Int J Endocrinol Metab. 2016 January; 14(1):e34726. doi: 10.5812/ijem.34726.

Published online 2016 January 30. **Research Article**

Chemical Composition and the Effect of Walnut Hydrosol on Glycemic Control of Patients With Type 1 Diabetes

Hossein Moravej,[1,*] Alireza Salehi,[2] Zahra Razavi,[3] Mahmood Reza Moein,[4] Hamed Etemadfard,[4] Forough Karami,[5] and Forough Ghahremani[6]

[1]Department of Pediatrics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, IR Iran
[2]Research Center for Traditional Medicine and History of Medicine, Shiraz University of Medical Sciences, Shiraz, IR Iran
[3]School of Medicine, Shiraz University of Medical Sciences, Shiraz, IR Iran
[4]Department of Pharmacognosy, Medicinal Plants Processing Research Centre, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, IR Iran
[5]Central Research Laboratory, School of Medicine, Shiraz University of Medical Sciences, Shiraz, IR Iran
[6]Bushehr University of Medical Sciences, Bushehr, IR Iran

[*]*Corresponding author*: Hossein Moravej, Department of Pediatrics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, IR Iran. Tel: +98-9171058371, Fax: +98-7136474298, E-mail: hmoravej@sums.ac.ir

Received 2015 November 21; **Revised** 2016 January 03; **Accepted** 2016 January 11.

Abstract

Background: Walnut hydrosol (WH) is used extensively by Iranian people with diabetes in order to control blood sugar (BS). There are few data regarding the effect of walnut on controlling diabetes.
Objectives: A pilot study to determine the efficacy and safety of WH in patients with type 1 diabetes.
Materials and Methods: Eight patients with diabetes mellitus (DM) type 1 were enrolled in the study. They did not use any medicine except insulin. They were advised to drink 250 mL WH after meals twice a day for four weeks. Their BS level was measured and their insulin dose was changed according to their BS. After four weeks, they discontinued WH use and their BS level was checked for two weeks. Descriptive statistics was used to analyze the data. Also, the essential oil of the sample was extracted using a liquid extractor and then analysis of the constituents was performed.
Results: The average daily BS level and insulin dose decreased in seven subjects. Two subjects developed generalized pruritic erythematous skin rash. One patient presented hypoglycemic coma. She had no other coma risk factor. Seven compounds were identified in the walnut essential oil and the rate of monoterpenoid and sesquiterpenes hydrocarbons were 53.45% and 5.95%, respectively. The main constituents of the oil were carvacrol (33.21%), thymol (16%) and homoveratrole (15.83%).
Conclusions: WH may control the glycemic level in people with diabetes, but it may be associated with minor and major side effects. Further in vitro studies, using these seven compounds, are recommended to determine the efficacy and complications of WH in people with diabetes.

Keywords: Diabetes Mellitus, Walnut, Alternative Medicine, Complementary Medicine, *Juglans regia* L.

1. Background

Walnut tree (*Juglans regia* L.), belongs to family *Juglandaceae* (1). Walnut contains up to 62% - 68% oil, containing a high amount of monounsaturated and polyunsaturated fatty acids (2). Walnut leaves are considered as a source of healthcare compounds and are intensely used in traditional medicine to treat venous insufficiency and haemorrhoidal symptoms; it is also used for its antidiarrheic, antihelminthic, depurative and astringent properties (3-5).

This valuable tree has a long history of medicinal use to treat a wide range of health complaints. Almost all parts of the plant are medicinally important. Dry seeds (nuts) are very popular and largely consumed as royal food in Iran. In addition, green walnuts, shells, bark, green husks (epicarps), and leaves are used in the cosmetic and pharmaceutical industries (6). The stem bark is reported to be alterative, anthelmintic, astringent, bactericidal, depurative, digestive, diuretic, laxative, detergent, stimulant, tonic and insecticidal (7).

Walnut oil is a component of dry skin creams, antiwrinkle and anti-aging products, because it presents moisturizing properties as well as free radical scavenging capacity (8).

In an experimental study, treatment of *J. regia* extracts in the experimental animal samples resulted in a significant decrease in blood glucose, glycosylated hemoglobin, low-density lipoprotein (LDL), triglyceride and total cholesterol and a significant increase in insulin and high-density lipoprotein (HDL) level (9).

Diabetes mellitus (DM) is a major public health problem. DM as well as its fatal complications is an important cause of death all over the world (10). For a very long time,

plants have played an important role in the treatment of many chronic diseases, including DM (11). Use of plants to treat DM is common in Iranian population too. World health organization (WHO) has recommended the evaluation of effective plants for diseases such as diabetes, for which there are few safe modern drugs.

Iranian people with diabetes extensively use the extract of walnut and its hydrosol to control their blood sugar (BS). Some of these patients are satisfied with this herbal product and tolerate it well. However, there are few data about its effectiveness and safety (12, 13). Therefore, more studies are required to determine the effects and side effects of walnut on diabetes.

The only study on the effect of walnut extract on glycemic control in patients with type 2 diabetes showed a significant increase of insulin level and decrease of glycosylated hemoglobin (13). According to the literature research, no such study is conducted on patients with type 1 diabetes.

2. Objectives

For this reason, authors conducted a pilot study on the efficacy and safety of walnut extract (WLE) in patients with type 1 diabetes.

3. Materials and Methods

3.1. Plant Material and Extraction of Essential Oils

Walnut hydrosol was obtained from a local market in Meymand, Fars province, Iran. The essential oil of the sample was extracted using a liquid extractor in two stages. At first, 500 mL of the sample was mixed with 500 mL of petroleum-ether as a solvent. The solvent was heated to 45°C for 150 minutes and the essential oil was transformed from an aqueous phase to a petroleum-ether phase. The remains of the organic phase from the first stage were removed and additional amount (500 mL) of fresh petroleum-ether was added to the system. Similar to the first stage, it was heated for 150 minutes. This stage was carried out to increase the yield of the essential oil in the organic phase.

3.2. Essential Oil Concentration

The extracted essential oil was concentrated by rotary evaporator equipped with a vacuum pump. In this process, 500 mL of petroleum-ether containing the essential oil was heated to 40°C and vacuumed by rotation of a pump motor at 60 rpm, evaporating the petroleum-ether and leaving the extract. This process produced approximately 50 mL of the sample dissolved in a small amount of organic phase.

3.3. Gas Chromatography-Mass Spectrometry

The concentrated essential oil was dehydrated and subjected to gas chromatography mass spectrometry (GC/MS) for analysis of the constituents (Agilent Technologies 7890 gas chromatograph coupled with an Agilent Technologies model 5975C mass detector, Palo Alto, CA) (14). The apparatus was equipped with a HP-5ms capillary column [phenylmethylsiloxane, length 30 m $\times$ inner diameter 0.25 mm, Agilent technologies (60 - 325/350°C)]. The oven temperature increased from 60°C (0 minute) to 220°C in increments of 5°C/minute and held at that temperature for 10 minutes. Helium was selected as the carrier gas and the flow rate was adjusted at a rate of 1 mL/minute. The mass spectrometer operated in EI mode at 70 eV. The interface temperature was 280°C and the mass range was 30 - 600 m/z. Retention indices were determined by using retention time of n-alkanes injected after the essential oil under the same chromatographic conditions. The components of the oil were identified by calculation of Kovat's indices (KI) and comparison of their mass spectra with those of Wiley library or the published ones (15, 16).

3.4. Patients

As a pilot study, eight patients with type 1 DM were enrolled in the study. Inclusion criteria were type 1diabetes, age over 12 years old, completed puberty, having diabetes for more than two years and use of insulin analogs including Lantus SoloSTAR® Pen and NovoRapid® FlexPen to control diabetes. Subjects who used medicines other than insulin, those afflicted with other associated diseases and those who used insulin types other than Lantus SoloSTAR® Pen and NovoRapid® FlexPen were excluded from the study. Written informed consent letters were taken from all subjects. They were advised to measure their BS level at least four times daily by glucometer and record their BS level and insulin doses for at least two weeks. Then, the patients were advised to drink 250 mL WLE after meals twice a day. They continued to measure and record their BS level and insulin doses. During the study, the subjects used just insulin as anti-diabetic medication.

The subjects were called frequently to evaluate compliance and any complications and also to adjust the dosage of insulin injection. After four weeks, the use of WLE was stopped, subjects were examined by a physician, and the data including BS levels and injected insulin doses were collected. The subjects continued measuring and recording their BS levels and insulin doses for two more weeks. At that time, the subjects were examined again and the data were collected. Insulin doses and BS levels were compared for each patient before and after consuming WLE.

4. Results

The constituents of the essential oil of *Juglans regia* L. are presented in Table 1. Several compounds were identified in the oil and the rate of monoterpenoid and sesquiterpenoid compounds were 53.45% and 5.95%, respectively; also, the rate of phenolic compound was 51.34%. The main constituents of the oil were carvacrol (33.21%), thymol (16%), and homoveratrole (15.83%).

Table 1. The Components of *Juglans regia* L. Essential Oil[a]

Components	Content, %	KI (HP-5)	Identification
Homoveratrole	15.83	1239	MS, KI
Piperitone	2.11	1257	MS, KI
Thymol	16	1291	MS, KI
Carvacrol	33.21	1301	MS, KI
Eugenol	2.13	1359	MS, KI
γ-eudesmol	5.95	1630	MS, KI
Nonadecane	3.12	1899	MS, KI
Total identified	78.35	-	-
Monoterpene hydrocarbons	53.45	-	-
Sesquiterpene hydrocarbons phenolic compounds	5.95 51.34	-	-

Abbreviations: KI, Kovat's indices; MS, mass spectroscopy.
[a]The compounds were sorted according to retention indices on HP-5mscapillary column.

All the eight subjects completed the study with no further dropout. Two of them were male and six were females with the age range of 13 - 25 years, and the mean age of 19.7 years. Table 2 shows the effect of WLE on average daily BS level and insulin dose of each subject. Five subjects had a decrease in both insulin dose and average daily BS level. Two other subjects showed a decrease only in average daily BS level, but because their BS level was in a favorite range, they did not decrease the insulin doses. In one other subject, the decrease in the average daily BS level was only 5 mg/dL, which was insignificant.

Five of the subjects tolerated the extract very well without any complications. However, a few days after WLE consumption, two subjects called one of the authors and complained about development of generalized pruritic erythematous rashes. The rashes were mild and non-progressive in one of them; therefore, she continued WLE consumption, but the other subject stopped using WLE after two weeks due to progressive and troublesome skin rashes. One subject had severe hypoglycemia leading to coma after three weeks of using WLE. The subject followed her usual program of eating, insulin injection and physical activity. She did not have any illness before the attack of hypoglycemia, and overall, there was no other risk factor for hypoglycemia for her. Therefore, hypoglycemia was probably the consequence of WLE consumption. The level of consciousness was improved in the subject after a few hours and she was discharged from hospital without any side effects.

Two weeks after stopping WLE use, insulin doses and average BS level of all subjects increased again to nearly the level before using WLE.

5. Discussion

Juglans regia belongs to the family Juglandaceae. It includes three species: *J. nigra*, *J. cinerea* and *J. regia* although only *J. regia* grows in Iran (17). The key chemical composition of walnut is Juglone (5-hydroxy-1, 4-naphthoquinone), the toxic compound which is found only in green and fresh walnuts, but such property disappears in dried leaves (18). Several other phenolic compounds with antioxidant properties are identified in *J. regia* leaves (19).

Walnut (*Juglans regia* L.) is a plant with a significant economic value and medicinal importance for human health. It is consumed in large quantities by people; therefore, it has a very important place in the public nutritive habits (20). Leaves of *J. regia* are widely used in folk medicine to treat venous insufficiency and haemorrhoidal symptoms, for their antidiarrheic, antihelmintic, depurative, and astringent properties; mix of leaves and stored-grains are also used as fungicide and insecticide (21, 22). Other properties such as antibacterial, human cancer cell antiproliferative, antioxidant, keratolytic, antifungal, hypoglycemic, hypotensive, anti-scrofulous and sedative are also reported for this plant (19, 23-25). The beneficial effect of walnut consumption against many diseases is reported, including protection from diabetes (26) or cardiovascular diseases (27). Researches have also shown that eating walnuts can improve the blood lipid profile (28). Other investigations showed that *J. regia* extract contains ellagitannins, which is an anti-cancer agent with anti-inflammatory effects (17).

There are several reports regarding the analysis of walnut tree from different places. Examples are phytochemical analysis of the leaf volatile oil of walnut tree (29), studying about protein fractionations, amino acid composition, molecular weight distribution and gel electrophoresis of *Juglans regia* L. (30), surveys on antioxidant and antibacterial activities of the leaf essential oil of *Juglans regia* L. and its constituents (31), determination of mineral contents of *Juglans regia* L. flowers and its anti-hemolytic activity. Also, another study reported that *J. regia* improves

Table 2. Efficacy and Side Effects of Walnut Extract in Patients With Type 1 Diabetes

Case No.	Average Daily BS		Average Daily Insulin Dose		Duration of Therapy, w	Side Effects
	Before Use	During Use	Before Use	During Use		
1	164	140	42	37	4	None
2	160	148	38	34	4	None
3	154	136	32	27	4	Skin rash
4	172	150	45	41	4	None
5	190	170	36	36	4	None
6	183	178	40	40	4	None
7	161	130	44	36	3	Hypoglycemic coma
8	208	194	24	24	2	Skin rash

Abbreviation: BS, blood sugar.

glycemic level in the patients with type 2 diabetes, without any adverse effects on the kidney and hepatic function (14).

In another study (29), the volatile oils of all 28 *J. regia* populations were analyzed by gas chromatography flame ionization detectors (GC/FID) and gas chromatography mass spectrometry (GC/MS). Major components of the essential oils in this research were (E)-caryophyllene (1.4% - 47.9%), β-pinene (4.5% - 39.5%), germacrene-D (5.0% - 23.3%), α-pinene (1.5% - 18.1%), α-humulene (1.1% - 11.8%), α-zingiberene (0.1% - 11.3%), α-copaene (0.0% - 10.1%), limonene (0.8% - 8.6%), caryophyllene oxide (0.1% - 8.6%), ar-curcumene (0.0 - 7.2%), δ-cadinene (0.3% - 6.7%), (E)-β-farnesene (0.0% - 5.9%), 1, 8-cineole (< 0.0 - 5.4%), γ-curcumene (0.0 - 4.2%), methyl salicylate (0.1% - 4.0%), (E)-myrtanol acetate (0.0 - 3.8%), (E)-muurola-3, 5-diene (0.0 - 3.8%), (E)-β-ocimene (0.6% - 3.9%), α-longipinene (0.0 - 3.0%), myrcene (0.2% - 2.6%), α-muurolene (0.1% - 2.5%), spathulenol (0.0 - 2.2%) and α-cadinol (0.1% - 2.0%) (29).

A single report on volatile compounds (head space analysis) of *J. regia* leaves from Egypt showed the presence of germacrene-D (28.6%), and methyl salicylate (16.8%) as the main constituents (32). β-pinene (30.5%), β-caryophyllene (15.5%), α-pinene (15.1%), germacrene-D (14.4%) and limonene (3.6%) were identified as the principal components of the essential oil of walnut leaves from Kashmir (31). In another research, the essential oil was obtained by headspace method and the volatile compounds were pentanal (0.07 - 0.12%), hexanal (0.26 - 0.80%), nonanal (0.34 - 0.89%) and 2-decenal (0.25 - 0.68%) and hexanol (0.21 - 1.58%) (33). This research indicated that the percentage of aldehyde compound is higher than that of alcoholic compound.

A difference was found between the current study data and those of other reports. These differences can be due to ecological factors or species variations.

According to the current study searches in database, this is the first pilot study to determine the effect of WH on glycemic control in subjects with type 1 diabetes. Previously, only one study was conducted on the effect of walnut extract on patients with type 2 diabetes and several similar studies on animals with diabetes. WLE caused improvement of glycemic control both in rats with diabetes and patients with type 2 diabetes (14, 34).

In the present study, glycemic level controlled in most of the subjects with type 1 diabetes. The mechanism of glucose lowering effect of walnut is not clear. Some in vitro studies are performed to find the mechanisms. Most of these studies are conducted on type 2 diabetes. They concluded that walnut may cause increased release of insulin from beta cells, increased insulin sensitivity, or may interfere with absorption of dietary carbohydrate (34, 35). The pathophysiology of T1DM is completely different from that of type 2 diabetes. In T1DM, most of beta cells are destroyed; therefore, many agents that can help patients with type 2 diabetes are not efficacious in T1DM.

The key compounds responsible for anti-hyperglycemic effect of WLE may be phenolic compounds. Phenolic acids and flavonoids are two major groups of phenolic compounds existing in walnut leaves. As it was mentioned, seven components were found in WH. According to the current study investigation on these components, two of them with anti-hyperglycemic effect have been mentioned in other studies. Therefore, carvacrol or thymol or both of them may have an anti-hyperglycemia role but further studies are needed to investigate the cases. In an animal study, carvacrol in combination with rosiglitazone caused improvement in blood glucose and glycosylated hemoglobin high fat diet induced type 2 diabetes in mice (36). Another animal study revealed the anti-hyperglycemic effect of methanol extract of Otoste-

gia persica Boiss (Labiatae) on rats with type 1 diabetes. Phytochemical analysis revealed thymol as the major component of the extract. More extensive studies are needed to determine the exact mechanism of anti-hyperglycemic effect of WLE.

Walnut extract was not associated with any complications in animals and patients with type 2 diabetes (14, 34), but its side effects were important among the current study subjects. Two of the subjects showed drug reactions as skin rashes, and one of them developed severe hypoglycemia. Drug reaction at least partially may be due to technical problems in providing the extract leading to an impure extract. Therefore, in leading studies, this process should be performed with more caution to prevent adding impurities. Hypoglycemia was a serious complication in one of the subjects. No other etiology was found for hypoglycemia in this subject and, therefore, hypoglycemia was probably the result of WH consumption. This complication can be an important limiting factor for extended use of the extract in human.

5.1. Conclusions

WH may control glycemic level in human, but it can be associated with minor and major side effects. Seven compounds were identified in walnut oil. According to the current study search, among these components two of them (thymol and carvacrol) may have an anti-hyperglycemia effect mentioned in other studies. Therefore, WH should not be advised extensively to people with diabetes until the knowledge is increased about its mechanism of action and potential side effects. Further studies are needed.

The most important limitation of this study was the low number of participants. According to the severe side effects in one of the subjects, it is not suggested to perform this study on larger population. However, new in vitro studies are recommended to determine the mechanism of WH effect on blood sugar in subjects with type 1 diabetes mellitus.

Acknowledgments

The authors would like to thank Shiraz University of Medical Sciences and also the clinical research development center of Nemazee hospital, Shiraz, Iran, and Nasrin Shokrpour for editorial assistance.

Footnote

Authors' Contribution: Study concept and design: Hossein Moravej and Mahmood Reza Moein; acquisition of data: Hossein Moravej, Zahra Razavi, Mahmood Reza Moein, Hamed Etemadfard, Forough Karami, and Forough Ghahremani; analysis and interpretation of data: Hossein Moravej; drafting of the manuscript: Hossein Moravej, and Zahra Razavi; critical revision of the manuscript for important intellectual content: Hossein Moravej; administrative, technical, and material support and study supervision: Hossein Moravej, and Alireza Salehi; final revision: Alireza Salehi, and Mahmood Reza Moein.

References

1. Tsamouris G, Hatziantoniou S, Demetzos C. Lipid analysis of Greek walnut oil (Juglans regia L.). *Z Naturforsch C.* 2002;**57**(1-2):51–6. [PubMed: 11926543].
2. Ros E, Nunez I, Perez-Heras A, Serra M, Gilabert R, Casals E, et al. A walnut diet improves endothelial function in hypercholesterolemic subjects: a randomized crossover trial. *Circulation.* 2004;**109**(13):1609–14. doi: 10.1161/01.CIR.0000124477.91474.FF. [PubMed: 15037535].
3. Van Hellemont J. Compendium de phytotherapie. Bruxelles: Association Pharmaceutique Belge; 1986. pp. 214–6.
4. Wichtl M, Anton R. Plantes Therapeutiques. 2 ed. Paris: TEC and DOC; 1999.
5. Oliveira I, Sousa A, Ferreira IC, Bento A, Estevinho L, Pereira JA. Total phenols, antioxidant potential and antimicrobial activity of walnut (Juglans regia L.) green husks. *Food Chem Toxicol.* 2008;**46**(7):2326–31. doi: 10.1016/j.fct.2008.03.017. [PubMed: 18448225].
6. Chopra RN, Varma BS, Chopra IC. Supplement to glossary of Indian medicinal plants. Publications and Information Directorate-CSIR; 1986.
7. Espin JC, Soler-Rivas C, Wichers HJ. Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-diphenyl-1-picrylhydrazyl radical. *J Agric Food Chem.* 2000;**48**(3):648–56. [PubMed: 10725129].
8. Mohammadi J, Saadipour K, Delaviz H, Mohammadi B. Anti-diabetic effects of an alcoholic extract of Juglans regia in an animal model. *Turk J Med Sci.* 2011;**41**:685–91.
9. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract.* 2010;**87**(1):4–14. doi: 10.1016/j.diabres.2009.10.007. [PubMed: 19896746].
10. Afifi-Yazar FU, Kasabri V, Abu-Dahab R. Medicinal plants from Jordan in the treatment of diabetes: traditional uses vs. in vitro and in vivo evaluations–part 2. *Planta Med.* 2011;**77**(11):1210–20. doi: 10.1055/s-0031-1279983. [PubMed: 21674438].
11. Hasani-Ranjbar S, Larijani B, Abdollahi M. A systematic review of Iranian medicinal plants useful in diabetes mellitus. *Arch Med Sci.* 2008;**4**(3):285–92.
12. Mohammadi J, Delaviz H, Malekzadeh JM, Roozbehi A. The effect of hydro alcoholic extract of Juglans regia leaves in streptozotocin-nicotinamide induced diabetic rats. *Pak J Pharm Sci.* 2012;**2**(25):407–11.
13. Hosseini S, Huseini HF, Larijani B, Mohammad K, Najmizadeh A, Nourijelyani K, et al. The hypoglycemic effect of Juglans regia leaves aqueous extract in diabetic patients: A first human trial. *Daru.* 2014;**22**(1):19. doi: 10.1186/2008-2231-22-19. [PubMed: 24447826].
14. Massada Y. Analysis of essential oil by gas chromatography and spectrometry. New York: John Wiley and Sons Inc; 1976. pp. 118–20.
15. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. Carol Stream, Illinois: Allured Publishing Co; 1995. p. 415.
16. Mozafarian V. Walnut tree. Identification of medicinal and aromatic plants of Iran. Tehran, Iran: Farhang Moaser press; 2012. pp. 473–5.

17. Amaral JS, Seabra RM, Andrade PB, Valentao P, Pereira JA, Ferreres F. Phenolic profile in the quality control of walnut (Juglans regia L.) leaves. *Food Chem Toxicol.* 2004;**88**(3):373–9.
18. Pereira JA, Oliveira I, Sousa A, Valentao P, Andrade PB, Ferreira IC, et al. Walnut (Juglans regia L.) leaves: phenolic compounds, antibacterial activity and antioxidant potential of different cultivars. *Food Chem Toxicol.* 2007;**45**(11):2287–95.
19. Caglarirmak N. Biochemical and physical properties of some walnut genotypes (Juglans regia, L.). *Food Nahrung.* 2003;**47**(1):28–32.
20. Cosmulescu S, Trandafir I. Seasonal variation of total phenols in leaves of walnut (Juglans regia L.). *J Med Plants Res.* 2011;**5**(19):4938–42.
21. Negi KS, Kanwal KS. Plants used as fish toxins in Garhwal region of Uttarakhand Himalaya. *Indian J Tradit Know.* 2009;**8**(4):535–8.
22. Carvalho M, Ferreira PJ, Mendes VS, Silva R, Pereira JA, Jeronimo C, et al. Human cancer cell antiproliferative and antioxidant activities of Juglans regia L. *Food Chem Toxicol.* 2010;**48**(1):441–7.
23. Girzu M, Carnat A, Privat AM, Fialip J, Carnat AP, Lamaison JL. Sedative effect of walnut leaf extract and juglone, an isolated constituent. *Pharm biol.* 1998;**36**(4):280–6.
24. Valnet J. Phytotherapie, traitement des maladies par les plantes [in French]. 6 ed. Paris: Maloine; 1992. pp. 476–8.
25. Kendall CWC, Esfahani A, Josse AR, Augustin LSA, Vidgen E, Jenkins DJA. The glycemic effect of nut-enriched meals in healthy and diabetic subjects. *Nutr Metab Cardiovas J.* 2011;**21**:S34–9.
26. Banel DK, Hu FB. Effects of walnut consumption on blood lipids and other cardiovascular risk factors: a meta-analysis and systematic review. *Am J Clin Nutr.* 2009;**90**(1):56–63.
27. Mukuddem-Petersen J, Oosthuizen W, Jerling JC. A systematic review of the effects of nuts on blood lipid profiles in humans. *J Nutr.* 2005;**135**(9):2082–9.
28. Verma RS, Padalia RC, Chauhan A, Thul ST. Phytochemical analysis of the leaf volatile oil of walnut tree (Juglans regia L.) from western Himalaya. *Ind Crops Prod.* 2013;**42**:195–201.
29. Rather MA, Dar BA, Dar MY, Wani BA, Shah WA, Bhat BA, et al. Chemical composition, antioxidant and antibacterial activities of the leaf essential oil of Juglans regia L. and its constituents. *Phytomedicine.* 2012;**19**(13):1185–90. doi: 10.1016/j.phymed.2012.07.018. [PubMed: 22951389].
30. Mao X, Hua Y, Chen G. Amino Acid Composition, Molecular Weight Distribution and Gel Electrophoresis of Walnut (Juglans regia L.) Proteins and Protein Fractionations. *Int J Mol Sci.* 2014;**15**(2):2003–14.
31. Farag MA. Headspace analysis of volatile compounds in leaves from the Juglandaceae (walnut) family. *J Essent Oil Res.* 2008;**20**(4):323–7.
32. Abdallah IB, Tlili N, Martinez-Force E, Rubio AGP, Perez-Camino MC, Albouchi A, et al. Content of carotenoids, tocopherols, sterols, triterpenic and aliphatic alcohols, and volatile compounds in six walnuts (Juglans regia L.) varieties. *J Food Chem.* 2015;**173**:972–8.
33. Jelodar G, Mohsen M, Shahram S. Effect of walnut leaf, coriander and pomegranate on blood glucose and histopathology of pancreas of alloxan induced diabetic rats. *Afr J Tradit Complement Altern Med.* 2008;**4**(3):299–305.
34. Pitschmann A, Zehl M, Atanasov AG, Dirsch VM, Heiss E, Glasl S. Walnut leaf extract inhibits PTP1B and enhances glucose-uptake in vitro. *J Ethnopharm.* 2014;**152**(3):599–602.
35. Ezhumalai M, Radhiga T, Pugalendi KV. Antihyperglycemic effect of carvacrol in combination with rosiglitazone in high-fat diet-induced type 2 diabetic C57BL/6J mice. *Mol Cell Biochem.* 2014(385):23–31.
36. Manzari-Tavakoli A, Pouraboli I, Yaghoobi MM, Mehrabani M, Mirtadzadini SM. Antihyperglycemic, antilipidperoxidation, and insulin secretory activities of Otostegiapersica shoot extract in streptozotocin-induced diabetic rats and in vitro C187 pancreatic beta-cells. *A Pharm Biol.* 2013;**2**(51):253–9.