http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2018.348.358

Research Article
Health Benefits of Fennel, Rosemary Volatile Oils and their Nano-Forms in Dyslipidemic Rat Model

[1]Sahar Y. Al-Okbi, [2]Ahmed M.S. Hussein, [1]Hagar F.H. Elbakry, [1]Karem Aly Fouda, [2]Khaled F Mahmoud and [3,4]Mohamed E Hassan

[1]Department of Nutrition and Food Sciences, National Research Centre, El-Buhouth Street, 12622, Dokki, Cairo, Egypt
[2]Department of Food Technology, National Research Centre, Cairo, Egypt
[3]Centre of Scientific Excellence-Group of Encapsulation and Nanobiotechnology, Cairo, Egypt
[4]Department of Chemistry of Natural and Microbial Products, National Research Centre, Egypt

Abstract

Background and Objective: Dyslipidemia is a major health problem that may lead to cardiovascular diseases (CVDs). In the present research, a biological experiment was run on dyslipidemic rats to study the health benefits of the volatile oils (VOs) of fennel and rosemary in its original and nano-form using chitosan as carrier. **Materials and Methods:** Rats were divided into 6 groups; normal control, dyslipidemic control and 4 test groups with dyslipidemia and treated by VOs of fennel and rosemary and their respective nano-forms separately. Glucose tolerance test was carried out after 4 weeks. Parameters reflecting oxidative stress/antioxidant; plasma catalase, malondialdehyde (MDA) and blood uric acid, were assessed. Plasma lipid profile and tumor necrosis factor alpha (TNF-α) as inflammatory biomarker were determined. Liver and kidney function were assessed as determinant of the safety of the different VO forms. Twenty four hour urinary volume was measured to assess creatinine clearance and to evaluate the possible diuretic activity of the VOs. **Results:** Dyslipidemic control rats showed dyslipidemia, increased CVDs risk, liver dysfunction, elevated MDA and TNF-α with marked increase in blood sugar after half an hour of glucose ingestion compared to normal control. Treatment with the four VOs forms improved the majority of the biochemical parameters. **Conclusion:** All treatment showed cardio and hepato- protective effect and safety towards kidney and blood sugar. Oxidative stress and inflammatory biomarkers were significantly improved by the different treatments; both VO forms of fennel were more efficient in ameliorating inflammation.

Key words: Fennel, rosemary, volatile oils, cardiovascular diseases, dyslipidemia, diuretic activity

Citation: Sahar Y. Al-Okbi, Ahmed M.S. Hussein, Hagar F.H. Elbakry, Karem Aly Fouda, Khaled F Mahmoud and Mohamed E Hassan, 2018. Health benefits of fennel, rosemary volatile oils and their nano-forms in dyslipidemic rat model. Pak. J. Biol. Sci., 21: 348-358.

Corresponding Author: Sahar Y. Al-Okbi, Department of Nutrition and Food Sciences, National Research Centre, El-Buhouth Street, 12622, Dokki, Cairo, Egypt Tel: +201003785152

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cardiovascular diseases are among the major health problems that lead to death worldwide. Dyslipidemia, oxidative stress and inflammation play an important role in prevalence of CVDs[1]. The use of natural bioactive constituents are increasing nowadays as potential protection and complementary remedy for chronic diseases due to their expected safety specially when they are derived from plant foods like condiments and herbal spices that used in human daily life. Nano-particles of such bioactive constituents were reported to have sustained release effect and enhanced absorption and bioavailability, thereby boost therapeutic potency that could permit reduction of dose level and frequency with subsequent reduced toxicity[2].

Rosemary herb is native to Mediterranean region; however it is cultivated Worldwide due to its importance as condiment for food flavoring and for medicinal purpose. In Folk medicine, rosemary is used as a stimulant, analgesic and for treating headaches, poor circulation and physical and mental fatigue. It is also used as hepatoprotective[3,4]. Anti-inflammatory, antinociceptive, antidepressant, cognition-enhancing, DNA protective and anticancer effects are among the health benefits of rosemary[5-9]. It was reported that rosemary possess high antioxidant activity due to its bioactive constituents like diterpenes, carnosol, carnosic acid, ursolic acid, rosmarinic acid and caffeic acid specially the constituents of VO[10,11]. The main antioxidant constituents in the essential oil are the monoterpenes such as 1,8-cineole, camphor and α-pinene[3].

Fennel volatile oil is used as flavoring agent in food products. Its VO was demonstrated previously to possess antioxidant, anti-inflammatory, antidiabetic, antimicrobial, antimutagenic and antiproliferative activity[12-16]. The reported bioactive constituents in fennel VO are trans-anethole, alpha-phellandrene, alpha-pinene, myrcene, delta-3-carene, cis-ocimene, gamma-terpinene, para cymene, fenchone, camphor and cis-anethole[13].

The cardioprotective and hypolipidemic effect of fennel and rosemary volatile oils were not studied well. In the current research it is hypothesized that being anti-inflammatory and antioxidant; fennel and rosemary volatile oils could possess health benefits towards dyslipidemia and cardiovascular diseases (CVDs).

The aim of the present study was to evaluate the protective effect and health benefits of the VOs of fennel and rosemary (in both the original and nano-forms) in dyslipidemic rats' model. This was accomplished through assessing the *in vivo* antioxidant, anti-inflammatory and lipid lowering potential. The safety of the VOs towards liver and kidney functions was evaluated in the same rat model. The potential glucose tolerance amelioration and diuretic effect of the VOs was also assessed in the aforementioned model.

MATERIALS AND METHODS

This Study was accomplished in August, 2018.

Materials: Fennel (*Foeniculum vulgare* Mill. family Umbelliferae) and rosemary (*Rosmarinus officinalis* L., family Lamiaceae) were purchased from local markets of Egypt. Chitosan of low MW was obtained from Sigma-Aldrich (St. Louis, MA, USA). Glucose was purchased from BDH, England.

Methods

Extraction of volatile oils of fennel and rosemary: One hundred gram of each crushed dried plant materials (fennel or rosemary) were subjected to 4 h of hydrodistillation using Clevenger-type apparatus[17]. The obtained volatile oils were dried over anhydrous sodium sulphate and filtered.

Preparation of nano-encapsulated fennel and rosemary volatile oils: In the current investigation, nano-encapsulation of fennel and rosemary volatile oils was carried out separately according to the modified method of Terjung *et al.*[18] via homogenization (Homogenizer PRO 400 PC, model, Germany) in a matrix comprised of chitosan (low molecular weight) and Tween 20 (T_{20}). Chitosan powder was prepared by ball milling (Ball milling Model: PQ-N2 Gear Drive 4-station-planetary Ball mill, 220 v) for 30 min at frequency converter 40 Hz to convert chitosan particles to the nano-scale. Then 2% chitosan was prepared in 1% acetic acid (2 g/100 mL). One gram of each VO was added to 100 mL solution containing 2 g chitosan and 0.1% T_{20}. The emulsion was created by mixing the solution in a high-pressure homogenizer at 18000 rpm for 30 min. The temperature was kept at 35°C. The resulted emulsions were stored at 4°C until used.

Transmission Electron Microscopy (TEM): The morphology of nano-encapsulated fennel VO in chitosan was obtained by transmission electron using transmission electron microscopy (JED 1230, JEOL Ltd. and Tokyo, Japan). About 20 µL of diluted sample (fennel VO in nano-encapsulated form) were placed on a film-coated 200-mesh copper specimen grid for 10 min and the excess fluid was eliminated using filter paper. The grid was

then stained with one drop of 3% phosphotungstic acid and allowed to dry for 3 min. The coated grid was dried and examined under the TEM microscope. The samples were observed by operating[19] at 160 kV. This procedure was carried out on rosemary in a previous research[20] which showed that rosemary essential oil fall within the nano-scale (37.51-58.6 nm).

Differential Scanning Calorimetry (DSC): The thermal stability of fennel and its nano-encapsulated form was determined using a Differential Scanning Calorimeter (DSC), model 823E from Mettler Toledo. Ten milligram samples were placed in aluminium crucibles. The samples were analyzed under a flow of nitrogen gas (40 mL min^{-1}). A dynamic scan was performed at a heating rate of 10°C min^{-1} over a temperature ranged from 0-300°C. Evaporation enthalpies were calculated by peak area integration of DSC profiles[21]. This procedure was applied on rosemary in a previous research and showed that the thermal peak of its original VO was 47.03 while that of its nano-form[20] was 84.05°C.

Preparation of dosage form for the animal experiment

For the original VO: To facilitate adjustment of doses; each original volatile oil was separately mixed with Tween 20 (10% of oils weight) then distilled water was added to form homogenous emulsion using vortex. The applied dose of rosemary original VO was 10 mg kg^{-1} rat body weight according to Raskovic *et al.*[22] while that of fennel was 30 mg kg^{-1} as demonstrated by Tognolini *et al.*[23].

For the nano-encapsulated VO: It is used as prepared under the previous step (Preparation of nano-encapsulation of fennel or rosemary volatile oils). The dose level of nano VO of rosemary that used in the present study was 3/4 the dose of its original VO (i.e., 7.5 mg kg^{-1} rat body weight). The dose of the nano-form of fennel VO was 1/2 that of its original VO (i.e., 15 mg kg^{-1}).

Preparation of diets: Balanced and dyslipidemic diets were prepared as in Table 1. The dyslipidemic diet was prepared according to Al-Okbi *et al.*[24] with some modifications.

Design of animal experiment: Forty eight male albino rats of 120-140 g body weight were purchased from Animal House of National Research Centre. Rats were kept individually in metabolic cages with free access to water and food. The animal experiment was carried out according to the Ethics Committee of the National Research Centre, Cairo, Egypt and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Rats were divided into 6 groups each of 8 rats. Rats of the first group were fed on balanced diet without any treatments and served as Normal Control (NC). Rats of groups 2 through 6 were fed on dyslipidemic diet. Group 2 received no treatments and served as dyslipidemic control (DC). Rats of group 3 and 4 received daily oral dose of fennel and rosemary original VO as 30 and 10 mg kg^{-1} rat body weight, respectively. Rats of group 5 and 6 were given nano-forms of fennel and rosemary VO as 15 and 7.5 mg kg^{-1} rat body weight orally thrice weekly (day after a day); respectively. Body weight and food intake were followed once weekly. For performing oral glucose tolerance test (OGTT), on the 28 day rats of all groups were fasted overnight and in the morning of the next day a drop of blood was taken from all rats' tails to measure blood glucose at zero time (fasting blood glucose) using glucose strips and their corresponding Glucometer (GlucoDr, Korea). The 4 tested volatile oils' forms were given orally to rats of groups 3-6 then all rats including the two control groups were given 1 g glucose kg^{-1} rat body weight in solution form[16]. Blood glucose was re-determined after 0.5, 1 and 2 h from glucose intake. After performing OGTT rats returned to their original diet and doses style of the biological experiment. At the end of the experiment (after 3 days from performing OGTT); 24 h urine was collected and measured. Then rats were fasted 16 h and uric acid was determined in blood drawn from rats' tail using uricometer (easytouch GCU, Taiwan). Fasting blood was collected in heparinized tubes from the eye vein orbital of anaesthetized rats. Blood was centrifuged at 4000 rpm for 15 min to separate plasma. Plasma oxidative stress/antioxidant biomarkers were assessed through determination of plasma catalase (CAT) according to Aebi[25], malondialdehyde (MDA) using the method of Ohkawa *et al.*[26] and blood uric acid by the uricometer. Lipids' profile represented by plasma total

Table 1: Composition of diets (g/100 g)

Ingredients	Balanced diet	Dyslipidemic diet
Casein	12*	20*
Sucrose	-	49.25
Starch	68.5	-
Cellulose	5	-
Cholesterol	-	1
Bile salt	-	0.25
Salt mixture	3.5	3.5
Vitamin mixture	1	1
Coconut oil	-	25
Sunflower oil	10	-
Total	100	100

*Casein contains 90% protein as determined by AOAC (2000)

cholesterol (T. Ch), high density lipoprotein cholesterol (HDL-Ch), low density lipoprotein cholesterol (LDL-Ch) and triglycerides (TGs) were determined adopting colorimetric methods[27-30]. Both T.Ch/HDL-Ch and TGs/HDL-Ch ratios were calculated as risk factor indicators for cardiovascular diseases. The inflammatory biomarker, plasma tumor necrosis factor alpha (TNF-α) was assessed using enzyme-linked immune-sorbent assay[31]. Liver Function was estimated through determination of plasma activity of Transaminases represented by AST and ALT[32]. Plasma and urinary creatinine were assessed by the method of Tobias *et al.*[33] with calculation of creatinine clearance as determinant of kidney function.

Statistical analysis: Statistical analysis was carried out using SPSS software version 16. One way analysis of ANOVA test was applied for inter-comparison between groups, followed by LSD at confidence interval of 95% ($p<0.05$).

RESULTS

TEM of nano-encapsulated fennel essential oil: Results in Fig. 1 showed the TEM of fennel oil encapsulated in chitosan nanoparticles with diameter range from 27.44-33.12 nm.

DSC thermal analysis of the VO of fennel (original and nano-encapsulated): Differential Scanning Calorimetry (DSC) was applied, in order to gain knowledge about thermal and oxidative stability of original and nano-encapsulated fennel VO. The observed changes in the thermodynamic properties of original fennel VO and its nano-encapsulated form in chitosan were shown in Fig. 2 and 3. The temperature programme was from 58.26-75.55°C in fennel original oil and the point of degradation was 72.91°C, while for nano-encapsulated fennel oil, the temperature was from 79.83-125.46°C and the point of degradation was 107.04°C. So, the nano-encapsulated form remained stable in temperatures as high as 107.04°C. Thermal analysis proved to be an excellent tool for fennel oil characterization. Solid restructuration and melting enthalpies for original oil and its nano-encapsulated form were -3.84 and -159.92 $J.g^{-1}$, respectively.

The thermal oxidation stability of original fennel volatile oil was lower compared to their chitosan encapsulated form. Figure 2 depicted exothermic peaks at approximately 72.05 and 75.55°C which can be interpreted as resulting from the hydrolysis or oxidation of original fennel volatile oil. These peaks are not detected in the thermogram of the nano-encapsulated form in chitosan (Fig. 3), inclusion complex, indicating that active compounds were protected within the cavity of the chitosan. Endothermic peaks detected at approximately 79.83°C were attributed to water evaporation and the exothermic peaks around 125.46°C for the nano-encapsulated form in chitosan might be due to melting and thermal decomposition of the chitosan itself.

Biological experiment: Nutritional parameters in Table 2 showed that feeding the dyslipidemic diet produced an increase in rat body weight gain and significant increase in

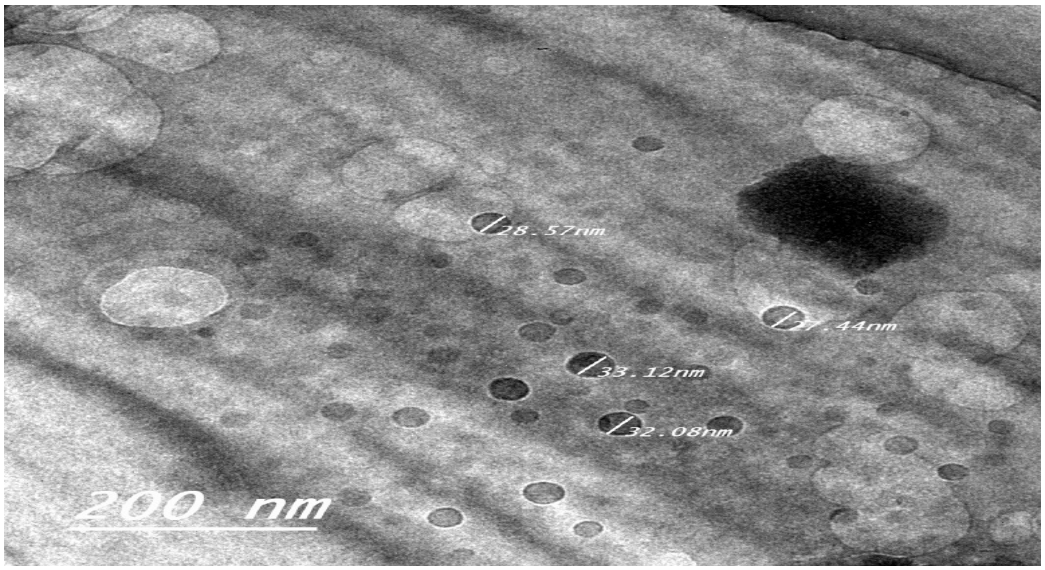

Fig. 1: Transmission Electron Microscopy (TEM) image of fennel oil nanoencapsulated in chitosan

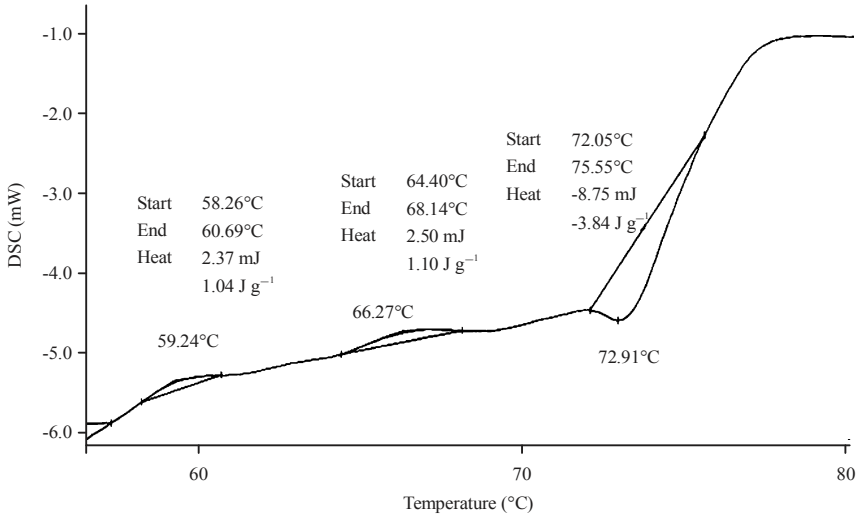

Fig. 2: DSC of original fennel volatile oil

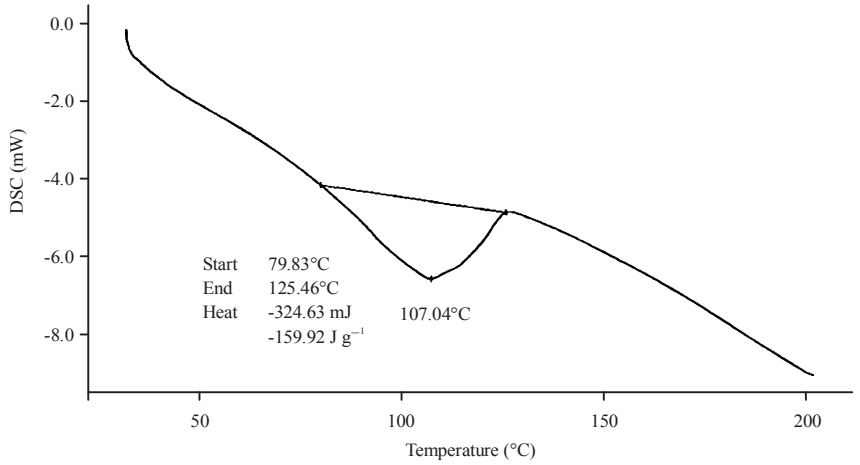

Fig. 3: DSC of nano-encapsulated fennel volatile oil in chitosan

Table 2: Nutritional parameters of different experimental groups (Mean±SE)

	Parameters				
Groups	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Total food intake (g)	Food efficiency ratio
NC	129.50±2.64^{a}	220.75±5.07^{a}	91.25±6.54^{a}	524.00±21.83^{b}	0.174±0.009^{a}
DC	129.00±11.92^{a}	244.63±11.25^{a}	110.00±4.89^{a}	470.63±17.50^{ab}	0.236±0.014^{b}
Fennel (original VO)	129.38±8.66^{a}	225.88±13.73^{a}	96.50±8.79^{a}	478.38±28.83^{ab}	0.204±0.017^{ab}
Rosmary (original VO)	129.50±7.84^{a}	232.38±14.36^{a}	102.88±14.65^{a}	452.38±26.77^{a}	0.222±0.025^{ab}
Fennel VO (Nano-form)	129.25±14.21^{a}	230.38±15.41^{a}	101.13±5.96^{a}	443.38±16.80^{a}	0.229±0.014^{b}
Rosmary VO (Nano-form)	129.50±7.44^{a}	239.50±13.13^{a}	110.00±8.91^{a}	464.13±17.70^{ab}	0.237±0.015^{b}

In each column, different superscript letters mean significant difference ($p<0.05$), NC: Normal control, DC: Dyslipidemic control

food efficiency ratio compared to balanced diet, this occurred without significant change in total food intake. Compared to DC group; rats fed on dyslipidemic diet showed insignificant reduction in body weight gain when treated with original fennel volatile oil to value more or less matches NC group. Rosemary original VO and fennel nano VO showed significant reduction in total food intake compared to NC group. Groups treated with either nano-formulas as well as the DC group exhibited significant increase in food efficiency ratio compared to normal control. Food efficiency of rats treated with the different tested 4 VO formulas showed no significant changes compared to dyslipidemic control group.

Results of glucose tolerance test (Table 3) showed no change in fasting blood glucose among all groups. After half an hour of glucose ingestion blood glucose reached the highest level for all groups. Although the dyslipidemic group showed the highest blood glucose level which account for 83% from its fasting level, it did not significantly differ from the control. Nano-volatile oil of fennel was the only treatment that produced significant reduction in blood glucose level after half an hour (45% increase from its FBG) compared to dyslipidemic group. After an hour; blood glucose showed insignificant change when DC group was compared with NC. Treatment with rosemary original VO and nano-VO of fennel produced significant reduction in 1 h blood glucose compared to DC. About 2 h glucose level of all treated groups showed significant reduction from NC and DC except for the group given nano VO of rosemary that showed insignificant change.

As Table 4 showed that DC group has significant high levels of plasma T.Ch, LDL-Ch, TGs, T.Ch/HDL-Ch and Tgs/HDL-Ch along with significant reduction of HDL-Ch compared to NC group. T.Ch was reversed to normal level on the four different VO treatments. HDL-Ch showed insignificant change when the different treated groups were compared with DC group. LDL-Ch was reduced significantly when the different treatments were compared to DC group but the level still not matching the control level. TGs of the different groups treated with VOs or their nano-form showed insignificant change from NC and DC except for nano-VO of rosemary that showed significant reduction from DC. T.Ch/HDL-Ch ratio was reduced significantly when the groups given the four VO treatments were compared to DC group but still not matching the control level. TGs/HDL-Ch was improved significantly on treatment with fennel VO and rosemary nano-form and it returned to the normal level in the latter case. TNF-α showed significant increase in the DC group compared to NC. Administration of the different forms of VO significantly reduced TNF-α which reached the normal level in case of fennel original VO and its nano-form. MDA and activity of catalase was significantly high in DC group compared to NC; different VO treatments produced significant reduction in their levels which was only similar to NC in case of catalase. Uric acid was significantly high in DC group compared to NC; treatment with the nano-form of both VOs produced significant reduction compared to DC group.

Table 3: Fasting blood glucose and blood glucose after 1/2, 1 and 2 h from glucose administration of different experimental groups (mg dL^{-1})

	BG							
		Blood glucose after 1/2 h (maximum absorption)		Blood glucose after 1 h		Blood glucose after 2 h		
Groups	FBG		Increase* (%)		Decrease** (%)		Decrease** (%)	Increase* (%)
NC	78.8±2.05[a]	124.30±1.59[ab]	58%	115.7±3.14[a]	-7%	106.00±5.51[a]	-15%	35%
DC	76.3±6.38[a]	140.00±9.86[a]	83%	107.67±5.1[ac]	-23%	105.00±4.65[a]	-25%	38%
Fennel VO	74.5±1.12[a]	120.83±4.09[ab]	62%	116.17±3.24[a]	-4%	83.00±3.08[b]	-31%	11%
Rosemary VO	74.83±4.26[a]	133.83±8.95[ab]	79%	89.67±4.84[b]	-33%	87.67±3.08[b]	-34%	17%
Nano VO of fennel	81.17±11.44[a]	117.83±7.1[b]	45%	94.5±3.66[b]	-20%	87.67±5.89[b]	-26%	9%
Nano VO of rosemary	74.5±4.76[a]	122.2±9.46[ab]	64%	98.33±4.19[bc]	-20%	94.83±2.48[a]	-22%	27%

NC: Normal control, DC: Dyslipidemic control, BG: Blood glucose, FBG: Fasting blood glucose, values are expressed as Mean±SE, where n = 8 rats per group, Within a column different superscript letters showed significance between groups ($p<0.05$), *Increase (%) in blood glucose from its FBG, **Decrease (%) in blood glucose from its maximal absorption (after 1/2 h)

Table 4: Plasma lipid profile and oxidative stress markers in different experimental groups

	Groups					
Parameters	NC	DC	Fennel VO	Rosemary VO	Fennel nano-VO	Rosemary nano-VO
Plasma lipid profile						
T.Ch (mg dL^{-1})	78.02±1.02[a]	97.43±2.38[b]	81.62±1.53[a]	82.34±1.89[a]	82.30±1.75[a]	79.00±1.7[a]
HDL-Ch (mg dL^{-1})	50.45±1.33[a]	37.18±1.84[b]	40.60±1.98[b]	38.30±2.29[b]	37.15±1.54[b]	42.21±1.98[b]
LDL-Ch (mg dL^{-1})	7.35±0.78[a]	21.31±1.19[b]	11.09±1.08[c]	12.39±1.06[c]	12.91±1.11[c]	11.42±1.45[c]
TGs (mg dL^{-1})	73.90±4.24[a]	93.49±4.06[b]	83.63±5.46[ab]	81.94±6.07[ab]	84.68±3.6[ab]	75.50±3.11[a]
T.Ch/HDL-Ch	1.56±0.05[a]	2.67±0.15[b]	2.04±0.09[cd]	2.20±0.14[cd]	2.24±0.1[d]	1.90±0.1[c]
TGs/HDL-Ch	1.46±0.06[a]	2.56±0.17[b]	2.08±0.15[cd]	2.22±0.25[bd]	2.31±0.14[bc]	1.80±0.07[ad]
Plasma inflammatory biomarker						
TNF-α (pg mL^{-1})	32.77±0.98[a]	92.71±1.95[b]	35.61±1.41[ac]	41.69±1.78[d]	34.91±1.25[ac]	39.05±1.46[cd]
Plasma oxidative stress/antioxidant biomarkers						
MDA (nmol mL^{-1})	6.28±0.4[a]	10.34±0.84[b]	8.01±0.44[c]	7.93±0.38[c]	8.49±0.47[c]	7.79±0.48[c]
Catalase (U L^{-1})	465.50±12.9[a]	712.20±26.3[b]	473.90±29.7[a]	470.40±30.4[a]	496.40±48.6[a]	449.80±28.7[a]
Uric acid (mg dL^{-1})	3.70±0.21[a]	7.50±0.22[d]	7.00±0.44[cd]	7.40±0.22[d]	5.00±0.2[b]	6.50±0.35[c]

Results were expressed as Mean±SE, where n = 8, different superscript letters in the same row indicate statistical significance at $p<0.05$. VO: Volatile oil, DC: Dyslipidemic control, NC: Normal control

Table 5: Parameters reflecting kidney and liver function in different experimental groups

	Groups					
Parameters	NC	DC	Fennel VO	Rosemary VO	Fennel nano-VO	Rosemary nano-VO
Kidney function						
Plasma creatinine (mg/dL)	0.29±0.03^{ac}	0.38±0.05^{c}	0.36±0.03^{ac}	0.27±0.02^{a}	0.27±0.03^{a}	0.30±0.03^{ac}
Urinary creatinine (mg/dL)	13.53±0.6^{a}	15.90±1.22^{ab}	13.88±0.48^{a}	13.87±1.33^{a}	20.08±2.33^{b}	18.88±2.43^{b}
24 h Urine volume (mL)	11.40±1.08^{ac}	14.90±1.13^{bc}	13.60±1.85^{abc}	18.10±3.07^{b}	9.69±083^{a}	12.88±0.58^{ac}
Creatinine Clearance (mL/min)	0.38±0.04^{ab}	0.50±0.08^{ab}	0.37±0.05^{a}	0.62±0.09^{ab}	0.52±0.07^{ab}	0.67±0.19^{b}
Liver function						
AST (U/L)	29.68±1.74^{a}	44.79±1.99^{c}	35.80±1.18^{b}	34.35±1.32^{b}	33.84±1.5^{b}	33.39±1.53^{b}
ALT(U/L)	11.82±1.79^{a}	21.70±2.21^{b}	12.11±0.78^{a}	13.00±0.46^{a}	12.95±0.62^{a}	13.47±0.6^{a}

Results were expressed as Mean±SE, where n = 8, Different superscript letters in the same row indicate statistical significance at p<0.05. VO: Volatile oil, DC: Dyslipidemic control, NC: Normal control

In Table 5; AST and ALT increased significantly in DC compared to NC. The different four VO treatments produced significant reduction in ALT compared to DC, where the ALT levels were matching the control. The AST was significantly improved in all VO treatments compared to DC but still higher than NC. No significant change was noticed in plasma and urinary creatinine, urine volume and creatinine clearance of DC group compared to NC. Treatment with original rosemary VO and nano-fennel VO produced significant reduction in plasma creatinine compared with DC. Urinary creatinine showed the highest level on treatment with either nano-VOs compared to all other experimental groups. Treatment with rosemary original VO showed the highest urine volume compared to all experimental groups. Creatinine clearance was at the highest level on treatment with original rosemary VO and its nano-form.

DISCUSSION

The present research investigated the comparative biological activity of the original VO of both fennel and rosemary with that of their nano-forms when applying the latter in reduced dose and frequency in rat model of dyslipidemia.

In the present study, FBG did not show any change among all experimental groups which mean that the dyslipidemic diet fed for 4 weeks in the present study has no influence on FBG and that the different VO treatments did not ameliorate the normal level of FBG reflecting their safety in this concern. The maximum absorption of blood glucose in all groups was shown after half an hour of glucose ingestion which agreed with previous studies[16,34]. The highest level of blood glucose after half an hour belonged to DC group which was significantly reduced by nano-fennel VO reflecting its hypoglycemic effect on this elevated level which could point to an inhibitory effect on glucose absorption. In the present study the original fennel VO only reduced the blood glucose after 1/2 an hour insignificantly whereas the nano-form was more efficient which could be related to increased bioavailability of the nano-particles. The same nano formula and rosmary original volatile oil possess significant hypoglycemic effect on blood glucose after an hour and 2 h while fennel original VO only produce reduction after 2 h indicating their beneficial effect on glucose utilization which could be related to stimulatory effect on insulin. Previously fennel essential oil was reported to possess anti diabetic effect which was ascribed to trans-anethole the major constituent of fennel VO that has the ability to enhance insulin secretion, improve glucose tolerance and inhibit the inflammation pathway of kappa B[16,35,36]. Terpenoids present in rosemary produced significant effect on dipeptidyl peptidase (DPP-4) enzyme inhibition with consequent antidiabetic effect by applying molecular docking[37].

The improvement in T.Ch and LDL-Ch on different oil treatments pointed to their ability as lipid lowering on these lipid components however they could not improve HDL-Ch. Only the nano-form of rosemary could improve TGs. Formulation of VO into nano-form showed no significant change in different plasma lipids compared to their original VO. This result reflected the high bioavailability of the nano-forms because although they were used as fraction from the original VO and was only given thrice weekly they showed equal response to that of the original VO. In dyslipidemic control; the increase in the ratios T.Ch/HDL-Ch as an atherogenic index and TGs/HDL-Ch was shown previously as risk factor for cardiovascular diseases[38,39]. Improvement of both ratios on the treatment with different forms of VO reflected their beneficial effect in this concern especially in case rosemary nano-form. The reduction of TNF-α by the different treatments showed reduction of inflammation which is one of the main factor of inducing CVDs.

The increased MDA on feeding dyslipidemic diet reflect the elevated lipid peroxidation and the enhanced oxidative stress. This high oxidative stress stimulates elevation of

catalase, the antioxidant enzyme, to counteract the reactive oxygen species as a self defense mechanism. So when MDA reduced by the different VO treatments this resulted in reduction in catalase. Uric acid was reported to have beneficial antioxidant activity[40] while when severely elevated it could be a biomarker of gout. Like catalase, uric acid was elevated on feeding dyslipidemic diet to resist the high free radicals reflected by the elevated MDA; however the increased uric acid level on consumption of dyslipidemic diet is a matter of discussion. The nano-form of both VOs significantly improved the level of uric. The high MDA together with the elevated T.Ch and LDL-Ch on feeding dyslipidemic diet indicates high risk of cardiovascular disease. Amelioration of these parameters by the different VO forms could lead to protection from the incidence of CVDs, though this protection is not complete due to the reduced HDL-Ch and the elevated TGs. The antioxidant bioactive constituents in rosemary VO was reported to be diterpenes, carnosol, carnosic acid, ursolic acid, rosmarinic acid, caffeic acid specially the constituents of VO[10,11].

Improvement of liver function by the different forms of VOs could be due to the role of volatile oil as antioxidant as could be seen from the reduced MDA. Raskovic *et al.*[22] demonstrated that rosemary essential oil exhibited free radical scavenging activity thereby mediates its hepatoprotective effects. Raskovic *et al.*[22] identified 29 chemical compounds of rosemary essential oil and the main constituents were 1,8-cineole (43.77%), camphor (12.53%) and α-pinene (11.51%) to which the antioxidant and hepatoprotective effect might be ascribed. Also, rosemary essential oil prevented carbon tetrachloride - induced increase of lipid peroxidation in liver homogenates and reversed the activities of antioxidant enzymes in liver homogenates[22]. The antioxidant activity of rosemary VO was also reported[41,42]. It was demonstrated that oxygenated monoterpenes, probably monoterpenoid ketones with established antioxidant properties, may have the greatest contribution to the antioxidant capacity of rosemary essential oil in addition of total phenolics as the minor components[22]. Renoprotective effect of rosemary essential oil was also reported in a dose of 10 mg kg^{-1} that produced significant reduction in urea and creatinine levels compared to control CCl4 group in the previous study[22] which agreed with the present study concerning plasma creatinine. Rosemary essential oil was also able to reduce hydrogen peroxide (H_2O_2) and 2,3-dimethoxy- 1,4-naphthoquinone (DMNQ)-induced oxidative damage of DNA in isolated rat hepatocytes and testicular cells[43,7].

The health benefits of Rosemary essential oil could be attributed to 1,8-cineole, the major compound. This monoterpene was reported to have anti-inflammatory, antioxidant and hypotensive effect. It was demonstrated that 1,8-cineole not only suppressed increase in liver weight and the elevation in serum transaminase activity but also prevented the necrosis and hemorrhage to a greater extent than dexamethasone in an *in vivo* murine model of septic shock[44]. Hepato-protection was suggested to be associated with a reduction in TNF-α serum concentration. 1,8-cineole increased glutathione peroxidase, catalase and reduced glutathione levels, while reducing MDA concentration close to the level of the control group, suggesting activation of antioxidant defense systems as one of the mechanisms of hepato-protection in a rat model of elevated oxidative stress[45]. Rosemary essential oil content from α-pinene could contribute to its antioxidant activity[46]. The increased urine volume on treatment with rosemary essential oil in the present study pointed to its potential diuretic activity which is parallel to a previous study[47] and that could have additional beneficial effect towards CVDs related to anti-hypertensive activity.

Fennel Essential Oil (FEO) possesses antioxidant and anti-inflammatory activity[15] which could explain the reduction of TNF-α and MDA in the present study. Fennel essential oil inhibits oxidative stress induced by cyclophosphamide through amelioration of MDA, catalase, superoxide dismutase and glutathione peroxidase[12]. Trans-anethole was the major compound found in fennel VO from Iraq variety (66.98%)[48]. In three Egyptian Varieties; 18 major monoterpenoids were identified in the essential oils. Trans-anethole, estragole, fenchone and limonene were highly abundant in all of the examined oils[49] which could impart synergistic bioactivity to the volatile oil.

The increase in rat body weight gain and significant increase in food efficiency ratio of rats fed dyslipidemic diet compared to balanced diet without significant change in total food intake might be attributed to the high caloric content of the dyslipidemic diet due to its high level of fat. The different VOs forms did not possess body weight reducing effect as could be seen from the nutritional parameters studied in the present research.

CONCLUSION

The different VO forms had anti-inflammatory, antioxidant and hepato- and cardio-protective effect and showed safety towards kidney function and fasting blood sugar. The nano VO of fennel improved glucose tolerance curve which could refer to its potential anti-diabetic effect. The nano-forms although were given in both reduced dose and frequency compared

to the original VO they elicited equal bioactivity and even in some cases superior effect referring to increased bioavailability of the nano-forms.

SIGNIFICANCE STATEMENTS

Volatile oil of fennel and rosemary and their respective nao-forms possess potential protective role towards dyslipidemia and CVDs in rat model. Nano-forms given in lower dose and frequency showed the same effect and even most efficient compared to original volatile oils. These VOs forms might be used as protective or complementary therapy for CVDs.

ACKNOWLEDGMENT

The study was supported by National Research Centre, Cairo, Egypt.

REFERENCES

1. Lozano, R., M. Naghavi, K. Foreman, S. Lim and K. Shibuya *et al*., 2012. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the global burden of disease study 2010. Lancet, 380: 2095-2128.
2. Dwivedi, P., R. Khatik, P. Chaturvedi, K. Khandelwal and I. Taneja *et al*., 2015. Arteether nanoemulsion for enhanced efficacy against *Plasmodium yoelii* nigeriensis malaria: An approach by enhanced bioavailability. Colloids Surf. B: Biointerfaces, 126: 467-475.
3. Al-Sereiti, M.R., K.M. Abu-Amer and P. Sen, 1999. Pharmacology of rosemary (*Rosmarinus officinalis* Linn.) and its therapeutic potentials. Indian J. Exp. Biol., 37: 124-130.
4. Yu, M.H., J.H. Choi, I.G. Chae, H.G. Im and S.A. Yang *et al*., 2013. Suppression of LPS-induced inflammatory activities by *Rosmarinus officinalis* L. Food Chem., 136: 1047-1054.
5. Moss, M., J. Cook, K. Wesnes and P. Duckett, 2003. Aromas of rosemary and lavender essential oils differentially affect cognition and mood in healthy adults. Int. J. Neurosci., 113: 15-38.
6. Takaki, I., L.E. Bersani-Amado, A. Vendruscolo, S.M. Sartoretto, S.P. Diniz, C.A. Bersani-Amado and R.K.N. Cuman, 2008. Anti-inflammatory and antinociceptive effects of *Rosmarinus officinalis* L. essential oil in experimental animal models. J. Med. Food, 11: 741-746.
7. Slamenova, D., E. Horvathova, Z. Kovacikova, K. Kozics and L. Hunakova, 2011. Essential rosemary oil protects testicular cells against DNA-damaging effects of H_2O_2 and DMNQ. Food Chem., 129: 64-70.
8. Wang, W., N. Li, M. Luo, Y. Zu and T. Efferth, 2012. Antibacterial activity and anticancer activity of *Rosmarinus officinalis* L. essential oil compared to that of its main components. Molecules, 17: 2704-2713.
9. Machado, D.G., M.P. Cunha, V.B. Neis, G.O. Balen and A. Colla *et al*., 2013. Antidepressant-like effects of fractions, essential oil, carnosol and betulinic acid isolated from *Rosmarinus officinalis* L. Food Chem., 136: 999-1005.
10. Bozin, B., N. Mimica-Dukic, I. Samojlik and E.A. Jovin, 2007. Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., Lamiaceae) essential oils. J. Agric. Food Chem., 55: 7879-7885.
11. Ngo, S.N.T., D.B. Williams and R.J. Head, 2011. Rosemary and cancer prevention: Preclinical perspectives. Crit. Rev. Food Sci. Nutr., 51: 946-954.
12. Tripathi, P., R. Tripathi, R.K. Patel and S.S. Pancholi, 2013. Investigation of antimutagenic potential of *Foeniculum vulgare* essential oil on cyclophosphamide induced genotoxicity and oxidative stress in mice. Drug Chem. Toxicol., 36: 35-41.
13. Zheljazkov, V.D., T. Horgan, T. Astatkie and V. Schlegel, 2013. Distillation time modifies essential oil yield, composition and antioxidant capacity of fennel (*Foeniculum vulgare* Mill). J. Oleo Sci., 62: 665-672.
14. Senatore, F., F. Oliviero, E. Scandolera, O. Taglialatela-Scafati, G. Roscigno, M. Zaccardelli and E. de Falco, 2013. Chemical composition, antimicrobial and antioxidant activities of anethole-rich oil from leaves of selected varieties of fennel [*Foeniculum vulgare* Mill. ssp. *vulgare* var. *azoricum* (Mill.) Thell]. Fitoterapia, 90: 214-219.
15. Aazza, S., B. Lyoussi, C. Megias, I. Cortes-Giraldo, J. Vioque, A.C. Figueiredo and M.G. Miguel, 2014. Anti-oxidant, anti-inflammatory and anti-proliferative activities of Moroccan commercial essential oils. Natl. Prod. Commun., 9: 587-594.
16. Mostafa, D.M., S.H. Abd El-Alim, M.H. Asfour, S.Y. Al-Okbi, A.M. Doha and A. Gamal, 2015. Transdermal nanoemulsions of *Foeniculum vulgare* Mill. essential Oil: Preparation, characterization and evaluation of antidiabetic potential. J. Drug Deliv. Sci. Technol., 29: 99-106.
17. Maisonneune, S.A., 1975. European Pharmacopoeia. Vol. 3. Saint-Ruffine, France.
18. Terjung, N., M. Loffler, M. Gibis, J. Hinrichs and J. Weiss, 2012. Influence of droplet size on the efficacy of oil-in-water emulsions loaded with phenolic antimicrobials. Food Funct., 3: 290-301.
19. Abd-Elsalam, K.A. and A.R., Khokhlov, 2015. Eugenol oil nanoemulsion: Antifungal activity against *Fusarium oxysporum* f. sp. *vasinfectum* and phytotoxicity on cottonseeds. Applied Nanosci., 5: 255-265.

20. Hussein, A.M.S., M.M. Kamil, S.N. Lotfy, K.F. Mahmoud, F.M. Mehaya and A.A. Mohammad, 2017. Influence of nano-encapsulation on chemical composition, antioxidant activity and thermal stability of rosemary essential oil. Am. J. Food Technol., 12: 170-177.
21. Hazra, A., K. Alexander, D. Dollimore and A. Riga, 2004. Characterization of some essential oils and their key components: Thermoanalytical techniques. J. Thermal Anal. Calorimetry, 75: 317-330.
22. Raskovic, A., I. Milanovic, N. Pavlovic, T. Cebovic, S Vukmirovic and M. Mikov, 2014. Antioxidant activity of rosemary (*Rosmarinus officinalis* L.) essential oil and its hepatoprotective potential. BMC Complement. Altern. Med., Vol. 14. 10.1186/1472-6882-14-225
23. Tognolini, M., V. Ballabeni, S. Bertoni, R. Bruni, M. Impicciatore and E. Barocelli, 2007. Protective effect of *Foeniculum vulgare* essential oil and anethole in an experimental model of thrombosis. Pharmacol. Res., 56: 254-260.
24. Al-Okbi, S.Y., A.G. Abdel-Razek, S.E. Mohammed and M. El-Sayed Ottai, 2017. Roselle seed as a potential new source of healthy edible oil. J. Biol. Sci., 17: 267-277.
25. Aebi, H., 1984. Catalase *in vitro*. Methods Enzymol., 105: 121-126.
26. Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.
27. Allain, C.C., L.S. Poon, C.S.G. Chan, W. Richmond and P.C. Fu, 1974. Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470-475.
28. Lopes-Virella, M.F., P. Stone, S. Ellis and J.A. Colwell, 1977. Cholesterol determination in high-density lipoproteins separated by three different methods. Clin. Chem., 23: 882-884.
29. Martin, M.J., S.B. Hulley, W.S. Browner, L.H. Kuller and D. Wentworth, 1986. Serum cholesterol, blood pressure and mortality: Implications from a cohort of 361 662 men. Lancet, 328: 933-936.
30. Megraw, R.E., D.E. Dunn and H.G. Biggs, 1979. Manual and continuous-flow colorimetry of triacylglycerols by a fully enzymatic method. Clin. Chem., 25: 273-278.
31. Duru, S., U. Koca, S. Oztekin, C. Olguner and A. Kar *et al*., 2005. Antithrombin III pretreatment reduces neutrophil recruitment into the lung and skeletal muscle tissues in the rat model of bilateral lower limb ischemia and reperfusion: A pilot study. Acta Anaesthesiol. Scand., 49: 1142-1148.
32. Reitman, S. and S. Frankel, 1957. Colorimetric methods for aspartate and alanine aminotransferase. Am. J. Clin. Pathol., 28: 55-60.
33. Tobias, G.J., Jr.R.F. McLaughlin and Jr.J. Hopper, 1962. *Endogenous creatinine* clearance: A valuable clinical test of glomerular filtration and a prognostic guide in chronic renal disease. N. Engl. J. Med., 266: 317-323.
34. Mohamed, D.A., I.M. Hamed and S.Y. Al-Okbi, 2008. *Ceratonia siliqua* pods as a cheap source of functional food components. Deutche Lebensmittel-Rundschau, 104: 25-29.
35. Aggarwal, B.B. and S. Shishodia, 2004. Suppression of the nuclear factor-kB activation pathway by spice-derived phytochemicals: Reasoning for seasoning. Ann. N. Y. Acad. Sci., 1030: 434-441.
36. Sheikh, B.A., L. Pari, A. Rathinam and R. Chandramohan, 2015. Trans-anethole, a terpenoid ameliorates hyperglycemia by regulating key enzymes of carbohydrate metabolism in streptozotocin induced diabetic rats. Biochimie, 112: 57-65.
37. Bouchentouf, S., H. Allali and G. Said, 2017. First study on anti-diabetic effect of rosemary and salvia by using molecular docking. J. Pharm. Res. Int., 19: 1-12.
38. Al-Okbi, S.Y., D.A. Mohamed, A.M.S. Hussein, S. Sabry, T.E. Hamed and N.H. Ahmed, 2013. Functional foods for reduction of cardiovascular risk in type 2 diabetic patients. J. Applied Sci. Res., 9: 2072-2084.
39. Al-Okbi, S.Y., D.A. Mohamed, T.E. Hamed and R.S.H. Esmail, 2014. Rice bran oil and pumpkin seed oil alleviate oxidative injury and fatty liver in rats fed high fructose diet. Pol. J. Food Nutr. Sci., 64: 127-133.
40. Fabbrini, E., M. Serafini, I.C. Baric, S.L. Hazen and S. Klein, 2013. Effect of plasma uric acid on antioxidant capacity, oxidative stress and insulin sensitivity in obese Diabetes, 63: 976-981.
41. Viuda-Martos, M., Y.R. Navajas, E.S. Zapata, J. Fernadez-Lopez and J.A. Perez-Alvarez, 2010. Antioxidant activity of essential oils of five spice plants widely used in a Mediterranean diet. Flavour Fragrance J., 25: 13-19.
42. Kadri, A., Z. Zarai, I.B. Chobba, A. Bekir, N. Gharsallah, M. Damak and R. Gdoura, 2011. Chemical constituents and antioxidant properties of *Rosmarinus officinalis* L. essential oil cultivated from South-Western Tunisia. J. Med. Plants Res., 5: 5999-6004.
43. Horvathova, E., D. Slamenova and J. Navarova, 2010. Administration of rosemary essential oil enhances resistance of rat hepatocytes against DNA-damaging oxidative agents Food Chem., 123: 151-156.
44. Santos, F.A., R.M. Silva, A.R. Tome, V.S. Rao and M.M. Pompeu *et al*., 2001. 1, 8-Cineole protects against liver failure in an *in vivo* murine model of endotoxemic shock. J. Pharm. Pharmacol., 53: 505-511.
45. Ciftci, O., I. Ozdemir, S. Tanyildizi, S. Yildiz and H. Oguzturk, 2011. Antioxidative effects of curcumin, β-myrcene and 1,8-cineole against 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced oxidative stress in rats liver. Toxicol. Ind. Health, 27: 447-453.

46. Turkez, H. and E. Aydin, 2016. *In vitro* assessment of cytogenetic and oxidative effects of α-pinene. Toxicol. Ind. Health, 32: 168-176.
47. De Melo, G.A.N., R. Grespan, J.P. Fonseca, T.O. Farinha and E.L. Silva *et al*., 2011. *Rosmarinus officinalis* L. essential oil inhibits *in vivo* and *in vitro* leukocyte migration. J. Med. Food, 14: 944-946.
48. Kiralan, M., A. Bayrak, O.F. Abdulaziz and T. Ozbucak, 2012. Essential oil composition and antiradical activity of the oil of Iraq plants. Natl. Prod. Res., 26: 132-139.
49. Shahat, A.A., A.Y. Ibrahim, S.F. Hendawy, E.A. Omer, F.M. Hammouda, F.H. Abdel-Rahman and M.A. Saleh, 2011. Chemical composition, antimicrobial and antioxidant activities of essential oils from organically cultivated fennel cultivars. Molecules, 16: 1366-1377.