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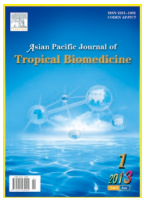

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Antioxidant properties and inhibitory effects of *Satureja khozestanica* essential oil on LDL oxidation induced-$CuSO_4$ *in vitro*

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PEER REVIEW

Peer reviewer

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Comments

This is a good study in which the authors showed that SKE is found to possess a good antioxidant activity and various concentrations of SKE have a dose-dependent antioxidant activity against LDL oxidation.

(Details on Page 26)

ABSTRACT

Objective: To assess various antioxidative activities of *Satureja khozestanica* essential oil (SKE) and its effect on oxidation of low density lipoprotein (LDL) induced by $CuSO_4$ *in vitro* by monitoring the formation of conjugated dienes and malondialdehyde (MDA). **Methods:** The formation of conjugated dienes, lag time and MDA were measured. Inhibition of this Cu-induced oxidation was studied in the presence of several concentrations of SKE. Also total antioxidant activity and free radical scavenging of SKE were determinated. **Results:** It was demonstrated that SKE was able to inhibit LDL oxidation and decrease the resistance of LDL against oxidation. The inhibitory effects of SKE on LDL oxidation were dose-dependent at concentrations ranging from 50 to 200 μg/mL. Total antioxidant capacity of SKE was (3.20±0.40) nmol of ascorbic acid equivalents/g SKE. The SKE showed remarkable scavenging activity on 2, 2-diphenyl-picrylhydrazyl, IC_{50} (5.30±0.11) ng/mL. **Conclusions:** This study shows that SKE is a source of potent antioxidants and prevents the oxidation of LDL *in vitro* and it may be suitable for use in food and pharmaceutical applications.

KEYWORDS

Antioxidant properties, *Satureja khozestanica*, Essential oil, Low density lipoprotein, Oxidation

1. Introduction

Cardiovascular disease is one of the leading causes of mortality in our society[1]. Although an increased concentration of plasma low density lipoprotein (LDL) is believed to be a major risk factor in this regard, the underlying mechanisms remain unclear and need more investigations. To date, considerable evidence supports a role for oxidatively modified LDL in the pathogenesis of atherosclerosis[2]. The uptake of oxidized LDL (Ox-LDL) by macrophages results in the formation of foam cells and cellular cholesterol accumulated in vascular endothelial cells, and promotes the development of the characteristic fatty streaks found in atherosclerotic lesions[1-4]. There is experimental evidence indicating that different antioxidant compounds given at high pharmacological doses are effective in decreasing both atherogenesis and LDL oxidation in animals. In humans, supplementation with antioxidants combined at physiological doses is not effective in decreasing atherogenesis[5]. Researchers are recently interested in investigation and research into extraction of natural antioxidants from medical herbs to replace synthetic antioxidants. Therefore, the research into the determination of the natural antioxidant source is very important to

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promote public health. *Satureja khuzestanica*, an endemic plant of Iran, decreases glucose and malondealdehyde in serum diabetic patients[6-9]. The components of this extract were analyzed with gas chromatography/mass spectrometry (GC/MS) in Research Center of Lorestan University as reported in our previous paper[9]. The main component of this extract is carvacrol. Since the various antioxidative activities of *Satureja khozestanica* essential oil (SKE) and inhibitory effects of its on LDL oxidation have not previously been reported, the objectives of the present study were to assess various antioxidative activities of SKE and investigate the effect of SKE on the oxidation of LDL induced by $CuSO_4$ *in vitro*.

2. Material and methods

2.1. Reagents and chemicals

Disodium ethylene diamine tetra acetate (Na_2EDTA), Potassium bromide (KBr), sodium chloride (NaCl), disodium hydrogen phosphate (Na_2HPO_4), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), trichloroacetic acid, ferric chloride, sodium acetate, 2, 6-di-tert-butyl-4-methyl phenol (BHT), ascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). All the solvents used were of analytical grade. 2-thiobarbituric acid (TBA) was obtained from Fluka Chemie (Buchs SG, Switzerland).

2.2. Isolation of the essential oil from *Satureja khozestanica*

SKE was prepared from cultivated *Satureja khozestanica* in Khoram Abad (Lorestan province, western Iran). The aerial parts of the plants were collected during flowering stage and were air-dried at ambient temperature in the shade. The aerial parts were hydro-distilled using a Clevenger apparatus for 4 h, giving yellow oil in 0.9% yield. The oil was dried over anhydrous sodium sulfate and stored at 4 °C. The plant was previously identified by the Department of Botany of the Research Institute of Forests and Rangelands (TARI) in Tehran, Iran. A voucher specimen (No. 58416) has been deposited at the TARI Herbarium[6,9].

2.3. Blood sampling

Blood samples were taken from ten men. The protocols for the blood sampling were approved by the Medical University of Lorestan Ethics Committee, and all the informed constants were taken from all the men. Fasting blood samples after an overnight fasting were collected in EDTA containing tubes (1.6 mg EDTA/mL blood). To obtain fresh plasma, the blood samples were centrifuged (3 000 r/min for 10 min at 4 °C) as soon as the samples were collected to avoid auto-oxidation. To minimize oxidation *in vitro*, sodium azide (0.06% wt/vol) was added to plasma samples immediately after separation.

2.4. Isolation of LDL

The LDL fraction was isolated from fresh plasma by single vertical discontinuous density gradient ultracentrifugation[10]. The density of the plasma was adjusted to 1.21 g/mL by the addition of solid KBr (0.365 g/mL). Centrifuge tubes were loaded by layering 1.5 mL of density-adjusted plasma under 3.5 mL of 0.154 mol/L NaCl, and centrifuged in a Beckman L7-55 ultracentrifuge at 40 000 r/min at 10 °C for 2.5 h. The isolated LDL was dialyzed for 48h at 4 °C against three changes of deoxygenated-PBS (0.01 mol/L Na_2HPO_4, 0.16 mol/L NaCl, pH 7.4).

2.5. DPPH free radical-scavenging activity

DPPH free radical-scavenging activity of the test samples was determinated according to the method of Blois[11]. In brief, 4 mL of DPPH radical solution in ethanol (1 mM) was mixed with 1 mL of essential oil solution in ethanol containing 0.0 001-3 000 μg/mL of SKE; and after 30 min, the absorbance was measured at 517 nm. This activity was given as percentage DPPH scavenging that is calculated as %DPPH scavenging=[(control absorbance-SKE absorbance)/(control absorbance)]×100.

The 50% inhibition concentration (IC_{50}), *i.e.* the concentration of SKE that was required to scavenge 50% of radicals, was calculated.

2.6. Total antioxidant activity test

Total antioxidant activity of the test samples was determinated according to the method of Prriteo *et al*. In brief, 0.3 mL of sample was mixed with 3.0 mL of reagent solution (0.6 mol/L sulfuric acid, 28 mmol/L sodium phosphate and 4 mmol/L ammonium molybdate). Reaction mixture was incubated at 95 °C for 90 min under water bath. Absorbance of all the sample mixtures was measured at 695 nm. The total antioxidant activity was expressed as the number of equivalents of ascorbic acid acid (μmol/g)[12] .

2.7. Oxidation of LDL test

2.7.1. Continuous monitoring of formation of conjugated dienes in LDL

After isolation of total LDL, the protein content of LDL was measured[13]. LDL was adjusted to 150 μg/mL of LDL protein with 10 mM PBS, pH7.4 and then aliquots of ESK were added to the solution. The oxidation of LDL was initiated by addition of freshly prepared 10μmol/L $CuSO_4$ solution at 37 °C in a water bath for 5 h. The kinetics of LDL oxidation was monitored every 10 min by measuring LDL samples absorbance at 234 nm. The lag phase was calculated from the oxidation profile of each LDL preparation by

drawing a tangent to the slope of the propagation phase and extrapolation into intercept the initial-absorbance axis. The lag phase represented the length of the antioxidant-protected phase during LDL oxidation by SKE *in vitro*. The lag time was measured as the time period until the conjugated dienes (CD) began to increase[14]. The formation of CD was calculated as CD equivalent content (nmol/mg protein) at 5 h. The CD concentration was calculated by using the extinction coefficient for diene conjugates at 234 nm.

2.7.2. Assay of the formation of malondialdehyde (MDA)

Lipid peroxidation end products were determined as MDA according to modified method of Buege and Aust[15]. After initiating the oxidation process with $CuSO_4$, the sample mixtures were incubated at 37 °C for 5 h in a water bath and the reaction was terminated by adding EDTA (2 mmol/L). MDA formation was measured in a spectrophotometer at 532 nm. The results were recorded as MDA equivalent content (nmol/mg LDL protein).

2.8. Statistical analysis

The data were presented as mean±SD of three experiments performed in duplicate. The variables used to describe the difference between the oxidation curves were lag time, CD and MDA. These parameters were obtained using the Mann-Whitney test (using SPSS 13.0 statistical software) for independent data and the differences were considered significant when $P<0.05$.

3. Results

3.1. Antioxidant activity

3.1.1. DPPH scavenging assay

The antioxidant activity of SKE was evaluated by the DPPH radical scavenging capacity. Figure 1 shows the percentage of DPPH radicals scavenging capacity with BHT as reference. In the DPPH scavenging assay, the IC_{50} (the concentration required to scavenge 50% of radical) values of SKE and BHT were (5.30±0.11) and (3.89±0.34) ng/mL, respectively.

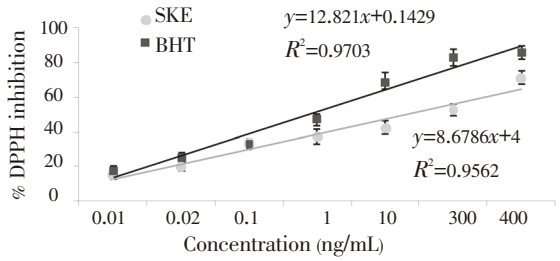

Figure 1. Free radical scavenging capacities of the SKE measured in DPPH assay. Each point represents the mean of five experiments.

3.1.1. Total antioxidant activity

The phosphomolybdenum method has been widely used in the assessment of total antioxidant activity of plant extracts, natural compounds and foods. Figure 2 shows the total antioxidant activity of ascorbic acid as standard. The total antioxidant activity of SKE was (3.20±0.40) nmol of ascorbic acid equivalents/g SKE.

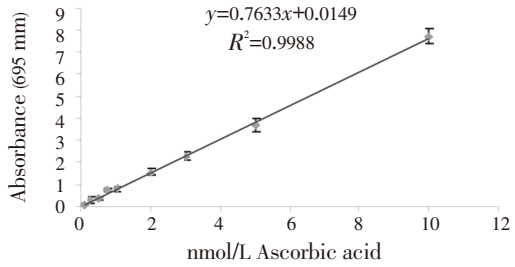

Figure 2. Total antioxidant activity free radical of ascorbic acid as standard measured in phosphomolybdenum method. Each point represents the mean of five experiments.

3.2. Oxidation of LDL

3.2.1. Continuous monitoring of formation of conjugated dienes in LDL and kinetics of $CuSO_4$-induced LDL oxidation

Figure 3 shows that $CuSO_4$ dramatically increased oxidation of LDL. The formation of CD, a marker of LDL oxidation, decreased by vitamin E and SKE. Figure 4 shows the levels of CD at 5 h in all the experimental groups. $CuSO_4$ increased the level of the CD in LDL about six-fold and was significantly different from the control LDL. Vitamin E and SKE (100, 200 μg/mL) inhibited the final levels of CD in LDL oxidation ($P<0.001$). SKE showed a dose-dependent inhibition in increasing of CD at 5 h. 50, 100 and 200 μg/mL concentrations. Figures 5 shows the levels of lag time in all the experimental groups. In the assay, various concentrations of SKE were confirmed to have a dose-dependent antioxidant activity by increasing lag time. 33.33% increased lag time by SKE at concentration of 50 μg/mL. At 100 μg/mL and 200 μg/mL, SKE showed an increase rate of 67% and 100%. Vitamin E, as the positive control, at concentrations of 100 μmol/L, showed an increase rate of 111% lag time, respectively.

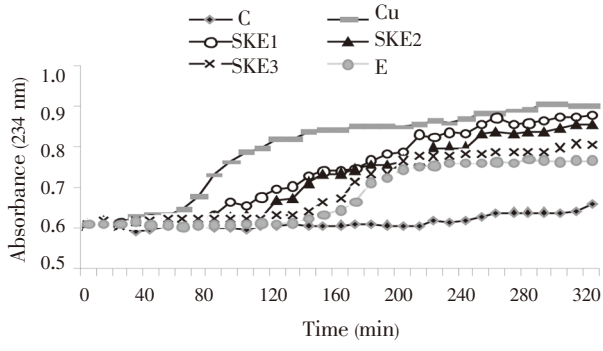

Figure 3. The effects of SKE on LDL oxidation in 10 mmol/L PBS, pH 7.4 at 37 °C for 5 h.
C: n-LDL; Cu: n-LDL+copper; SKE1: n-LDL+SKE (50 μg/mL); SKE2: n-LDL+ SKE (100 μg/mL; SKE3: n-LDL+SKE (200 μg/mL); E1: n-LDL+Vitamin E (100 μmol/L). Each point represents the mean of five experiments.

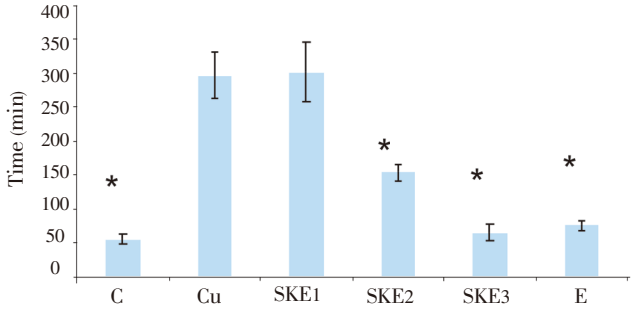

Figure 4. The effects of SKE on the formation of conjugated dienes of LDL oxidation.
C: n-LDL; Cu: n-LDL+copper; SKE1: n-LDL+SKE (50 μg/mL); SKE2: n-LDL+SKE (100 μg/mL; SKE3: n-LDL+SKE (200 μg/mL); E1: n-LDL+Vitamin E (100 μmol/L). Each point represents the means of five experiments. **significant compared to Cu and SKE1, by man whitny test (P<0.01).

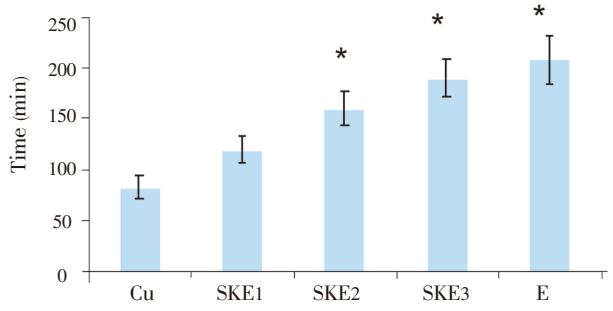

Figure 5. The effects of SKE on lag time of $CuSO_4$-induced LDL oxidation.
C: n-LDL; Cu: n-LDL+copper; SKE1: n-LDL+SKE (50 μg/mL); SKE2: n-LDL+SKE (100 μg/mL; SKE3: n-LDL+SKE (200 μg/mL); E1: n-LDL+Vitamin E (100 μmol/L). Each point represents the mean of five experiments. *significant compared to Cu by man whitny test (P<0.01).

3.2.2. Assay of the formation of MDA

The levels of MDA after 5 h of incubation in all experiment groups are shown in Figure 6. Vitamin E significantly inhibited MDA formation (P<0.01). SKE (100, 200 μg/mL) significantly was inhibited by the MDA production in LDL (P<0.01 and P<0.001) respectively.

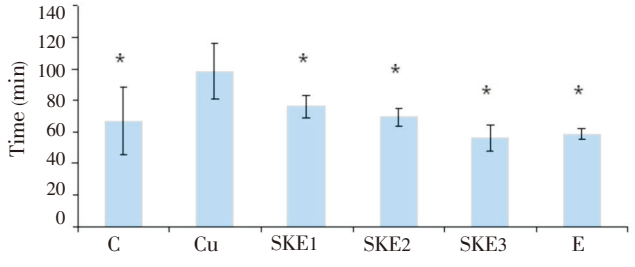

Figure 6. The effects of SKE on the formation of MDA.
C: n-LDL; Cu: n-LDL+copper; SKE1: n-LDL+SKE (50 μg/mL); SKE2: n-LDL+SKE (100 μg/mL; SKE3: n-LDL+SKE (200 μg/mL); E1: n-LDL+Vitamin E (100 μmol/L). Each point represents the means of five experiments.

4. Discussion

Researchers are recently interested in investigation and research into extraction of natural antioxidants from medical herbs to replace synthetic antioxidants. Natural antioxidants are healter and more beneficial and have fewer side effects than synthetic antioxidants[16]. Phytochemicals with antioxidant effects include some cinnamic acids, olorpein, coumarins, falvonoids, carvacrol, lignans and tannins[17]. Therefore, the herbs which have high amount of these compounds are taken into consideration in order to inhibit diseases related to oxidative stress such as coronary heart disease and diabetes mellitus[18]. *Satureja khuzestanica*, an endemic plant of Iran, is useful in diabetes, cardiovascular diseases and other complications[7]. Conducting research on herbal antioxidants and evaluating and comparing their antioxidant effects, as well as newer and more valuable sources of natural antioxidants can be found and used in special cases.

A stable free radical 2, 2′-diphenyl-1-pycrylhydrazyl (DPPH) has widely been used in the assessment of radical scavenging activity of plant extracts, natural compounds and foods[19]. The antioxidant activity of SKE was evaluated by the DPPH radical scavenging capacity. In the DPPH scavenging assay, the IC_{50} (the concentration required to scavenge 50% of radical) values of SKE and BHT were (5.30±0.11) ng/mL and (3.89±0.34) ng/mL, respectively. The data obtained show that SKE is a free radical scavenger and may act as a primary antioxidant, which can react with free radicals by donating hydrogen. Haeri *et al.* showed the presence of different hydroxyl containing compounds such as carvacrol, tannins, triterpenoids, steroids and flavoniods in the SKE[7]. These compounds may be the main cause of its considerable radical-scavenging activity.

The phosphomolybdenum method has been widely used in the assessment of total antioxidant activity of plant extracts, natural compounds and foods. The total antioxidant activity of SKE was (3.20±0.40) nmol of ascorbic acid equivalents/g SKE. The difference in the amount of antioxidant of extracts may be attributed to the differences in the amount and kind of existing antioxidant compounds in them such as carotenoids, phenol and ascorbic acid[20]. The antioxidant activity shown by the SKE may be due to the presence of carvacrol, tannins, triterpenoids, steroids and flavoniods. Our recent results indicated that SKE is found to possess a good antioxidant activity.

Recently, it has become widely accepted that diet may play an important role in health promotion and disease prevention. Objective data are required by consumers and health care professionals to improve daily diets and, consequently, reduce the risk of chronic diseases such as coronary heart disease[21]. Lipid peroxidation is one of principal factors in causing atherosclerosis[22]. Oxidized LDL

is atherogenic-it causes arterial cell death, accumulation of growth factors and cytokine release. In addition, oxidized LDL contributes to platelet aggregation, smooth muscle cell proliferation, and LDL oxidation was shown in patients with hypercholesterolemia, hypertension, diabetes mellitus, chronic renal failure, and in smokers[22]. The formation of CD, a marker of LDL oxidation, decreased by vitamin E and SKE. CD formation serves as a marker of the early stages of LDL oxidation process[14]. $CuSO_4$ increased the level of the CD in LDL about six-fold and was significantly different from the control LDL. Vitamin E and SKE (100, 200 μg/mL) inhibited the final levels of CD in LDL oxidation (P<0.001). SKE showed a dose-dependent inhibition in increasing of CD at 5 h. 50, 100 and 200 μg/mL concentrations. In the assay, various concentrations of SKE were confirmed to have a dose-dependent antioxidant activity by increasing lag time. So SKE has highly strong resistance to peroxidation. Some compounds in SKE extract can provide hydroxyl to accept electrons and scavenge OH induced by $CuSO_4$. The results are considered to be noteworthy when compared to the findings of other studies concerning medicinal plants[23,24].

TBARS is a secondary product from lipid peroxidation in LDL. MDA analysis measures the formation of secondary products of lipid oxidation, mainly MDA, which may contribute off-flavour to oxidized oil[25]. The antioxidative effect of SKE on LDL was determined and expressed by measurement of MDA equivalent content. Vitamin E significantly inhibited MDA formation. SKE (100, 200 μg/mL) significantly was inhibited by the MDA production in LDL . SKE showed a dose-dependent inhibition of MDA formation in the dose of 50, 100 and 200 μg/mL concentrations.

This result suggests that SKE is a good antioxidant and may be used in suppressing LDL oxidation *in vivo*. The reduction of LDL oxidation *in vivo* may delay the progress of atherosclerosis and reduce the risk of heart diseases. The protection of LDL by SKE in a copper-induced oxidation system could be due to both metal-chelating and radical scavenging capacity. However, the mechanism by which the extracts inhibit LDL oxidation *in vitro* remains unclear. Laranjinha *et al*. suggested possible explanations for the protecting effects of compounds of extracts on LDL: "(i) scavenging of various radical species in the aqueous phase, (ii) interaction with peroxyl radicals at the LDL surface, (iii) partitioning into the LDL particle and terminating chain-reactions of lipid peroxidation by scavenging lipid radicals, and (iv) regenerating endogenous α-tocopherol back to its active antioxidative form"[26]. The results showed that SKE similar to vitamin E is a potent antioxidant and may be protects LDL in plasma against oxidation.

The results of the present study clearly showed that SKE is found to posses a good antioxidant activity and various concentrations of SKE have a dose-dependent antioxidant activity against LDL oxidation by inhibiting the formation of conjugated diene and MDA, and increasing lag time. In conclusion, *Satureja khuzestanica* is a potent antioxidant and may be a good alternative to reduce the risk of atherosclerosis and coronary heart disease and other free radical associated health problems.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

Cardiovascular disease is one of the leading causes of mortality in our society. Although, an increased concentration of plasma low density lipoprotein (LDL) is believed to be a major risk factor in this regard, the underlying mechanisms remain unclear and need more investigations. To date, considerable evidence supports a role for oxidatively modified LDL in the pathogenesis of atherosclerosis. "The uptake of oxidized LDL (Ox-LDL) by macrophages results in the formation of foam cells and cellular cholesterol accumulated in vascular endothelial cells, and promotes the development of the characteristic fatty streaks found in atherosclerotic lesions". There is experimental evidence indicating that different antioxidant compounds given at high pharmacological doses are effective in decreasing both atherogenesis and LDL oxidation in animals. In humans, supplementation with antioxidants combined at physiological doses is not effective in decreasing atherogenesis. Researchers are recently interested in investigation and research into extraction of natural antioxidants from medical herbs to replace synthetic antioxidants. Therefore, the research into the determination of the natural antioxidant source is very important to promote public health.

Research frontiers

The present study was to assess various antioxidative activities of SKE and investigate the effect of SKE on the oxidation of LDL induced by $CuSO_4$ *in vitro*.

Related reports

The components of *Satureja khozestanica* essential oil with gas chromatography/ mass spectrometry (GC/MS) in Research Center of Lorestan University have previously been analyzed and reported. Also hypolipidemic, antiatherogenic and liver protective effects of SKE have previously been reported.

Innovations and breakthroughs

This study showed that SKE is a source of potent

antioxidants and prevents the oxidation of LDL *in vitro*.

Applications

Satureja khuzestanica is a potent antioxidant and may be a good alternative to reduce the risk of atherosclerosis and coronary heart disease and other free radical associated health problems.

Peer review

This is a good study in which the authors showed that SKE is found to possess a good antioxidant activity and various concentrations of SKE have a dose-dependent antioxidant activity against LDL oxidation.

References

[1] Soriano JB, Lamprecht B. Chronic obstructive pulmonary disease: a worldwide problem. *Med Clin North Am* 2012; **96**(4): 671-680.

[2] Castelao JE, Gago-Dominguez M. Risk factors for cardiovascular disease in women: relationship to lipid peroxidation and oxidative stress. *Med Hypotheses* 2008; **71**(1): 39-44.

[3] Miller YI, Choi SH, Fang L, Tsimikas S. Lipoprotein modification and macrophage uptake: role of pathologic cholesterol transport in atherogenesis. *Subcell Biochem* 2010; **51**: 229-251.

[4] Ahmadvand H, Bagheri SA, Boshtam M, abdolahpour F. Effects of olive leaves extract on LDL oxidation induced-$CuSO_4$ *in vitro*. *Pak J Pharm Sci* 2012; **25**(3): 571-575.

[5] Yekini I, Hammoudi F, Martin-Nizard F, Yous S, Lebegue N, Berthelot P. Antioxidant activity of benzoxazolinonic and benzothiazolinonic derivatives in the LDL oxidation model. *Bioorg Med Chem* 2009; **17**(22): 7823-7830.

[6] Abdollahi M, Salehnia A, Mortazavi SH, Ebrahimi M, Shafiee A, Fouladian F, et al. Antioxidant, antidiabetic, antihyperlipidemic, reproduction stimulatory properties and safety of essential oil of *Satureja khuzestanica* in rat *in vivo*: a oxicopharmacological study. *Med Sci Monit* 2003; **9**(9): 331-335.

[7] Haeri S, Minaie B, Amin G, Nikfar S, Khorasani R, Esmaily H. Effect of *Satureja khuzestanica* essential oil on male rat fertility. *Fitoterapia* 2006; **77**(7-8): 495-499.

[8] Mahboubi M, Kazempour N. Chemical composition and antimicrobial activity of *Satureja hortensis* and *Trachyspermum copticum* essential oil. *Iran J Microbiol* 2011; **3**(4): 194-200.

[9] Tavafi M, Ahmadvand H, Tamjidipoor A, Delfan B, Khalatbari AR. *Satureja khozestanica* essential oil ameliorates progression of diabetic nephropathy in uninephrectomized diabetic rats. *Tissue Cell* 2011; **43**(1): 45-51.

[10] Jantan I, Saputri FC. Benzophenones and xanthones from *Garcinia cantleyana* var. cantleyana and their inhibitory activities on human low-density lipoprotein oxidation and platelet aggregation. *Phytochemistry* 2012; **80**: 58-63.

[11] Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature* 1958; **181**: 1199-1200.

[12] Kishore RK, Halim AS, Syazana MS, Sirajudeen KN. Tualang honey has higher phenolic content and greater radical scavenging activity compared with other honey sources. *Nutr Res* 2011; **31**(4): 322-325.

[13] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal Biochem* 1979; **72**(1/2): 248-254.

[14] Hui SP, Sakurai T, Ohkawa F, Furumaki H, Jin S, Fuda H, et al. Detection and characterization of cholesteryl ester hydroperoxides in oxidized LDL and oxidized HDL by use of an Orbitrap mass spectrometer. *Anal Bioanal Chem* 2012; **404**(1): 101-112.

[15] Ahmadvand H, Ani M, Moshtagie. Inhibitory effect of *Allium cepa* extract on LDL oxidation induced by $CuSO_4$ *in vitro* compared with *Allium sativum* and *Allium ascalonicom*. *Iran J Pharmacol Therap* 2011; **10**: 67-71.

[16] Hajhashemi V, Zolfaghari B, Yousefi A. Antinociceptive and anti-inflammatory activities of *Satureja hortensis* seed essential oil, hydroalcoholic and polyphenolic extracts in animal models. *Med Princ Pract* 2012; **21**(2): 178-182.

[17] Tatiya AU, Surana SJ, Sutar MP, Gamit NH. Hepatoprotective effect of poly herbal formulation against various hepatotoxic agents in rats. *Pharmacognosy Res* 2012; **4**(1): 50-56.

[18] Mosaffa F, Behravan J, Karimi G, Iranshahi M. Antigenotoxic effects of *Satureja hortensis* L. on rat lymphocytes exposed to oxidative stress. *Arch Pharm Res* 2006; **29**(2): 159-164.

[19] Morsy MD, Mostafa OA, Hassan WN. A potential protective effect of alpha-tocopherol on vascular complication in spinal cord reperfusion injury in rats. *J Biomed Sci* 2010; **7**: 17-55.

[20] Jana K, Chatterjee K, Ali KM, De D, Bera TK, Ghosh D. Antihyperglycemic and antioxidative effects of the hydro-methanolic extract of the seeds of Caesalpinia bonduc on streptozotocin-induced diabetes in male albino rats. *Pharmacogn Res* 2012 ; **4**(1): 57-62.

[21] Leopold JA, Loscalzo J. Oxidative risk for atherothrombotic cardiovascular disease. *Free Radic Biol Med* 2009; **47**(12): 1673-1706.

[22] Mahfouz MM, Zhou Q, Kummerow FA. Effect of curcumin on LDL oxidation *in vitro*, and lipid peroxidation and antioxidant enzymes in cholesterol fed rabbits. *Int J Vitam Nutr Res* 2011; **81**(6): 3783-3791.

[23] Fu L, Xu BT, Xu XR, Qin XS, Gan RY, Li HB. Antioxidant capacities and total phenolic contents of 56 wild fruits from South China. *Molecules* 2010; **15**(12): 8602-8617.

[24] Chan EW, Soh EY, Tie PP, Law YP. Antioxidant and antibacterial properties of green, black, and herbal teas of *Camellia sinensis*. *Pharmacogn Res* 2011; **3**(4): 266-722.

[25] Rababah T, Hettiarachchy NS, Horax R, Cho MJ, Davis B, Dickson J. Thiobarbituric acid reactive substances and volatile compounds in chicken breast meat infused with plant extracts and subjected to electron beam irradiation. *Poult Sci* 2006; **85**(6): 1107-1113.

[26] Laranjinha JA, Almeida LM, Madeira VM. Reactivity of dietary phenolic acids with peroxyl radicals: antioxidant activity upon low density lipoprotein peroxidation. *Biochem Pharmacol* 1994; **48**(3): 487-494.