Coronary angiogenesis diabetic rats during consumption of *Petroselinum crispum*

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Background and objectives
Diabetes mellitus is one of the most prevalent metabolic disorders worldwide. Cardiac angiogenesis disruption occurs in diabetes. *Petroselinum crispum* has antioxidant properties leading to its therapeutic attributes.
Materials and methods
Sixty-four male Wistar rats were randomly sorted into eight groups: control, extract groups (50, 100, and 150 mg/kg of *P. crispum*), diabetic, and diabetic+extract groups. Diabetes was induced by intraperitoneal injection of streptozotocin (50 mg/kg), and different doses of extract were administered intraperitoneal for 30 days. The serum level of vascular endothelial growth factor was determined by enzyme-linked immunosorbent assay method; nitrite oxide was measured by Griess assay; and capillary density in the heart was evaluated by immunohistochemistry assay.
Results and conclusion
The values of all parameters were reduced significantly in the diabetic group compared with the control group ($P<0.001$). No significant modifications were observed in all extract groups compared with the control group as well as diabetic +extract groups compared with diabetic rats ($P>0.05$). The hydroalcoholic extract of *P. crispum* did not affect cardiac angiogenesis in normal and diabetic patients, which probably is due to the insufficient amounts of active ingredients, especially flavonoids available in *P. crispum*.

Keywords:
angiogenesis, diabetes mellitus, *Petroselinum crispum*, rat

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Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by increased blood sugar levels due to insulin deficiency or reduced body responsiveness [1]. In chronic states of hyperglycemia, complications such as degeneration of very small veins may influence the various organs like the kidneys and the eyes. Diabetes is also directly associated with an increased risk of cardiovascular disease [2]. Angiogenesis, the development of new blood vessels from the preexisting ones, is essential for the growth and development of body tissues that can be affected by diabetes [3,4]. Diabetes, by disruption in the balance of stimulatory factors and angiogenic inhibitors, can decrease or increase this process in some organs of the body like heart [5]. In fact, diabetes reduces the rate of angiogenesis by alteration in the expression angiogenic genes such as vascular endothelial growth factor (VEGF) [6]. About 80% of the people live in developing countries where they are more willing to use medicinal plants for the treatment of the disease due to the high cost, lack of access, and the complications of synthetic drugs [7]. *Petroselinum crispum* is one of the herbs used in traditional medicine due to its high phenolic content, strong antioxidant effect, and various therapeutics properties such as antidiabetic, anti-inflammatory, antiaging, and sedative [8]. The active ingredients of *P. crispum* are kaempferol, quercetin, geraniol, eugenol, citronella, myrcene, phenylethyl alcohol, nonadecane, eicosane, triclosan, geranyl acetate, and carboxylic acid [9]. *P. crispum* is a biennial plant from the Umbelliferae family, which is widely used in nutritional and pharmacological interventions [10]. Since the antioxidants play a crucial role in the modification of the angiogenesis [11], it seems that the *P. crispum* plant can protect the angiogenesis in diabetic-induced stress. Moreover, a review of the literature indicated that there is no study in the field on the effects of *P. crispum* on angiogenesis in diabetic rats. Thus, this study aimed to determine the effects of hydroalcoholic extract of the *P. crispum* on the angiogenesis process following induction of diabetes in male Wistar rats.

Materials and methods

Chemicals and reagents

P. crispum was purchased from a local herbal store (Kermanshah, Iran). Ethanol, formalin solution, paraffin, and xylol were purchased from the Merck Co. (Merck, Darmstadt, Germany). Streptozotocin (STZ) was purchased from Sigma Co. (Sigma; St. Louis, USA). Sodium chloride 0.9% was obtained from the DarouPakhsh Co. (Tehran, Iran). H_2O_2 (5%) solution was purchased from the Behsaman Co. (Behsaman, Tehran, Iran). CD31 primary antibody, EnVision secondary antibody, phosphate-buffered saline (PBS) solution, Ringer's lactate solution (RS) solution, diaminobenzidine (DAB) solution, and hematoxylin solution were purchased from the Dako Co. (Dako, Copenhagen, Denmark). CK-E30634 enzyme-linked immunosorbent assay (ELISA) kit was purchased from the BioPharm Co. (Leeches; Westbury, UK).

Animals

All animal procedures were conducted in accordance with the guidelines established by the Research Ethics Committee at Kermanshah University of Medical Sciences. Sixty-four male Wistar rats (200–250 g) were purchased from the Pasteur Institute (Tehran, Iran) and were housed in animal homes at 22±2°C under a 12 h light/dark photocycle. Rats had free access to water and standard chow. They were randomly divided into eight groups (eight animals in each): control group, extract groups (100, 150, and 200 mg/kg), diabetic group, and diabetic+extract groups (100, 150, and 200 mg/kg). The extract group (1 g/kg of body weight) was administered daily by intraperitoneal injection for 1 month [12].

Preparation of hydroalcoholic *Petroselinum crispum* extract

The *P. crispum* was purchased from a local store (Kermanshah, Iran, spring 2018). After confirmation by a botanist, the plant was purged, and the impurities were removed. The leaves and stems were dried in shade and then ground to produce the *P. crispum* powder. Then it was dissolved in 98% ethanol solution, and it was extracted using distilled water and ethanol (1 : 1 v/v) as a solvent. It was filtered and concentrated under reduced pressure on a rotary evaporator. It was finally freeze-dried at −80°C. The extract was dissolved in water and prepared fresh for daily experimental use. The hydroalcoholic extract of *P. crispum* was administrated 1 g/kg of body weight daily intraperitoneal for 1 month, by the way, in each injection, 1 ml of the intended volume was prescribed for the extract-treated groups (doses of 100, 150, and 200 mg/kg) [9,10].

Induction of diabetes

Type I diabetes group was induced intraperitoneally by a single dose of STZ (60 mg/kg). The STZ causes partial or total degradation of pancreatic beta cells. At 48 h later, subsequent to the blood sampling from the tail of rats, they were considered as diabetic animals if the blood glucose was greater than 250 mg/dl. To confirm diabetes induction, the blood sugar levels were evaluated twice, before and several days after the STZ injection. The blood sugar was also measured weekly to the end of the experiment [13].

Heart sampling and serum preparation

After anesthesia induction, the rats underwent thoracotomy and the blood samples were gathered directly from the heart. The blood serum isolation was carried out to measure the VEGF. Then, the heart was removed, washed with normal saline, and fixed in formalin solution. The heart tissue underwent tissue processing by an Autotechnicon machine, and they were embedded in paraffin in order to measure the capillary density using the immunohistochemistry technique [14].

Measurement of vascular endothelial growth factor

VEGF concentration in blood serum was measured by ELISA and CK-E30634 kit (BioPharm Co.). After preparation of samples and standards, 40 µl of the rat standard serum sample was added, followed by 10 µl of biotin solution, and 50 µl of Horseradish peroxidase (HRP)-linked polyclonal antibody, which was added to each well of the microplate coated with monoclonal antibodies. The microplate was then placed at 37°C for 60 min. Then they were washed with a washing solution for five times, and the chromogen A and B solutions were added to the wells and incubated at 37°C for 10 min. A measure of 50 µl of Stop solution was added to the wells for color resuscitation. Finally, the optimum density was determined at the 450 nm wavelength using a microplate reader and the concentration of VEGF is expressed in ng/l [14].

Measurement of capillary density

The primary antibody CD31 was used as an indicator of endothelial cells in cardiac capillaries. Paraffin blocks of the heart tissue were sliced into 3 µm sections. The slides were incubated in the oven and then in xylol. The slides were placed in solutions of PBS and RS. After placing in distilled water, the slides were also placed in

H_2O_2 solution (5%), then distilled water and in PBS solutions. An appropriate amount of antibody was placed on the slices while adding the primary antibody. Then the slides were washed. In the next step, the secondary antibody was added to the slices, and they were washed and placed in a PBS buffer. In the next step, the chromogen was placed on the slices and washed in distilled water and PBS solutions. Hematoxylin was added to the slices, and they were washed in the next stage. Ultimately, the slides were dehydrated and placed in xylol, and were prepared for morphology detection. Ten microscopic fields from each tissue preparation were selected, and the capillary density was expressed as the number of endothelial cells per mm^2 by counting the number of endothelial cells [14].

Griess technique

Nitrite oxide was measured by the Griess assay using the microplate technique. Through this process, a mixture of zinc sulfate powder (6 mg) and serum samples (400 µl) was vortexed for 1 min. The samples were centrifuged at 4°C for 10 min at 12 000 rpm, and the supernatant was used to measure the nitrite oxide level. A measure of 50 µl of the sample was added to 100 µl of Griess reagent (Sigma Co.), and the reaction mixture was incubated for about 30 min at room temperature. The optical density of the sample was measured according to the manufacturer protocol by an ELISA reader (Hyperion, Washington, USA) at a wavelength of 540 nm [15].

Statistical analysis

Data were analyzed using statistical package for the social sciences software (version 16.0) (IBM, New York, USA). The unpaired *t* test was used, and one-way analysis of variance was followed by Tukey's post-hoc tests. A *P* value less than 0.05 was considered statistically significant. All data were expressed as mean ±SEM.

Results

Levels of blood glucose

A comparison of mean blood glucose in different groups at the beginning of the experiment and from the first to the fourth week of treatment is shown. The blood glucose levels in diabetic and diabetic+extract groups were significantly higher than the control and extract groups ($P<0.001$). However, there was no significant difference in blood glucose levels in the control group in comparison with the extract groups as well as the diabetic group compared with the diabetic +extract groups ($P>0.05$) (Fig. 1).

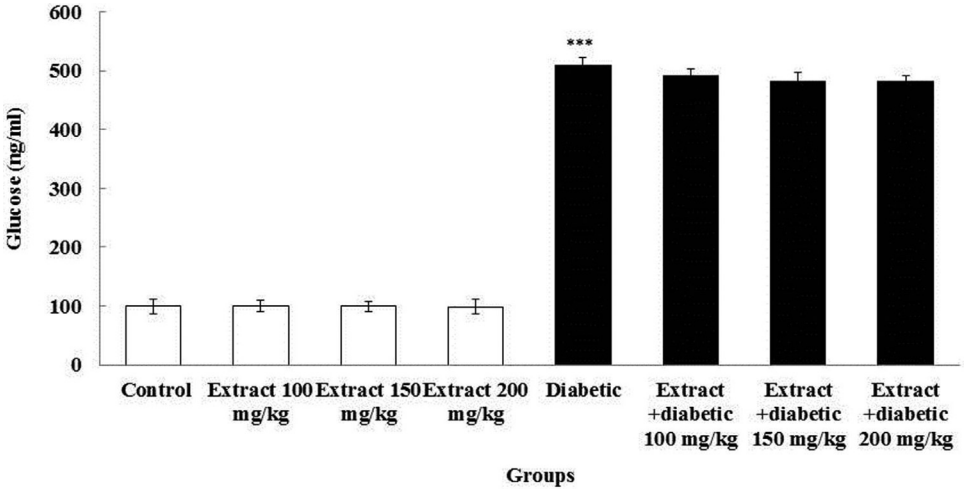
Serum level of vascular endothelial growth factor

Figure 2 shows the mean serum concentrations of VEGF in the control and diabetic groups. The level of serum VEGF in the diabetic group was reduced significantly compared with the control group ($P<0.001$). Administration of the extract at doses of 100, 150, and 200 mg/kg had no significant effect on the level of serum VEGF in the extract groups in comparison to the control group. Also, no significant differences were detected among the diabetic group and the diabetic +extract groups in terms of mean serum levels of VEGF ($P>0.05$) (Fig. 2).

Capillary density in the heart

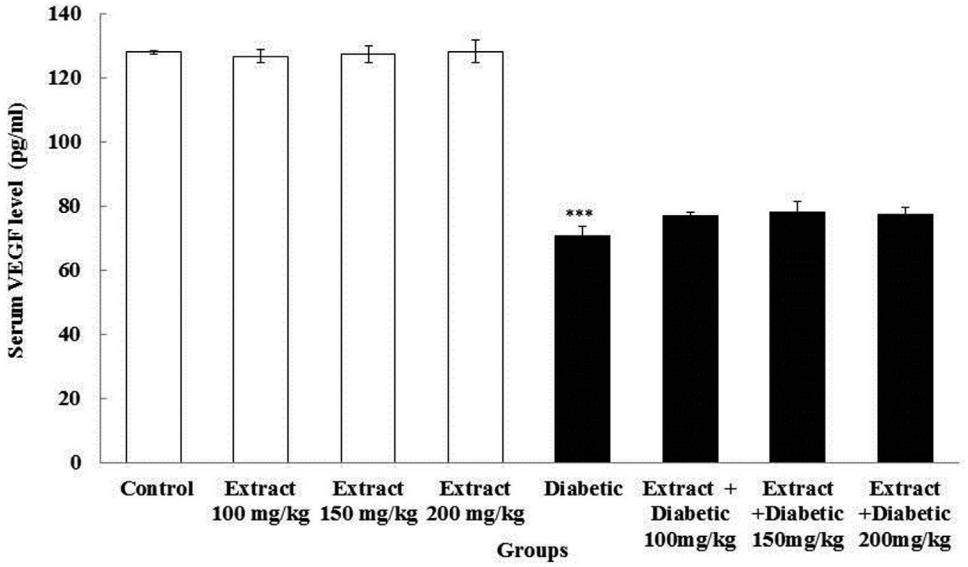
Results showed a significant alteration of capillary density among the diabetic group compared with the

Figure 1



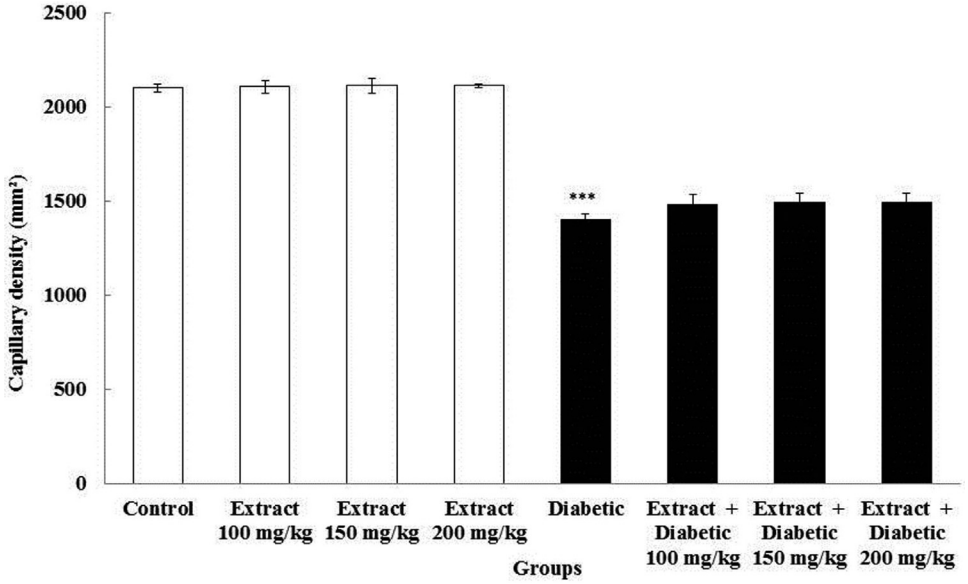
Effects of diabetes and *Petroselinum crispum* on the mean blood glucose levels in rats (n=8 for each group). ***Significant difference in the diabetic group compared with the control group ($P<0.001$).

Figure 2



Mean concentrations of serum VEGF in experimental groups. ***Significant difference in the diabetic group compared with the control group ($P<0.001$). VEGF, vascular endothelial growth factor.

Figure 3



Effect of diabetes and *Petroselinum crispum* on myocardial capillary density. ***Significant difference in the diabetic group compared with the control group ($P<0.001$).

control group ($P<0.001$). Findings showed no significant differences in the field of capillary density between the extract and control groups as well as the diabetic+extract groups compared with diabetic rats ($P>0.05$) (Fig. 3).

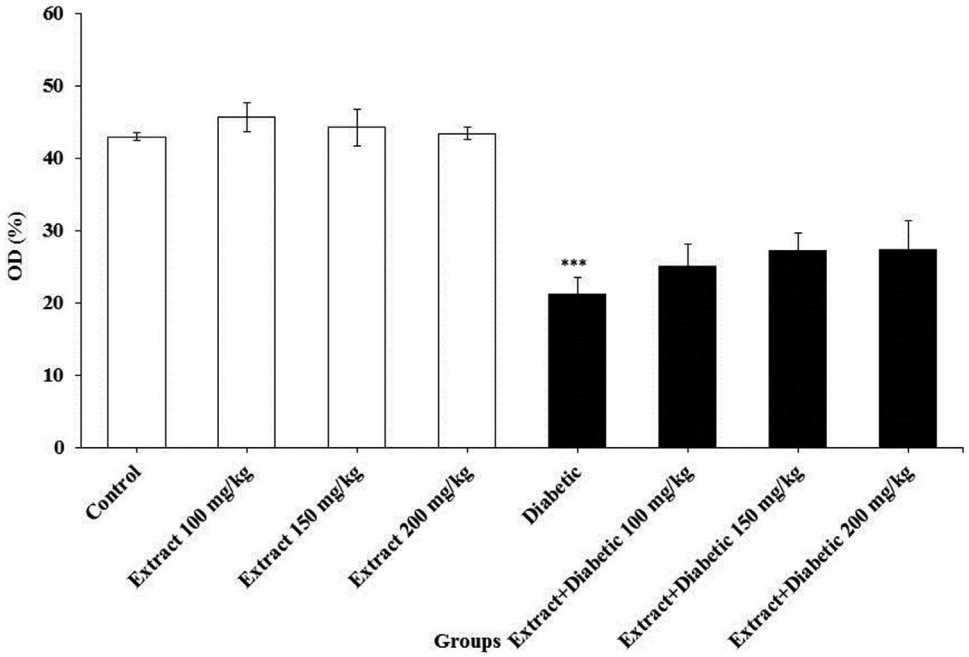
Nitrite oxide

The results of blood serum nitrite oxide analysis showed a significant decrease in the diabetic group compared with the control group ($P<0.001$). The mean nitrite oxide level in the blood serum was not significant in all extract groups compared with the control group ($P>0.05$). Also, the mean of nitrite oxide in blood serum was not significant in diabetic +extract groups in all doses compared with the diabetic group ($P>0.05$) (Fig. 4).

Discussion

In this study, diabetes reduced the cardiac capillary density and serum levels of VEGF and nitrite oxide compared with the control group. Although the *P.*

Figure 4



Effects of *Petroselinum crispum* and diabetes on the mean nitrite oxide levels. ***Significant difference compared with the control group ($P<0.001$).

crispum extract at the doses of 100, 150, and 200 mg/kg increased the cardiac capillary density in diabetic groups receiving the extract, this incremental trend was not statistically significant. Also, the capillary density rate in the extract groups was not significantly different compared with the control group. The findings of this study showed no significant changes in serum VEGF of diabetic +extract group compared with the diabetic group as well as the extract groups compared with the control group, which is consistent with some studies [16]. Hazarika *et al.* [17] have shown that in the absence of ischemia, the VEGF signaling in diabetic mice was lower than the control group, which is in line with the results of this study. The results of Gao and Yu [18] were in agreement with the findings of the present research which found that the capillary density and the rate of revascularization in the limbs of diabetic rats were decreased, with reduced mRNA and protein expression of Endothelial nitric oxide synthase (eNOS), VEGF, and Basic fibroblast growth factor (bFGF). There are various chemical compounds in the *P. crispum*, including terpenes, glycosides, and flavonoids, such as kaempferol and quercetin [19,20]. Studies have shown that the flavonoids reduce the rate of angiogenesis by reduction in the expression of VEGF or inhibition of the hypoxia-induced VEGF [21,22]. Another study showed that quercetin induces antiangiogenesis which was restricted by the inhibition of proliferation and migration of vascular endothelial cells (by reduction in the expression and activity of the matrix metalloproteinase-2 of matrix) [23]. The results of this study also showed that intraperitoneal administration of *P. crispum* hydroalcoholic extract increased the cardiac capillary density in diabetic rats, while it slightly reduced the serum VEGF in the experimental groups compared with the control group in the nonstatistically significant analysis. It has been shown that the VEGF is an important inducer of angiogenesis in the various in-vivo models [24]. The findings of Li *et al.* [25] confirmed the results of this study in that diabetes could decrease the plasma VEGF level. According to previous studies, this consequence returns to VEGF tolerance of diabetes-induced vascular complications [14]. The possible reason for this phenomenon is the type of diabetes (type I) used for animals, which caused more severe complications and disabilities and less tolerance against diabetes. Thus, the extract could not exert its effects to alleviate the complications of diabetes. It should also be noted that the amount of nontoxic intraperitoneal doses used in this study were the same as the dose of the extract to make a significant change in the capillary density due to the presence of sufficient flavonoids, and also the insignificant difference between the experimental groups may be due to the insufficient time of extract consumption [26]. Also, this study has shown that the use of *P. crispum* extract at doses of 100, 150, and 200 mg/kg did not significantly increase the blood glucose levels of the extract groups compared with the control group and had no effects on the

decrease of blood glucose levels in diabetic rats. However, its administration in the diabetic+extract groups at the specified doses reduced the blood glucose level at the end of the experiment (fourth week) which is a nonsignificant result. The researches have shown that the polysaccharides, flavonoids, polypeptides, and alkaloids available in medicinal plants can justify the nature of hypoglycemic and hypolipidemic effects [27,28]. Also, several studies have reported the positive effects of medicinal plants on antioxidant properties [29]. The results of the study by Su *et al.* [30] are consistent with the results of the present study indicating that the administration of flavonoids may increase the glucose uptake by hepatocytes, adipocytes, and muscle cells. A part of the hypoglycemic effects of flavonoids can be attributed to increased activity of hexokinase and glucokinase in the liver, as well as their insulin-like effect which reduces the symptoms of diabetes mellitus [31]. Our results have shown that the serum nitrite oxide level in diabetic rats was lower than the control. Statistical analysis demonstrated that the capillary density in the myocardial tissue was correlated with high serum angiogenic markers. Angiogenesis is controlled by a number of proangiogenic and antiangiogenic factors which are released in tissues. Nitrite oxide and VEGF have crucial roles during angiogenesis [32]. Nitrite oxide not only has a direct effect on angiogenesis, but also affects other angiogenic growth factors by increasing nitrite oxide production [33]. A decrease in nitrite oxide production in endothelial nitrite oxide synthase gene-deficient rats disrupts the development of coronary vessels and angiogenesis [34]. The results of Silva *et al.* [35] were in agreement with the findings of the present research, indicating that serum nitrite oxide concentration in diabetic rats was lower than the concentration in the control group. Roshankhah *et al.* [9] reported that *P. crispum* administration for male Wistar rats attenuated the nitrite oxide level compared with the control group, which did not confirm the results of the current research. The lack of effect of *P. crispum* extract on blood glucose reduction may be due to nontoxic intraperitoneal doses; the amount of flavonol-active ingredients, and the duration of the extract administration. It is also possible that the active ingredients of *P. crispum* have not entered into the blood circulation due to less than effective levels or not being absorbed through the intraperitoneal administration, or they have been metabolized into inactive metabolites in the liver. Another possibility is that the slight reduction in blood glucose is neutralized by the absorbable carbohydrates which are normally found in the *P. crispum* extract. It is also possible that the blood glucose-reducing substances along with their additives exist in *P. crispum* extract to prevent the rise in blood glucose levels in experimental rats.

Conclusion

The hydroalcoholic extract of *P. crispum* at specified doses did not show significant effects on blood glucose level, nitrite oxide, serum capillary density, and VEGF in control and diabetic groups. Thus, according to the results of this study, the type of diabetes, *P. crispum* dose level, the amount of *P. crispum*-active ingredient, and its duration of prescription were considered as the factors leading to inefficiency of hydroalcoholic extract of *P. crispum* in preventing cardiovascular complications caused by diabetes in male Wistar rats.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Karunakaran U, Park K-G. A systematic review of oxidative stress and safety of antioxidants in diabetes: focus on islets and their defense. Diabetes Metab J 2013; 37:106–112.

2 Mehrabzadeh M, Pasalar P, Karimi M, Abdollahi M, Daneshpour M, Asadolahpour E, *et al.* Association between ELMO1 gene polymorphisms and diabetic nephropathy in an Iranian population. J Dia Met Dis 2016; 15:43–51.

3 Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature 2000; 407:249–456.

4 Zarei M, Khazaei M, Sharifi MR, Pourshanazari AA. Coronary angiogenesis during experimental hypertension: is it reversible? J Res Med Sci 2011; 16:269.

5 Martin A, Komada MR, Sane DC. Abnormal angiogenesis in diabetes mellitus. Med Res Rev 2003; 23:117–145.

6 Gan LM, Lagerström-Fermér M, Carlsson LG, Arfvidsson C, Egnell AC, Rudvik A, *et al.* Intradermal delivery of modified mRNA encoding VEGF-A in patients with type 2 diabetes. Nature 2019; 10:871–877.

7 Silva N, Fernandes Júnior A. Biological properties of medicinal plants: a review of their antimicrobial activity. J Venom Anim Toxins Incl Trop Dis 2010; 16:402–413.

8 Roshankhah S, Jalili C, Salahshoor MR. The effects of *Petroselinum crispum* extract on milk production parameters in female rats. SJKU 2019; 24:11–23.

9 Roshankhah S, Jalili C, Salahshoor MR. Protective effects of *Petroselinum crispum* on ischemia/reperfusion-induced acute kidney injury in rats. Physiol Pharmacol 2019; 23:129–139.

10 Jalili C, Salahshoor MR, Naderi T. The effects of hydroalcoholic extract of *Petroselinum crispum* on sperm parameters, testis tissue and serum nitric oxide levels in mice. Adv Biomed Res 2015; 4:40–47.

11 Kim J-D., Liu L, Guo W, Meydani M. Chemical structure of flavonols in relation to modulation of angiogenesis and immune-endothelial cell adhesion. J Nutr Biochem 2006; 17:165–176.

12 Ghorbani R, Mokhtari T, Khazaei M, Salahshoor MR, Jalili C, Bakhtiari M. The effect of walnut on the weight, blood glucose and sex hormones of diabetic male rats. Int J Morphol 2014; 32:58–62.

13 Salahshoor MR, Mohammadi MM, Roshankhah SH, Najari N, Jalili C. Effect of *Falcaria vulgaris* on oxidative damage of liver in diabetic rats. J Diabetes Metab Disord 2019; 18:15–23.

14 Khazaei M, Fallahzadeh AR, Sharifi MR, Afsharmoghaddam N, Javanmard SH, Salehi E. Effects of diabetes on myocardial capillary density and serum angiogenesis biomarkers in male rats. Clinics 2011; 66:1419–1424.

15 Jalili C, Salahshoor MR, Hoseini M, Roshankhah S, Sohrabi M, Shabanizadeh A. Protective effect of thymoquinone against morphine injuries to kidneys of mice. Iran J Kidney Dis 2017; 11:142–149.

16 El-Naggar AR, Zaafar D, Elyamany M, Hassanin S, Bassyouni A, Abdel-Latif H. The role of vildagliptin in treating hypertension through modulating serum VEGF in diabetic hypertensive patients. J Cardiovasc Pharmacol Ther 2019; 24:254–261.

17 Hazarika S, Dokun AO, Li Y, Popel AS, Kontos CD, Annex BH. Impaired angiogenesis after hindlimb ischemia in type 2 diabetes mellitus: differential regulation of vascular endothelial growth factor receptor 1 and soluble vascular endothelial growth factor receptor 1. Circ Res 2007; 101:948–956.

18 Gao L, Yu DM. Molecular mechanism of limbs' postischemic revascularization improved by perindopril in diabetic rats. Chin Med J (Engl) 2008; 121:2129–2133.

19 Wong PY, Kitts DD. Studies on the dual antioxidant and antibacterial properties of parsley (*Petroselinum crispum*) and cilantro (Coriandrum sativum) extracts. Food Chem 2006; 97:505–515.

20 Petrolini FVB, Lucarini R, Souza MGMD, Pires RH, Cunha WR, Martins CHG. Evaluation of the antibacterial potential of *Petroselinum crispum* and *Rosmarinus officinalis* against bacteria that cause urinary tract infections. Braz J Microbiol 2013; 60:1007–1020.

21 Mojzis J, Varinska L, Mojzisova G, Kostova I, Mirossay L. Antiangiogenic effects of flavonoids and chalcones. Pharmacol Res 2008; 57:259–265.

22 Anso E, Zuazo A, Irigoyen M, Urdaci MC, Rouzaut A, Martínez-Irujo JJ. Flavonoids inhibit hypoxia-induced vascular endothelial growth factor expression by a HIF-1 independent mechanism. Biochem Pharmacol 2010; 79:1600–1609.

23 Tan W-F, Lin L-P, Li M-H, Zhang Y-X, Tong Y-G, Xiao D, *et al.* Quercetin, a dietary-derived flavonoid, possesses antiangiogenic potential. Eur J Pharmacol 2003; 459:255–262.

24 Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling – in control of vascular function. Nat Rev Mol Cell Biol 2006; 7:359–371.

25 Li Y, Hazarika S, Xie D, Pippen AM, Kontos CD, Annex BH. In mice with type 2 diabetes, a vascular endothelial growth factor (VEGF)-activating transcription factor modulates VEGF signaling and induces therapeutic angiogenesis after hindlimb ischemia. Diabetes 2007; 56:656–665.

26 Ramezani R, Moghimi A, Rakhshandeh H, Ejtehadi H, Kheirabadi M. The effect of *Rosa damascena* essential oil on the amygdala electrical kindling seizures in rat. Pak J Biol Sci 2008; 11:746–751.

27 Murray-Lyon I, Eddleston A, Williams R, Brown M, Hogbin B, Bennett A, *et al.* Treatment of multiple-hormone-producing malignant islet-cell tumour with streptozotocin. Lancet 1968; 292:895–898.

28 Oktarina Y, Mulyani S. The difference in the activity of ethanol extracts of leaves of anti diabetic tin (*Ficus Carica* l.) leaf, rambutan (*Nephelium lappaceum*) and Persimmon (Diospyros Ft L) on the white mancit on streptozotocin induced. Caring Indonesian J Nurs Sci 2019; 1:49–55.

29 El-Far YM, Zakaria MM, Gabr MM, El Gayar AM, El-Sherbiny IM, Eissa LA. A newly developed silymarin nanoformulation as a potential antidiabetic agent in experimental diabetes. Nanomedicine 2016; 11:2581–2602.

30 Su H-C, Hung L-M, Chen J-K. Resveratrol, a red wine antioxidant, possesses an insulin-like effect in streptozotocin-induced diabetic rats. Am J Physiol Endocrinol 2006; 290:E1339–E1346.

31 Vessal M, Hemmati M, Vasei M. Antidiabetic effects of quercetin in streptozocin-induced diabetic rats. Comparative biochemistry and physiology part C. Pharmacol Toxicol 2003; 135:357–364.

32 Vimalraj S, Pichu S, Pankajam T, Dharanibalan K, Djonov V, Chatterjee S. Nitric oxide regulates intussusceptive-like angiogenesis in wound repair in chicken embryo and transgenic zebrafish models. Nitric Oxide 2019; 82:48–58.

33 Gao Y, Zhou S, Pang L, Yang J, Li HJ, Huo X, *et al.* Celastrol suppresses nitric oxide synthases and the angiogenesis pathway in colorectal cancer. Free Radic Res 2019; 53:324–334.

34 Martínez-Ruiz M, Vargas-Robles H, Rios A, Sánchez D, Escalante B. Comparative effects of nitric oxide dependent and independent vasodilation on impaired hindlimb revascularization in eNOS–/– mice. Can J Physiol Pharmacol 2019; 97:377–385.

35 Silva AM, Penno Lde M, Bertoluci MC, Irigoyen MC, Schaan BD. Insulin therapy does not interfere with venous endothelial function evaluation in patients with type 2 diabetes mellitus. Clinics 2010; 65:1139–1142.