E-only Article

Antidiabetic and Antihyperlipidemic Effects of Ethanol Extract of *Rosa canina* L. fruit on Diabetic Rats: An Experimental Study With Histopathological Evaluations

Journal of Evidence-Based Complementary & Alternative Medicine
2016, Vol. 21(4) NP25-NP30

DOI: 10.1177/2156587215612626
cam.sagepub.com
SAGE

Mohsen Taghizadeh, PhD[1], Ali Akbar Rashidi, MsC[2], Ali Akbar Taherian, PhD[1], Zarichehr Vakili, PhD[1], Mohammad Sajad Sajadian, MD[1], and Mostafa Ghardashi, MD[1]

Abstract

Rosa canina L. (Rosaceae) has been traditionally used as a medicinal plant. This study was undertaken to evaluate the antidiabetic and antihyperlipidemic effects of *Rosa canina* fruit extract in streptozotocin induced diabetic rats. The results showed oral administration of *Rosa canina* fruit extract significantly ameliorated the high levels of blood glucose compared with the control group. Serum triglyceride levels significantly decreased by the administration of *Rosa canina* extract compared with control. Histopathological examinations showed that the *Rosa canina* extract improved islets necrotic and regenerated pancreatic islet cells. *Rosa canina* extract has the antihyperglycemic and antihyperlipidemic effects in streptozotocin-induced diabetic rats.

Keywords

diabetes mellitus, hyperglycemia, medicinal plants, lipids

Received June 29, 2015. Received revised September 21, 2015. Accepted for publication September 27, 2015.

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.[1] Diabetese-related chronic hyperglycemia is associated with long-term damage, dysfunction, and failure of different organs, especially in eyes, kidneys, nerves, heart, and blood vessels.[2] According to the World Health Organization, the population of diabetes is likely to increase to 300 million or more by the year 2025.[3] Approximately about 2 million of Iranian adults (7.7%) aged between 25 and 64 years have developed diabetes mellitus and about 4.4 million (16.8%) of diabetes-related Iranian people have impaired fasting glucose. Nowadays, there is an increased global interest in traditional medicine, and remedies could be defined as potential medicaments for treating of different ailments especially diabetes mellitus.[4] Although, more than 400 plants in traditional medicine have been reported to be effective in diabetes treatment, only a few plants have been scientifically evaluated.[5]

Rosa canina L. (Rosaceae family) as a prickly shrub with white flowers and bright red hips has been distributed in some parts of the world. *Rosa canina* (*Nastaran* or *Nasrin* in Persian) has a long history of use in traditional Persian medicine. For example, according to the *Canon of Medicine* written by Avicenna (*Ibn-e-Sina*) (AD 980-1037).[6] It can be used for headache and some neural and gastrointestinal diseases.[7] Also, Aghili in *The Storehouse of Medicaments* (written in AD 1772)[8] has recommended *Nasrin* as a tonic for heart and brain, and for treatment of some hepatic disorders.[9]

Rosa canina fruit with common name of "rose hip" has been used as diuretic, laxative, antigout and antirheumatism agent in traditional medicine.[10]

During World War II, rose hips were widely used for preventing scurvy because of their vitamin C content.[11] Rose hips have several active components such as phenolic acids, proanthocyanidins, tannins, flavonoids, unsaturated and polyunsaturated fatty acids, phospholipids, minerals, galactolipids, and carotenoids.[10,12] Some pharmacological activities of rose hips, including the anti-inflammatory and antioxidant properties have been confirmed.[13-15] It has been shown that the administration of rose hips with seeds may reduce plasma triglyceride and free fatty acids.[16]

[1] Kashan University of Medical Sciences, Kashan, I.R.Iran
[2] Fasa University of Medical Sciences, Fasa, Iran

Corresponding Author:
Ali Akbar Rashidi, Non-Communicable Disease Research Center, Fasa University of Medical Sciences, Ebne Sina Square, PO Box 7461686688, Fasa, Iran.
Email: rashidi_al@yahoo.com

Literature surveys showed a few studies on the effects of *Rosa canina* on glycemic and lipid profile levels.[17] Therefore, the objective of this study was to evaluate the antihyperglycemic and antihyperlipidemic activities of *Rosa canina* fruit extracts in streptozotocin-induced diabetic rats.

Materials and Methods

Preparation of Extract

Rosa canina fruits were purchased from a local herb store in Kashan, Iran and powdered by a grinder. A voucher specimen (No. R116) of the flower was identified and authenticated by the taxonomist at the Barij Research Institute, Kashan, Iran.

Two kilograms of this powder was mixed with 5 L ethanol + water (70:30) mixture and was placed on a shaker for 48 hours. Then, the supernatant fluid of mixture was separated and filtered and placed in a sterile container.[15] The extract was concentrated under reduced pressure in a rotary evaporator at 30°C to 40°C. Then, it was stored until the analysis.

Determination of Total Phenolic Content

The total phenolic content in extract was determined using the Folin-Ciocalteu reagent and gallic acid was used as standard. Calibration line was $y = 0.0017x - 0.0016$ and $R^2 = 0.9995$. The sample (25 µL) and 1.25 mL of sodium carbonate (75 g/L) were added to 2.5 mL of Folin-Ciocalteu reagent (10% v/v). After 1.5 hours of incubation at room temperature, the absorbance was measured at 765 nm by spectrophotometer. The results were given as gallic acid equivalent per 100 g of extract.

Animals

Male Wistar albino rats aged 2 to 3 months wiith a body weight ranging from 180 to 200 g, were purchased from Central Animal House, Physiology Research Center, Kashan University of Medical Sciences, Iran and were maintained in an air conditioning room (25°C ± 1°C) with a 12-hour light/12-hour dark cycle. Food and water was provided ad libitum. All the animal experiments were approved by the Animal Research Ethics Board at Kashan University of Medical Sciences (No. 9144).

Induction of Diabetes

Diabetes was induced in male Wistar albino rats by intraperitoneal administration of streptozotocin (single dose of 55 mg/kg body weight). Streptozotocin was dissolved in freshly prepared 0.01 M citrate buffer, pH 4.5.[18] In order to prevent the initial drug-induced hypoglycemic mortality, 20% glucose solution was given to streptozotocin-injected animals for 24 hours. Seventy-two hours after the streptozotocin injection, the overnight fasting blood glucose levels were taken from tail arteries of rats. Animals with fasting blood glucose levels higher than 250 mg/dL were selected as diabetic rats. All the animals were allowed free access to water and pellet diet and were maintained at room temperature (25°C ± 1°C).

Experimental Design

Rats were divided into 5 groups (n = 10 in each group). Diabetes was induced in all groups except the normal control group.

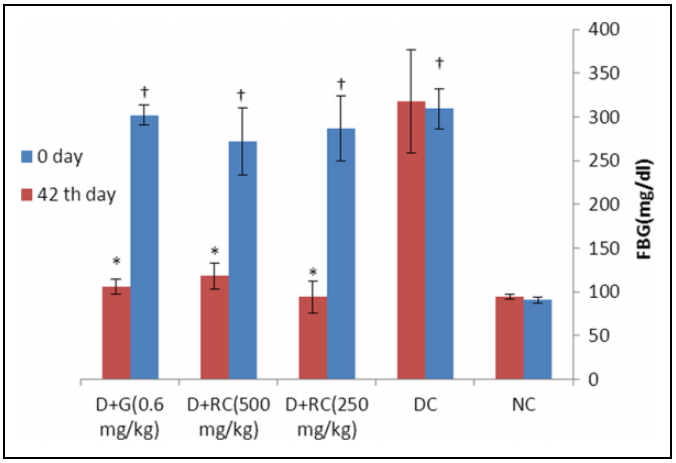

Figure 1. Fasting blood glucose levels in different animal groups during the experimental period. NC, normal control; DC, diabetic control; RC 250 and 500, *Rosa canina* at dose 250 and 500 mg/kg body weight; G, glibenclamide. Values are means ± standard error of the mean. *$P < .05$. **$P < .01$ compared with DC group. †$P < .001$ compared with NC group.

Group I: Normal nondiabetic control
Group II: Diabetic control
Group III: Diabetic rats + *Rosa canina* extract (250 mg/kg body weight/d)
Group IV: Diabetic rats + *Rosa canina* extract (500 mg/kg body weight/d)
Group V: Diabetic rats + glibenclamide (600 µg/kg body weight/d).

Normal and diabetic controls rats were fed with distilled water, whereas *Rosa canina* extract or glibenclamide was administered orally to treated rats once a day for 6 weeks.

Biochemical Analysis

Fasting blood glucose levels and body weights were measured on the days 0 and 42. At the end of the experimental period, rats were fasted for 12 hours with free access to drinking water and then were sacrificed by anesthetic ether and further cervical dislocation. Blood samples were collected into sterile plastic tubes and allowed to clot at room temperature for 20 minutes, and were then centrifuged for 15 minutes and the platelet-free unhemolyzed serums were collected and were stored at −30°C for later measurements. Pancreas and liver were immediately removed and placed in labeled beakers in 10% formaldehyde solution. Stored serum samples were analyzed for glucose levels by glucose oxidase-peroxidase method. Cholesterol was measured by enzymatic colorimetric method. The amount of triglycerides was determined by colorimetric method (enzymatic hydrolysis of triglycerides). All the biochemical analyses were performed using commercial kits (Parsazmun Company, Iran).

Histopathological Examinations

The pancreases were excised, fixed in 10% formaldehyde solution and processed in an Auto-Technicon machine and paraffin blocks were prepared. Microscopic sections (5 µm) were stained with hematoxylin and eosin and examined under a light microscope with a computer-connected camera.

Table 1. Effect of Ethanol Extract of *Rosa canina* on the Lipid Profile Levels in Streptozotocin-Induced Diabetic Rats.[a]

Groups	TG (mg/dL)	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
NC	83.37 ± 10.19	69.77 ± 2.79	23.11 ± 1.18	28.00 ± 3.70
DC	90.11 ± 12.49	66.22 ± 2.42	24.55 ± 1.44	23.64 ± 2.19
D + RC (250 mg/kg)	59.08 ± 8.83[b]	65.00 ± 5.43	25.83 ± 1.76	27.35 ± 3.18
D + RC (500 mg/kg)	69.37 ± 9.67	70.25 ± 4.34	27.12 ± 1.63	29.25 ± 1.79
D + G (0.6 mg/kg)	86 ± 7.58	66.75 ± 3.01	23.81 ± 1.36	26.37 ± 2.42

Abbreviations: DC, diabetic control; D + G, diabetic + glibenclamide; D + RC, diabetic+ *Rosa canina*; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NC, normal control; TG, triglycerides.
[a]Values are means ± standard errors of the mean.
[b]$P = .041$.

Statistical Analysis

The results were expressed as mean ± standard error of mean. The statistical significance was estimated by 1-way analysis of variance followed by Tukey's post hoc test. *P* values less than .05 were considered as significant.

Results

The Total Phenolic Content of Rosa canina *Extract*

Total phenolic content of extract was determined as gallic acid equivalent using Folin-Ciocalteau reagent. Total phenolic content was 5.9% ± 0.4% gallic acid equivalent/100 g fraction.

The Effect of Rosa canina *Extract on the Level of Fasting Blood Glucose*

The effect of *Rosa canina* extracts on the fasting blood glucose levels in streptozotocin-induced diabetic rats is summarized in Figure 1. Fasting blood glucose levels were significantly elevated after the induction of diabetes in comparison with normal rats ($P < .001$). Administration of *Rosa canina* extract significantly decreased fasting blood glucose levels at the dose of 250 mg/kg body weight (94 ± 18.19 vs 317.71 ± 59.28 mg/dL) and at the dose of 500 mg/kg body weight (117.87 ± 15.41 vs 317.71 ± 59.28 mg/dL) in comparison with control ($P < .001$ for both).

Effect of Rosa canina *Extract on Serum Lipid Profile*

The effect of *Rosa canina* extract on the serum lipid profile in streptozotocin-induced diabetic rats has been reported in Table 1.

The levels of serum triglycerides significantly decreased by the administration of *Rosa canina* extract at a dose of 250 mg/kg body weight (59.08 ± 8.83 vs 90.11 ± 12.49 mg/dL) compared with diabetic control group ($P = .041$). Serum cholesterol, low-density lipoprotein, and high-density lipoprotein levels did not change significantly in treated rats compared with control group.

Histopathological Findings

Microscopic examinations of the pancreas sections showed the normal histology of the pancreas in normal nondiabetic rats of control group (Figure 2). The islets of Langerhans in diabetic rats showed sever necrotic changes. In the necrotic islets, changing in nucleus of cell, disappearing of nucleus, karyolysis were seen and in some places residue of destructed cells were visible (Figure 2).The improving effect of *Rosa canina* extract (250 mg/kg body weight) on diabetic rats was clearly obvious and the pancreas histology of the extract treated diabetic rats did not show a significant difference compared with the normal nondiabetic rats (Figure 2). Under the light microscope, most extract treated rats (250 mg/kg body weight) showed many small islets of Langerhans with normal histology. Although, the higher dose of extract (500 mg/kg) also could reverse the diabetic effects of streptozotocin (Figure 2), but the slides related to low dose (250 mg/kg body weight) of extract had more normal cells than that of high dose (500 mg/kg) of extract (Figure 3).

Discussion

Diabetes mellitus as chronic metabolic disorder is characterized by chronic hyperglycemia as a result of deficiency in insulin secretion or insulin resistance.[19] This study evaluated the antihyperglycemic and antihyperlipidemic effect of *Rosa canina* fruit extract in streptozotocin-induced diabetic rats.

The results of our study showed the reducing effects of oral administration of *Rosa canina* extract on fasting blood glucose levels significantly, and also the low dose of *Rosa canina* extract had more effect than the high doses. On the basis of our knowledge, this is the first study that has confirmed the reducing effect of *Rosa canina* ethanol extract on fasting blood glucose levels in diabetic rats. Can et al[17] demonstrated that the aqueous and ethanol extracts of *Rosa canina* fruits had no hypoglycemic activity in normal rabbits. In another study, after intraperitoneally loading of 1 g/kg glucose, oral administration of 10 mg/kg body weight trans-tiliroside, an isolated compound from *Rosa canina* seeds, had hypoglycemic activity in normoglycemic mice.[16] Our results showed that the effect of *Rosa canina* was similar to glibenclamide, a standard hypoglycemic drug. Histopathological examinations of organs showed sever

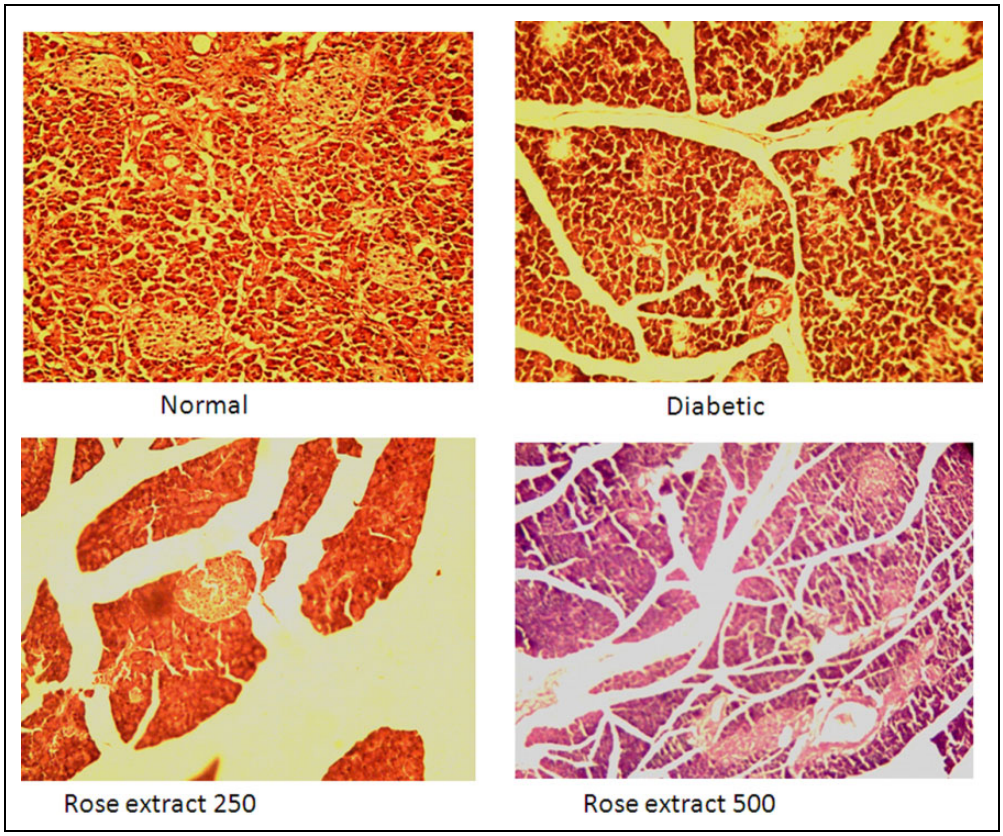

Figure 2. The pancreas microscopic sections of normal (A), diabetic (B), diabetic rats treated with *Rosa canina* extract 250 mg/kg body weight (C), and 500 mg/kg body weight (D) (hematoxylin and eosin, 100×).

necrosis changes in islets of pancreas of streptozotocin-induced diabetic rats, while low dose of *Rosa canina* extract (250 mg/kg body weight) significantly improved the histology of necrotic islets of pancreas and increased the number of islets in comparison with diabetic rats of control group. To date, there is no histopathological study examining the pancreatic structure of *Rosa canina* extract–treated diabetic rats. Therefore, for the first time, this study evaluated the beneficial effects of *Rosa canina* extract on pancreatic cells, histopathologically.

The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia. On the other hand, hypertriglyceridemia is associated with some metabolic disorders such as hypercoagulability, hyperinsulinemia, insulin resistance, and glucose intolerance.[3,20] The results of our study showed that the best decreasing effects of *Rosa canina* extract (250 mg/kg body weight) on serum triglycerides. Ninomiya et al[16] reported that the 14 days oral administration of *Rosa canina* seeds (25 mg/kg/d) significantly reduces the serum triglycerides and frees fatty acids of normal mice. These results suggest that the *Rosa canina* may decrease the complications of diabetes (cardiovascular disease risks) by reducing plasma triglycerides levels. The present study is one of the few studies that demonstrate the beneficial effects of *Rosa canina* extract in diabetic disorders.

streptozotocin, an *N*-nitro derivative of glucosamine, is an antibiotic and cytotoxic agent that has been generally used to induce diabetes in experimental animals. It is selective for pancreatic β-cells that destruct the cells after injection, while it has no effects on the exocrine parts of pancreas. Therefore, streptozotocin induces hyperglycemia.[21,22] Moreover, studies showed that the antioxidant capacity was decreased after administration of streptozotocin in diabetic rats.[23] On the other hand, hyperglycemia generates reactive oxygen species, which in turn play an important role in the production of secondary complications in diabetes mellitus, including cardiovascular disease, liver and kidney failure, blindness, and nerve injury.[24,25]

It is known that *Rosa canina* fruits contain biologically active compounds, some of which such as vitamin C, phenolic acids, and flavonoids have antioxidant properties.[10-15] The biochemical and histopathological findings of this study suggest that the hypoglycemic activity of *Rosa canina* could be due to reduction of oxidative stress, thus preserving pancreatic β-cell integrity leading to insulinotrophic action.

The limitation of our study was some financial restriction that limited us from measuring active components of *Rosa canina* fruit by high-performance liquid chromatography and to investigate on some related enzymes and the expressing of glucose metabolism–related genes in order to determine the mechanism of the *Rosa canina* extract in diabetic disorder.

Thus, the authors suggest further studies on hypoglycemic mechanism of *Rosa canina* extract in animal model and then plan some randomized placebo-controlled clinical trials for

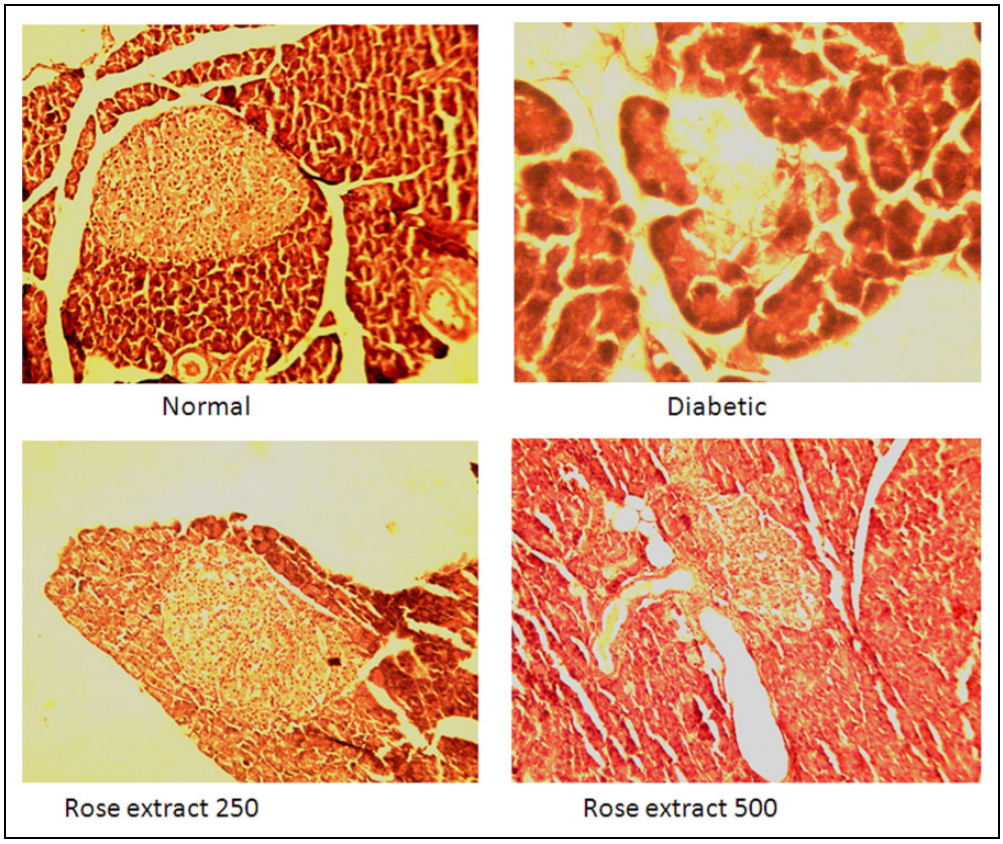

Figure 3. Higher magnification of pancreas microscopic sections of normal (A), diabetic (B), diabetic rats treated with *Rosa canina* extract 250 mg/kg body weight (C) and 500 mg/kg body weight (D) (hematoxylin and eosin, 400×).

evaluation of the effects of *Rosa canina* in treatment of diabetes.

Conclusions

The results of present study and histopathological examinations indicate that the administration of *Rosa canina* extract markedly reduces hyperglycemia and hyperlipidemia in streptozotocin-induced diabetic rats.

Acknowledgments

Authors are thankful to the Research Center of Barij Essence Pharmaceutical Company and Dr Dolati for providing the laboratory space and equipments for the research.

Author Contributions

MT assisted experimental planning and analysis of data. AAR contributed to the experimental design, analysis of data, and preparation of the article. AAT and ZV contributed to the histopathological examinations. SMSS and MG contributed to the animal trials and experiments.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by Vice Chancellor of Research, Kashan University of Medical Sciences, Iran.

Ethical Approval

All the animal experiments were approved by the Animal Research Ethics Board at Kashan University of Medical Sciences (No. 9144).

References

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2010;33(suppl 1):S62-S69.
2. Nabi SA, Kasetti RB, Sirasanagandla S, Tilak TK, Kumar MV, Rao CA. Antidiabetic and antihyperlipidemic activity of *Piper longum* root aqueous extract in STZ induced diabetic rats. *BMC Complement Altern Med*. 2013;13:37.
3. Patel DK, Kumar R, Laloo D, Hemalatha S. Diabetes mellitus: an overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. *Asian Pac J Trop Biomed*. 2012;2:411-420.
4. Hashempur MH, Homayouni K, Ashraf A, Salehi A, Taghizadeh M, Heydari M. Effect of *Linum usitatissimum* L. (linseed) oil on mild and moderate carpal tunnel syndrome: a randomized, double-blind, placebo-controlled clinical trial. *Daru*. 2014;22:43.

5. Rashidi AA, Mirhashemi SM, Taghizadeh M, Sarkhail P. Iranian medicinal plants for diabetes mellitus: a systematic review. *Pak J Biol Sci*. 2013;16:401-411.
6. Mosavat SH, Ghahramani L, Sobhani Z, Haghighi ER, Chaijan MR, Heydari M. The effect of leek (*Allium iranicum* (Wendelbo) Wendelbo) leaves extract cream on hemorrhoids patients: a double blind randomized controlled clinical trial [published online September 1, 2015]. *Eur J Integr Med*. doi:10.1016/j.eujim.2015.08.008.
7. Avicenna. *The Canon of Medicine*. Beirut, Lebanon: al-'Alami Beirut Library Press; 2005.
8. Shoara R, Hashempur MH, Ashraf A, Salehi A, Dehshahri S, Habibagahi Z. Efficacy and safety of topical *Matricaria chamomilla* L. (chamomile) oil for knee osteoarthritis: a randomized controlled clinical trial. *Complement Ther Clin Pract.* 2015;21:181-187.
9. Shirazi Alavi. *Makhzan Al-adviyah (The Storehouse of Medicaments)* [in Persian]. Tehran, Iran: Intisharat va Amoozesh Enghelab Press; 2009.
10. Chrubasik C, Roufogalis BD, Müller-Ladner U, Chrubasik S. A systematic review on the *Rosa canina* effect and efficacy profiles. *Phytother Res*. 2008;22:725-733.
11. Lattanzio F, Greco E, Carretta D, Cervellati R, Govoni P, Speroni E. In vivo anti-inflammatory effect of *Rosa canina* L. extract. *J Ethnopharmacol*. 2011;137:880-885.
12. Montazeri N, Baher E, Mirzajani F, Barami Z, Yousefian S. Phytochemical contents and biological activities of *Rosa canina* fruit from Iran. *J Med Plant Res*. 2011;5:4584-4589.
13. Kharazmi A, Winther K. Rose hip inhibits chemotaxis and chemilumine scence of human peripheral blood neutrophils in vitro and reduces certain inflammatory parameters in vivo. *Inflammopharmacology*. 1999;7:377-386.
14. Larsen E, Kharazmi A, Christensen LP, Christensen SB. An antiinflammatory galactolipid from rose hip (*Rosa canina*) that inhibits chemotaxis of human peripheral blood neutrophils in vitro. *J Nat Prod*. 2003;66:994-995.
15. Deliorman Orhan D, Hartevioğlu A, Küpeli E, Yesilada E. In vivo anti-inflammatory and antinociceptive activity of the crude extract and fractions from *Rosa canina* L. fruits. *J Ehnopharmacol*. 2007;112:394-400.
16. Ninomiya K, Matsuda H, Kubo M, Morikawa T, Nishida N, Yoshikawa M. Potent anti-obese principle from *Rosa canina*: structural requirements and mode of action of transtiliroside. *Bioorg Med Chem Lett*. 2007;17:3059-3064.
17. Can A, Akev N, Sótlópinar N. Effect of rose hips on blood glucose level. *Acta Pharm Turcica*. 1992;34:17-22.
18. Gupta S, Kataria M, Gupta PK, Murganandan S, Yashroy RC. Protective role of extracts of neem seeds in diabetes caused by streptozotocin in rats. *J Ethnopharmacol*. 2004;90:185-189.
19. Heydari M, Homayouni K, Hashempur MH, Shams M. Topical *Citrullus colocynthis* (bitter apple) extract oil in painful diabetic neuropathy: a double-blind randomized placebo-controlled clinical trial [published online March 20, 2015]. *J Diabetes*. doi:10.1111/1753-0407.12287.
20. Sachdewa A, Khemani LD. Effect of *Hibiscus rosa sinensis* L. ethanol flower extract on blood glucose and lipid profile in streptozotocin induced diabetes in rats. *J Ethnopharmacol*. 2003;89:61-66.
21. Mythili MD, Vyas R, Akila G, Gunasekaran S. Effect of streptozotocin on the ultrastructure of rat pancreatic islets. *Microsc Res Tech*. 2004;63:274-281.
22. Yin H, Miao J, Zhang Y. Protective effect of β-casomorphin-7 on type 1 diabetes rats induced with streptozotocin. *Peptides*. 2010;31:1725-1729.
23. Evelson P, Susemihl C, Villarreal I, et al. Hepatic morphological changes and oxidative stress in chronic streptozotocin-diabetic rats. *Ann Hepatol*. 2005;2:115-120.
24. Aslan M, Orhan N, Orhan DD, Ergun F. Hypoglycemic activity and antioxidant potential of some medicinal plants traditionally used in Turkey for diabetes. *J Ethnopharmacol*. 2010;128:384-389.
25. Nyenwe EA, Jerkins TW, Umpierrez GE, Kitabchi AE. Management of type 2 diabetes: evolving strategies for the treatment of patients with type 2 biabetes. *Metabolism*. 2011;60:1-23.