Suppressive Impact of *Anethum Graveolens* Consumption on Biochemical Risk Factors of Atherosclerosis in Hypercholesterolemic Rabbits

Mahbubeh Setorki, Mahmoud Rafieian-Kopaei[1], Alireza Merikhi[2], Esfandiar Heidarian[3], Najmeh Shahinfard[1], Roya Ansari[1], Hamid Nasri[4], Nafiseh Esmael[5], Azar Baradaran[6]

Department of Biology, Izeh Branch, Islamic Azad University, Izeh, Iran, [1]Medical Plants Research Center, Shahrekord University of Medical Sciences, Sharekord, Iran, [2]Department of Pediatric Nephrology, Isfahan University of Medical Sciences, [3]Clinical Biochemistry Research Center, Shahrekord University of Medical Sciences, Sharekord, Iran, [4]Department of Internal Medicine Isfahan Kidney Disease Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, [5]Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran, [6]Department of Pathology, Isfahan University of Medical Sciences, Isfahan, Iran

Correspondence to:
Prof. Mahmoud Rafieian-Kopaei,
Medical Plants Research Center,
Shahrekord University of Medical Sciences, Sharekord, Iran.
E-mail: rafieian@yahoo.com

Date of Submission: Sep 01, 2012

Date of Acceptance: May 28, 2013

How to cite this article: Setorki M, Rafieian-Kopaei M, Merikhi A, Heidarian E, Shahinfard N, Ansari R, Nasri H, Esmael N, Baradaran A suppressive impact of anethum graveolens consumption on biochemical risk factors of atherosclerosis in hypercholesterolemic rabbits. Int J Prev Med 2013;4:889-95.

ABSTRACT

Background: We aimed to determine the effects of *Anethum graveolens* (Dill) powder on postprandial lipid profile, markers of oxidation and endothelial activation when added to a fatty meal.

Methods: In an experimental study, 32 rabbits were randomly designated into four diet groups: normal diet, high cholesterol diet (1%), high cholesterol diet plus 5% (w/w) dill powder and high cholesterol diet plus lovastatin (10 mg/kg, bw). The concentrations of glucose, total cholesterol (TC), low-density lipoproteins-cholesterol (LDL-C), alanine aminotransferase (alt), aspartate aminotransferase (ast), fibrinogen, factor VII, apolipoprotein B (ApoB), nitrite and nitrate were measured in blood samples following 15 h of fasting and 3 h after feeding.

Results: Concurrent use of *A. graveolens* powder or lovastatin significantly decreased ALT, TC, glucose, fibrinogen and LDL-C values in comparison with hypercholesterolemic diet group ($P < 0.05$). Consumption of *A. graveolens* or lovastatin did not change factor VII, ApoB, nitrite and nitrate levels significantly in comparison with hypercholesterolemic diet group. Intake of *A. graveolens* significantly decreased serum AST compared to hypercholesterolemic diet.

Conclusions: *A. graveolens* might have some protective values against atherosclerosis and that it significantly affects some biochemical risk factors of this disease. Our findings also confirm the potential harmful effects of oxidized fats and the importance of dietary polyphenols in the meal.

Keywords: *Anethum graveolens*, atherosclerosis, hypercholesterolemia, rabbits

Original Article

INTRODUCTION

Hyperlipidemia promotes atherosclerosis, which is a syndrome affecting arterial blood vessels, a chronic inflammatory response in the walls of arteries, caused largely by the accumulation of macrophage white blood cells and promoted by low-density lipoproteins (LDL) without adequate removal of fats and

cholesterol from the macrophages by functional high-density lipoproteins (HDL). It is caused by the formation of multiple plaques within the arteries.[1]

Atherosclerosis is a chronic disease that remains asymptomatic for a long time.[2] Upon formation, intraluminal thrombi can occlude arteries outright (i.e., coronary occlusion), but more often they detach, move into the circulation and eventually occlude smaller downstream branches causing thromboembolism. Apart from thromboembolism, atherosclerotic lesions can cause complete closure of the arteries. Interestingly, chronically expanding lesions are often asymptomatic until the artery stenosis is so severe that the blood supply to downstream tissue (s) is insufficient and results in ischemia.[3]

Various, physiological, anatomic and behavioral risk factors for atherosclerosis are known. Diabetes or impaired glucose tolerance, dyslipoproteinemia, high serum concentration of LDL and/or very low density lipoprotein (VLDL), Low serum concentration of functioning HDL, LDL:HDL ratio greater than 3:1, elevated serum C-reactive protein concentrations, elevated lipid peroxidation, are among the most important modifiable risk factors of atherosclerosis.[4-6]

Hyperlipidemia and lipid peroxidation are more than others implicated in induction of atherosclerosis. As humans are predominantly in a postprandial state, attention has been paid, recently, on postprandial hyperlipidemia and its abnormalities on the contributing factors leading to atherosclerosis.[7-9]

Evidence suggests that postprandial hypertriglyceridemia is a risk factor for cardiovascular disease (CVD)[10] and that it has a negative effect on endothelial function in normal subjects.[7,11,12]

It has also shown that medicinal plants, rich in antioxidants, may ameliorate hyperlipidemia,[13-15] other than reduction of diabetic or atherosclerosis complications.[16] These plants mostly include phenolic and flavonoid components with antioxidant activities.[15]

It has also been shown that flavonoid-containing food such as fruits, vegetables and tea are protective against CVD through their free radical-scavenging quality.[17] As it was mentioned scientists mostly attribute these effects to flavonoids potential role in decreasing blood lipid levels and therefore changing the postprandial state.[10,18]

Dill is a short-lived perennial herb and is the sole specie of the genus *Anethum*. Its seeds contain 3% oil, carotene, flandrenin, limonene and tannin. In traditional Iranian medicine, Dill has been used as sedative, carminative, antispasmodic, lactogogue, diuretic and home remedy for hyperlipidemia.[19]

It has been found that other than glucose, dill significantly may reduce triglyceride, total cholesterol (TC), VLDL, LDL-cholesterol (LDL-C) and increases HDL-cholesterol (HDL-C) in diabetic rats. These effects have also been attributed to antioxidant contents of dill.[20] It seems that if dill reduces postprandial hyperlipidemia, it would be beneficial to use in these patients. In this study, therefore, we assessed the effects of *Anethum graveolens* powder on postprandial risk hyperlipidemia and some other factors leading to atherosclerosis in rabbits, fed a high cholesterol diet.

METHODS

Plant preparation

A. graveolens was purchased from the local market in Shahrekord, Iran. The genus and species were authenticated by a botanist in the Medical Plants Research Centre of Shahrekord University of Medical Sciences and a voucher specimen was deposited in the institution herbarium (number 236). The plants were dried, powdered and anthocyanins, flavonoids and phenolic contents were measured for standardization as follows.

Measurement of total anthocyanins

A total of anthocyanins were assayed by spectrophotometeric method at 535 nm.[21] A total of 3 g of the sample was weighed in a 50-mL centrifuge tube and 24 mL of acidified ethanol (ethanol and HCl 1.0 N, 85:15, v/v) was added. The solution was mixed and adjusted to pH 1 with 4 N HCl. The resulting solution was shaken for 15 min, readjusted to pH 1 if necessary and the solution was shaken for an additional 15 min. The tube was centrifuged for 15 min, at 27,200 × g and the supernatant was poured into a 50-mL volumetric flask and made up to volume with acidified ethanol. Cyanidin 3-glucoside was used as a standard pigment. A series of cyanidin 3-glucoside standard solutions was prepared at 0-0.02 mmol (0-27 mg/3 mL). Absorbance was read at 535 nm against a reagent blank.

Measurement of total flavonoids

The amount of flavonoid components in the *Anethum* extract was evaluated by colorimetric method.[22] Thus, 0.5 mL of rutin (standard flavonoid compound) or garlic extract was added to a flask containing 1.5 mL of methanol, 2.8 mL of distilled water, 0.1 mL of 1 M potassium acetate, 0.1 mL of 10% aluminum chloride and left at room temperature for 30 min. The reaction absorbance was measured at 415 nm after preparing a standard curve from rutin solutions at concentrations of 25-500 ppm in methanol. The experiment was repeated in triplicate. The total flavonoid components were expressed in terms of rutin equivalents (in mg/g).

Measurement of total phenolic compounds

The amount of total phenolic components in the *Anethum* extract was determined colorimetrically using the Folin-Ciocalteu reagent.[23,24] in brief, 5 mL of gallic acid (standard phenolic compound) or *Anethum* extract was mixed with Folin-Ciocalteu reagent diluted with distilled water (1:10) and aqueous Na_2CO_3 (4 mL, 1 M). The mixture was left to stand for 15 min and the total phenolic compounds were determined by colorimetry at 765 nm. A standard curve was prepared from different concentrations (0-250 mg/L) of gallic acid in methanol: water (50:50, vol/vol). The experiment was repeated in triplicate and the total phenolic values were expressed in in mg/g of gallic acid equivalent.

Treatment of rabbits

This study was approved by the ethics committee of Shahrekord University of Medical Sciences. 32 male white New Zealand rabbits weighing 2010 ± 275 g were obtained from Razi Vaccine and Serum Research Institute in Iran. They were kept in an air-conditioned room for 2 weeks and allowed water and standard rabbit chow containing 16% protein, 4-5% vegetable fat, 13% fiber, 1.4% calcium, 0.8% phosphor, 0.5% cysteine and 0.5% tryptophan.[25]

The rabbits were randomized into four groups of eight and fed a diet as follows: group I: normal diet, Group II: diet containing 1% cholesterol, Group III: a high cholesterol diet containing 5% dill powder and Group IV: high cholesterol (1%) plus lovastatin (10 mg/kg bw).

After fasting for 12-15 h, venous blood samples were taken to obtain baseline data. Venous blood samples were also obtained 3 h after the diet to determine the acute effects of *A. graveolens*.[10]

Measurement of biochemical factors in rabbits

Serum and plasma samples were collected by centrifuging blood samples at 3500 rpm for 20 min. Fibrinogen and factor VII values were obtained through the plasma and the serum was used for other biomarker measurements.

Serum TC, LDL-C, apolipoprotein B (ApoB), alanine aminotransferase (alt), aspartate aminotransferase (ast) and serum glucose were determined using standard enzymatic kits (Pars Azmoon Co, Iran) and an auto-analyzer (Hitachi 902, Japan). Factor VII was measured using clotting time, in the presence of the STA-Neoplastine reagent of a system in which all factors were present, constant and in excess except factor VII, which is derived from the sample being tested (Diagnostic Stago, French). The serum levels of nitrite and nitrate were measured using a colorimetric assay kit (R and D Systems, USA), which involves the Griess reaction.

Statistical analysis

Results were analyzed using Instat 3 software. To investigate the biochemical results and comparison of the experimental groups, Kruskal-Wallis and Dunn tests were used and $P < 0.05$ was considered statistically significant.

RESULTS

Determination of some physiochemical factors in *A. graveolens*

Analyzing *A. graveolens* factors showed that the amounts of total anthocyanins, flavonoids and phenolic compounds in the extract were 94 mg/100 g, 62 mg/100 g (equivalent to gallic acid) and 95 mg/100 g (equivalent to gallic acid), respectively.

Measurement biochemical factors in rabbit

Consumption of *A. graveolens* powder or lovastatin significantly decreased ALT, TC, glucose, fibrinogen and LDL-C values in comparison with hypercholesterolemic diet group [Table 1]. Concurrent use of *A. graveolens* or lovastatine did not change factor VII, ApoB, nitrite

Table 1: Comparison of LDL-C, factor VII, ApoB, TC, AST, ALT, glucose, nitrite, nitrate and fibrinogen values at the end of experiment

Biochemical factors	Groups			
	Cholesterolemic diet	Lovastatin	*Anethum graveolens*	Normal diet
LDL-C (mg/dl)	39.31±3.20	15.81±2.50*	15.10±2.16*	24.31±1.26*
TC (mg/dl)	91±3.37	65.43±3.21*	67.25±2.76*	56.63±0.68*
ALT (mg/dl)	40±1.34	36.43±2.25*	32.25±1.33*	26.63±0.50*
AST (mg/dl)	43.22±2.63	35.86±1.03	32.22±1.42*	29.75±0.53*
Glucose (mg/dl)	132±3.57	113.71±4.96*	103.13±4.26*	51.25±3.12*
Factor VII	298.1±5.7	292.5±2.5	293.8±1.8	295.7±2.5
Nitrite (µmol/l)	249.3±10.4	214.1±30	252±13.7	250.4±10
Nitrate (µmol/l)	430±36.7	324.6±44.8	439±39.7	305.6±108.8
Fibrinogen	251±4.6	221.3±3.2*	220.3±2.9*	216.6±2.6*
ApoB (mg/dl)	30.78±1.02	34.43±1.09	32.86±2.07	27.88±0.88

Mean total cholesterol, ApoB100=Apolipoprotein B100, nitrite, nitrate, factor VII, glucose, LDL=Low-density lipoprotein, fibrinogen, alt=Alanine aminotransferase, ast=Aspartate aminotransferase, SEM=Standard error of mean, in each group (n=8 for each experimental group) *P<0.05, comparison between cholesterolemic diet group and each of other three groups (*Anethum graveolens*, lovastatin and normal diet). Results are expressed as mean±SEM

and nitrate levels significantly in comparison with hypercholesterolemic diet group. Intake of *A. graveolens* significantly decreased serum AST compared with hypercholesterolemic diet [Table 1].

DISCUSSION

The association between postprandial triglyceride and atherosclerosis has been confirmed by various studies.[10,26] Evidence suggests that postprandial lipoproteins induce the expression and release of endothelial mediators *in vitro* and that this metabolic syndrome is associated with humeral risk markers of endothelial origin.[27-30]

Recently, the importance of therapeutic plants has become the focus of many studies. Vegetables, fruits and their juices contain phenolic compounds, which have antioxidant properties and are protective against atherosclerosis.[31,32]

In a study, the hypolipidemic effects of powdered dill and its special oil (which is the main constituent of this plant) was investigated in male rats fed a high cholesterol diet. The results of this study showed that adding powdered dill to rat's diet for 2 weeks significantly reduced TC, triglyceride and LDL-C and also significantly increased HDL-C levels.[33] In the present study, we assessed the effects of *A. graveolens* powder on postprandial risk factors leading to atherosclerosis in rabbits fed a high cholesterol diet. In this study, lovastatin was used to compare the effects of standard drug with dill. Statins reduce the cholesterol content from lipoporoteins by their hypercholestric effects and also deplete the amount of oxidable compounds.[32]

Lovastatin attaches to phospholipids on LDL surface and therefore prevents the diffusion of free radicals under oxidative pressure to the core of lipoproteins.[34]

By significantly reducing LDL-C, cholesterol, glucose, ALT, AST values in *A. graveolens* seems to have the potential to protect against coronary artery disease. It decreases LDL-C and cholesterol levels by either upregulating lipoprotein lipase, which hydrolyses triglycerides or reducing the hepatic VLDL production or by reducing the activity of two enzymes involved in cholesterol metabolism (3-hydroxy-3 methyl glutaryl coenzyme A reductase and/or acyl cholesterol acyl transferase). Acyl cholesterol acyl transferase catalyses the intracellular esterification of cholesterol and plays a role in cholesterol absorption and hepatic secretion of VLDL and ApoB.[35]

The glucose reducing effects of powdered dill can be due to the following:[36,37]

- The presence of flavonoids, which inhibits glucose absorption in the intestine
- Flavonoids block the enzyme glucose 6 phosphatase and therefore reduce blood glucose levels. This enzyme mediates separation of phosphate from phosphorylated glucose and therefore releases glucose into the blood stream.

Nitric oxide (NO) is the most important vasodilator derived from the endothelium. NO produced by endothelial nitric oxide synthase (eNOS) has a significant role in the initiation of coronary vessel hemostasis, whereas NO produced by inducible nitric oxide synthase (iNOS) causes CVD. iNOS is an enzyme produced by various cells and mediated by cytokines in inflammatory conditions.[38-44] Anthocyanins reduce iNOS expression and increase the expression of eNOS and thus play a role in the equilibrium between iNOS and eNOS in different pathologic systems.[45] In the present study, its blood level was not changed, perhaps due to shortage of the experiment.

Studies indicate the role of vegetarian diets in the hemostasis of coagulation and fibrinolysis. These compounds are effective in reducing coagulation factors, increasing fibrinolysis and decreasing blood coagulation by reducing fibrinogen, inhibiting platelet aggregation and increasing prothrombin time.[46] In the present study consumption of *A. graveolens* powder, similar to lovastatin, significantly decreased fibrinogen compared with hypercholesterolemic diet group.

The results of this study indicate that consumption of *A. graveolens* can decreases the negative effects of postprandial cholesterol rich diet. Considering the price and the availability of *A. graveolens,* this plant seems to be an effective choice for preventing some of the risk factors of atherosclerosis.

CONCLUSIONS

A. graveolens decreases the postprandial atherosclerosis risk factors and might be beneficial in hyperchlesterolemic patients. Considering the price and availability of *A. graveolens,* this plant seems to be an effective choice for hyperlipidemia, especially for reducing postprandial risk factors of atherosclerosis. However, the mechanism of action and the structures of the active ingredients should be established. These are our focus for future studies. Further studies are also need to be performed to determine similar effects of *A. graveolens* in human.

ACKNOWLEDGMENTS

This work was supported by the Research Fund of Shahrekord University of Medical Sciences, Shahrekord, Iran. This paper has been derived from a MD thesis.

REFERENCES

1. Ross R. Atherosclerosis – An inflammatory disease. N Engl J Med 1999;340:115-26.
2. Ross R. The pathogenesis of atherosclerosis: A perspective for the 1990s. Nature 1993;362:801-9.
3. Rodondi N, Auer R, de Bosset Sulzer V, Ghali WA, Cornuz J. Atherosclerosis screening by noninvasive imaging for cardiovascular prevention: A systematic review. J Gen Intern Med 2012;27:220-31.
4. Mitchell RS, Kumar V, Abbas AK, Fausto N. Robbins Basic Pathology: With Student Consult Online Access. 8th ed. Philadelphia: Saunders; 2007. p. 345.
5. Baradaran A. Lipoprotein (a), type 2 diabetes and nephropathy; the mystery continues. J Nephropathology 2012;1:126-9.
6. Kari J. Epidemiology of chronic kidney disease in children. J Nephropathology 2012;1:162-3.
7. Bae JH, Bassenge E, Kim KB, Kim YN, Kim KS, Lee HJ, *et al*. Postprandial hypertriglyceridemia impairs endothelial function by enhanced oxidant stress. Atherosclerosis 2001;155:517-23.
8. Rahimi Z. ACE insertion/deletion (I/D) polymorphism and diabetic nephropathy. J Nephropathology 2012;1:143-51.
9. Setorki M, Nazari B, Asgary A, Azadbakht L, Rafieian-Kopaei M. Anti atherosclerotic effects of verjuice on hypocholesterolemic rabbits. Afr J Pharm Pharmacol 2011;5:1038-45.
10. Asgari S, Setorki M, Rafieian-Kopaei M, Heidarian E, Shahinfard N, Ansari R, *et al*. Postprandial hypolipidemic and hypoglycemic effects of *Allium hertifolium* and *Sesamum indicum* on hypercholesterolemic rabbits. Afr J Pharm Pharmacol 2012;6:1131-5.
11. Solati M, Mahboobi HR. Paraoxonase enzyme activity and dyslipidemia in chronic kidney disease patients. J Nephropathology 2012;1:123-5.
12. Anderson RA, Evans ML, Ellis GR, Graham J, Morris K, Jackson SK, *et al*. The relationships between post-prandial lipaemia, endothelial function and oxidative stress in healthy individuals and patients with type 2 diabetes. Atherosclerosis 2001;154:475-83.
13. Asgary S, Rafieian-Kopaei M, Adelnia A, Kazemi S, Shamsi F. Comparing the effects of lovastatin and cornus MAS fruit on fibrinogen level in hypercholesterolemic rabbits. ARYA Atheroscler 2010;6:1-5.
14. Khosravi-Boroujeni H, Mohammadifard N, Sarrafzadegan N, Sajjadi F, Maghroun M, Khosravi A, *et al*. Potato consumption and cardiovascular disease risk factors among Iranian population. Int J Food Sci Nutr 2012;63:913-20.
15. Rafieian-Kopaei M, Asgary S, Adelnia A, Setorki M, Khazaei M, Kazemi S. The effects of cornelian cherry on atherosclerosis and atherogenic factors

in hypercholesterolemic rabbits. J Med Plants Res 2011;5:2670-6.
16. Kazemi S, Asgary S, Moshtaghian J, Rafieian M, Adelnia A, Shamsi F. Liver-protective effects of hydroalcoholic extract of allium hirtifolium boiss. In rats with alloxan-induced diabetes mellitus. ARYA Atheroscler 2010;6:11-5.
17. Hollman PC, Hertog MG, Katan MB. Role of dietary flavonoids in protection against cancer and coronary heart disease. Biochem Soc Trans 1996;24:785-9.
18. de Whalley CV, Rankin SM, Hoult JR, Jessup W, Leake DS. Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. Biochem Pharmacol 1990;39:1743-50.
19. Evans WC, Trease GE. Trease and Evans' Pharmacognosy. 14th ed. London: WB Saunders Company; 1996. p. 264-5.
20. Madani H, Ahmadi M, Abadi N, Vahdati A. Effect of hydroalcoholic extract of *Anethum graveolens* L. on glucose and lipids in diabetic rats. Diabet Lipid 2006;???:109-16.
21. Abdel-Aal ES, Hucl P. A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheats. Cereal Chem 1999;76:350-4.
22. Shirzad H, Taji F, Rafieian-Kopaei M. Correlation between antioxidant activity of garlic extracts and WEHI-164 fibrosarcoma tumor growth in BALB/c mice. J Med Food 2011;14:969-74.
23. Sharafati R, Sherafati F, Rafieian-Kopaei M. Biological characterization of Iranian walnut (*Juglans regia*) leaves. Turk J Biol 2011;35:635-9.
24. Bahmani M, Rafieian-Kopaei M, Parsaei P, Mohsenzadegan A. The anti-leech effect of *Peganum harmala* L. extract and some anti-parasite drugs on *Limnatis nilotica*. Afr J Microbiol Res 2012;6:2586-90.
25. Akhlaghi M, Shabanian G, Rafieian-Kopaei M, Parvin N, Saadat M, Akhlaghi M. Citrus aurantium blossom and preoperative anxiety. Rev Bras Anestesiol 2011;61:702-12.
26. Amini FG, Rafeian-Kopaei M, Nematbakhsh M, Baradaran A, Nasri H. Ameliorative effects of metformin on renal histologic and biochemical alterations of gentamicin-induced renal toxicity in Wistar rats. J Res Med Sci 2012;17:621-5.
27. Jagla A, Schrezenmeir J. Postprandial triglycerides and endothelial function. Exp Clin Endocrinol Diabetes 2001;109:S533-47.
28. Gheissari A, Hemmatzadeh S, Merrikhi A, Fadaei Tehrani S, Madihi Y. Chronic kidney disease in children: A report from a tertiary care center over 11 years. J Nephropathology 2012;1:177-82.
29. Shahni N, Gupta KL. Dietary antioxidents and oxidative stress in predialysis chronic kidney patients. J Nephropathology 2012;1:134-42.
30. Assadi F. The epidemic of pediatric chronic kidney disease: The danger of skepticism. J Nephropathology 2012;1:61-4.
31. Khajehdehi P. Turmeric: Reemerging of a neglected Asian traditional remedy. J Nephropathology 2012;1:17-22.
32. Jafarzadeh L, Rafieian-Kopaei M, Ansari Samani R, Asgari A. The effect of hydroalcoholic extract of *Stachys lavandulifolia* Vahl on pregnant mice. EXCLI J 2012;11:357-62.
33. Hajhashemi V, Abbasi N. Hypolipidemic activity of *Anethum graveolens* in rats. Phytother Res 2008;22:372-5.
34. Aviram M, Hussein O, Rosenblat M, Schlezinger S, Hayek T, Keidar S. Interactions of platelets, macrophages, and lipoproteins in hypercholesterolemia: Antiatherogenic effects of HMG-CoA reductase inhibitor therapy. J Cardiovasc Pharmacol 1998;31:39-45.
35. Hoffman R, Brook GJ, Aviram M. Hypolipidemic drugs reduce lipoprotein susceptibility to undergo lipid peroxidation: *In vitro* and *ex vivo* studies. Atherosclerosis 1992;93:105-13.
36. Armulik A. Splice variants of human beta 1 integrins: Origin, biosynthesis and functions. Front Biosci 2002;7:d219-27.
37. Neuschwander-Tetri BA, Brunt EM, Wehmeier KR, Oliver D, Bacon BR. Improved nonalcoholic steatohepatitis after 48 weeks of treatment with the PPAR-gamma ligand rosiglitazone. Hepatology 2003;38:1008-17.
38. Gheissari A, Mehrasa P, Merrikhi A, Madihi Y. Acute kidney injury: A pediatric experience over 10 years at a tertiary care center. J Nephropathology 2012;1:101-8.
39. Kam-Tao Li PK, Burdmann EA, Mehta RL. Acute kidney injury: Global health alert. J Nephropathology 2013;2:90-7.
40. Nematbakhsh M, Ashrafi F, Pezeshki Z, Fatahi Z, Kianpoor F, Sanei MH, *et al*. A histopathological study of nephrotoxicity, hepatoxicity or testicular toxicity: Which one is the first observation as side effect of cisplatin-induced toxicity in animal model. J Nephropathology 2012;1:190-3.
41. Rouhi H, Ganji F. Effect of N-acetyl cysteine on serum lipoprotein (a) and proteinuria in type 2 diabetic patients. J Nephropathology 2013;2:61-6.
42. Tolouian R, Hernandez GT. Prediction of diabetic nephropathy: The need for a sweet biomarker. J Nephropathology 2013;2:4-5.
43. Tavafi M. Diabetic nephropathy and antioxidants. J Nephropathology 2013;2:20-7.
44. Triggle CR, Samuel SM, Ravishankar S, Marei I,

Arunachalam G, Ding H. The endothelium: Influencing vascular smooth muscle in many ways. Can J Physiol Pharmacol 2012;90:713-38.
45. Okopień B, Krysiak R, Madej A, Belowski D, Zieliński M, Kowalski J, *et al*. Effect of simvastatin and fluvastatin on plasma fibrinogen levels in patients with primary hypercholesterolemia. Pol J Pharmacol 2004;56:781-7.
46. Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L. Dietary polyphenols and the prevention of diseases. Crit Rev Food Sci Nutr 2005;45:287-306.

Source of Support: This work was supported by the Research Fund of Shahrekord University of Medical Sciences, Shahrekord, Iran, **Conflict of Interest:** None declared.