINT (*Mentha piperrita*)	*5.76±0.16	3.51	2.73	*3.67±0.05	3.6	2.3
G4: PENNYROYAL (*Menth pulegium*)	*4.36±0.43	2.66	2.07	*3.01±0.17	3.0	1.89
G5: BASIL (*Ocimum basilicum*)	*4.05±4.05	2.47	1.91	*2.51±0.09	2.48	1.57
G6: LIMONENE	*4.15±0.05	2.53	1.91	*2.14±0.16	2.12	1.34

Table 7: Effect of volatile oils of some plants on superoxid dismutase (SOD) in target tissues of female mice treated with DMBA

	Organs					
	Liver			Kidney		
Groups	Sp. activity	Ratio a	Ratio b	Sp. activity	Ratio a	Ratio b
G1: Not treated control	2.54±0.056	1.0		1.65±0.09	1.0	
G2: TREATED control	2.95±0.31	1.16	1.0	2.0±0.16	1.21	1.0
G3: PEPPERMINT (*Mentha piperrita*)	*9.97±0.54	3.93	3.38	*5.12±0.56	3.1	2.56
G4: PENNYROYAL (*Menth pulegium*)	*9.23±0.95	3.63	3.13	*4.85±0.44	2.94	2.42
G5: BASIL (*Ocimum basilicum*)	*8.45±0.75	3.33	2.86	*4.72±0.51	2.86	2.36
G6: LIMONENE	*8.1±0.16	3.18	2.74	*4.65±0.09	2.81	2.33

Sp. activity: Specific activity = μmol/min/mg protein Ratio a: Test/control Ratio b: Test/treated control * : $P<0.05$

The SOD activity of DMBA control group (G2) did not exhibit any significant changes as compared with normal control mice (G1). The administration of essential oil and limonene to DMBA-mice induced significant ($P<0.05$) increase of SOD activity in hepatic and renal tissues as compared with DMBA control group (Table 7). However, hepatic SOD activity for the oils groups were higher several folds than that in the untreated group (G1). The increase of SOD activity (administration of basil, pennyroyal, peppermint and limonene groups) were 2.86, 3.13, 3.38 and 2.74, respectively over than that of the DMBA control group. Also, their values for increase in the renal SOD activity were 2.36, 2.42, 2.56 and 2.33, respectively. The comparison between the ratio of increasing hepatic SOD due to administration of essential oils and limonene led to conclusion that the peppermint oil caused significant increase in SOD activity than the limonene (G6). While, administration of basil and pennyroyal oils did not possess any significant increase in renal SOD activity as compared with the limonene group (G6). In general, the increase in SOD activity in the liver and kidney tissues were in the following order: limonene < basil< pennyroyal < peppermint

The change in enzymes activity of liver and kidney indicated that the administration of DMBA-induced carcinogenesis and lipid peroxidation caused several cellular change in cytocolic enzyme activity. Thus, the inhibitory effects of essential oils (riched in ketonic and phenolic compounds) on DMBA-induced carcinogenesis may be explained by alteration of the cytosolic metabolism of the carcinogens, other mechanisms exist, such as a direct reaction between active form of the carcinogen and the inhibitors (Wattenberg and Lam, 1984). The essential oils were able to induced increase the activity of enzymatic antioxidant defense: components: SOD, peroxidase and catalase in liver and kidney. The change in activity of liver and kidney enzymes indicated that the administration of DMBA caused several cellular change in microsomal enzyme activity (Kodam *et al.*, 1996; Govindwar and Adav, 1999). However, increase of enzymatic activity and level of non-enzymatic defense system can be explained by the finding of lower MDA levels in liver and kidney tissues of DMBA–mice. The peroxidase which can catalyze the reduction of lipid hydroperoxides to their corresponding alcohols via glutathione, whereas glutathione serves as a hydrogen donor to lipoxy radicals, resulting in their conversion to hydroperoxides and preventing their propagation of a lipid free radical chain reaction, could be caused by DMBA (Zheng *et al.*, 1993). However, Padmaja *et al.* (1997) and Ozturk-Urek *et al.* (2001) mentioned that the increase of enzymatic defense system components prevents the lipid peroxidation and protect cell membrane structure from oxidation. The active ingredients found in their essential oils were ketonic and phenolic compounds have been shown to inhibit the chemical carcinogens in many animals models (Wattenberg, and Lam, 1984; Wattenberg *et al.*, 1986). However, cancer prevention with dietary terpenes intervention occurred only during promotion/progression of NMU-induced carcinomas, not during initiation. The terpenes could prevent the cancer associated with directly acting carcinogens suggested that the terpenes were not only blocking agents but were also suppressing agents in their prevention role (Maltzman *et al.*, 1991). Gould (1993) reported that dietary terpenes could prevent mammary carcinomas induced by the direct transfer of activated ras. In this respect, limonene caused a significant increase in regression of tumors initiated by both DMBA and NMU when compared to the occurrence of spontaneously regressing tumors (Gould *et al.*, 1994).

In general, as aromatic plant, basil and mint species were regularly consumed by humans over most of the world and used in perfumery and pharmaceutical preparation (Masada, 1976). Therefore, the essential oils isolated from the same source should be readily acceptable as diet supplements which the toxicity and side effects are minimal (Zheng *et al.*, 1993).

So, it may be concluded that, the essential oils of peppermint, basil and pennyroyal can be effective inhibitors of DMBA-induced lipid peroxidation and carcinogens. Such inhibitory effect on environmental exposure of human to benzo-ananthracene-type carcinogens could be important. Consequently, it can be

considered their essential oils as a source of chemoprevented agent.

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