

Effect of *Thymus Vulgaris* Extract against Toxic Effects of Diabetes on the Coronary Heart Angiogenesis of Rats

SHIVA ROSHANKHAH¹, MAHDI TAGHADOSI², AMIR ABDOLMALEKI³, ARASH ZIAPOUR⁴, MOHAMMAD REZA SALAHSHOOR⁵

ABSTRACT

Introduction: *Thymus vulgaris* (*T. vulgaris*), with its antioxidant properties has therapeutic applications. Diabetes causes disruption in process of cardiac angiogenesis.

Aim: To evaluate the therapeutic effects of *T. vulgaris* against destructive effects of diabetes in the field of coronary angiogenesis in rats.

Materials and Methods: In this experimental study, 64 male rats were randomly sorted into 8 groups: 1) control group; 2-4) three extract groups (4.5, 9 and 18 mg/kg of *T. vulgaris*); 5) diabetic group; and 6-8) three diabetic + extract groups (4.5, 9 and 18 mg/kg of *T. vulgaris*) from April 2019 to November 2019. Diabetes was induced and various doses of *T. vulgaris* were administered for 60 consecutive days intraperitoneally. The serum level of Vascular Endothelial Growth Factor (VEGF)

was determined by ELISA method, Nitrite Oxide (NO) measured by Griess assay, and the cardiac capillary density evaluated by immunohistochemistry assay. Data were analysed by one-way ANOVA test.

Results: The values of VEGF, nitrite oxide, glucose and capillary density reduced significantly in diabetic group compared to the control group ($p < 0.05$). No significant differences were detected among the studied variables (VEGF, nitrite oxide, glucose and capillary density) in all extract groups, compared to the control group as well as diabetic + extract groups compared to the diabetic rats ($p > 0.05$).

Conclusion: The hydroalcoholic extract of *T. vulgaris* represented no cardiac angiogenesis effects in normal and diabetic groups. This is due to the insufficient amounts of active biochemical ingredients, especially flavonoids in *T. vulgaris*.

Keywords: Antioxidant, Biochemical ingredients, Cardiovascular system diabetes

INTRODUCTION

The heart as a member of cardiovascular system provides a continuous systematic and pulmonary circulation of blood flow [1]. Coronary artery disease leads to reduction of blood flow to the heart [2]. Chronic hyperglycaemia causes degeneration of small veins affecting optimised function of various organs like kidneys and eyes [3]. Diabetes is directly associated with an increased risk of cardiovascular disease [4]. Angiogenesis is defined as development of new blood vessels from the pre-existing ones. This process is crucial for growth and development of tissues, while it can be affected by diabetes [5,6]. Diabetes disrupts the balance which exists in the activity of inhibitory and stimulatory factors of angiogenesis, especially in cardiac tissue [7]. In fact, diabetes by alteration of VEGF gene expression can reduce the rate of angiogenesis [8]. About 80% of people in developing countries prefer medicinal plants for treatment of disease [9].

T. vulgaris is a herb which is widely used in traditional medicine. The application of this plant is very common due to the high phenolic content, vigorous antioxidant effects and various therapeutics properties like anti-diabetic, anti-inflammatory, anti-ageing, and sedative effects [10]. The active ingredients available in *T. vulgaris* are kaempferol, quercetin, geraniol, eugenol, citronelle, myrcene, phenylethyl alcohol, nonadecane, eicosane, triclosan, geranyl acetate, and carboxylic [11]. *T. vulgaris* is a biennial plant from the Umbelliferae family [12].

Antioxidants have crucial impacts on modification of angiogenesis [13]. It seems that the *T. vulgaris* plant can protect all the stages of angiogenesis process occurring in diabetic-induced stress [14]. Moreover, no study has been conducted to study the effect of *T. vulgaris* on angiogenesis, in diabetic rats. Thus, this study was aimed to determine the effects of hydroalcoholic extract of the *T. vulgaris* on angiogenesis following diabetes induction in male Wistar rats.

MATERIALS AND METHODS

This experimental study was performed in Department of Anatomical Sciences of Kermanshah University of Medical Sciences from April 2019 to November of 2019. All animals were treated in accordance with guidelines of National Institute of Health for the Care and Use of Laboratory Animals approved by Research Deputy at Kermanshah University of Medical Sciences (Department of Anatomical Sciences, Medical School, Iran) based on WMA Declaration Ethic of Helsinki (Ethic number; IR.KUMS.REC.1397.564).

Sixty-four male Wistar rats (200-250 gm) were prepared from the Pasteur Institute (Tehran, Iran) and housed in animal home under normal conditions ($22 \pm 2^\circ\text{C}$, 12 hours light/dark photocycle). They had free access to the water and standard food. All 64 animals were divided into 8 groups (8 animals in each) by random; control group, extract groups (4.5, 9 and 18 mg/kg), diabetic group, and diabetic + extract groups (4.5, 9 and 18 mg/kg). The extract was administered for 60 consecutive days by i.p injection.

Chemicals and Reagents

T. vulgaris was purchased from the centre of medicinal plants (Natural pharmacy health school traditional medicine, Faculty of Traditional Medicine, University of Tehran, Iran). Ethanol, formalin, paraffin and xylol were purchased from the Merck Co. (Germany). STZ was prepared from Sigma Co. (USA). Sodium chloride 0.9% was obtained from the Darou Pakhsh Co. (Tehran, Iran). H_2O_2 (5%) solution was provided from the Behsaman Co. (Iran). CD31 primary antibody, EnVision secondary antibody, PBS solution, R.S solution, DAB solution, and haematoxylin solution were purchased from the Dako Co. (Denmark). CK-E30634 ELISA kit was purchased from the Biopharm Co. (USA).

Induction of Diabetes Type I

It was achieved by a single i.p dose of STZ (60 mg/kg). This destructive chemical, causes partial or total degradation of pancreatic β cells. Two days later the animals were considered diabetic if the blood

glucose was greater than 250 mg/dL. This procedure was repeated twice to confirm the diabetes. The blood sugar was also measured weekly by the end of experiment [15].

Preparation of Hydroalcoholic *T. vulgaris* Extract

This plant was purchased from a local store (Kermanshah, Iran, spring 2019) and confirmed by a botanist. The plant was cleaned and leaves and stems were dried in the shade and then grounded to produce the PC powder. It was dissolved in 98% ethanol solution, and it was extracted using distilled water and ethanol (1:1 V/V) as a solvent. The solution was filtered and concentrated under the reduced pressure on a rotary evaporator. It was finally frozen at -80°C for future application. If needed, it was dissolved in water and prepared freshly for daily experimental use. The hydroalcoholic extract of *T. vulgaris* was administered (doses of 4.5, 9, and 18 mg/kg) for one month by i.p injection [11,12].

Measurement of VEGF: VEGF serum concentration was measured by ELISA technique and CK-E30634 kit (Biopharm, USA). Samples and standards were prepared. A 40 μL of rat standard serum sample was added, followed by 10 μL of biotin solution and 50 μL of HRP linked polyclonal antibody. They were added to each well of microplate coated with monoclonal antibodies and incubated at 37°C for 60 minutes. They were washed 5 times with washing solution, and the solution of chromogen A and B were added to the wells and incubated at 37°C for 10 minutes. A 50 μL of Stop solution was added to the wells for colour resuscitation. Finally, the optimum density of VEGF concentration was determined (ng/L) at the 450 nm wavelength using a Microplate Reader [16].

Heart sampling and blood serum preparation: Under anaesthesia, the thoracotomy procedure was done and the blood samples were gathered directly from the heart for measurement of serum VEGF. The heart was removed, washed in normal saline solution and fixed in formalin. The cardiac tissues underwent processing by an autotechnicon machine, and they were embedded in paraffin to assess the capillary density using immunohistochemistry technique [16].

Griess technique: Nitrite oxide was measured by the Griess assay using the microplate technique. The mixture of zinc sulfate powder (6 mg) and the serum samples (400 μL) was vortexed for one minute and then centrifuged (4°C , 10 minutes, 12,000 rpm). The supernatant was used for nitrite oxide measurement. A 50 μL of sample was added to 100 μL of Griess reagent (Sigma; USA) and incubated (30 minutes at room temperature). As per manufacturer protocol by an ELISA reader (Hyperion; USA), Optical Density (OD) of the sample at wavelength of 540 nm was measured [17].

Measurement of capillary density: The primary antibody CD31 was used as an indicator of endothelial cells in cardiac capillaries. Cardiac paraffin blocks were cut into 3 μm sections. The slides were passed through incubator and various solutions respectively as follows; incubated in oven and then in Xylol, placed in PBS and R.S solutions, placed in distilled water, H_2O_2 solution (5%), and finally washed in distilled water and PBS solutions. An appropriate amount of antibody was placed on the slices while adding the primary antibody. Then the slides were washed. The secondary antibody was also 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 washing process was applied with distilled water and PBS solutions. Haematoxylin was added to the slices. Finally, the slides were dehydrated and placed in xylol. Ten microscopic fields from each tissue were selected, and the capillary density was expressed as the number of endothelial cells per mm^2 [16].

STATISTICAL ANALYSIS

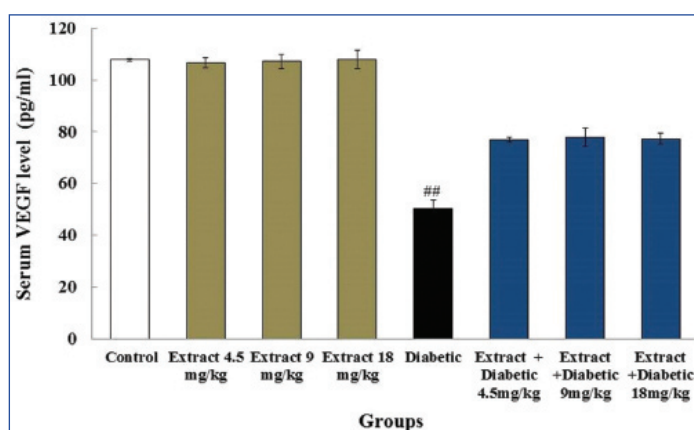
The Kruskal-Wallis test was used for examination of data normality. Data were analysed using SPSS software (version 16.0). The Unpaired t-test was used, and one-way analysis of variance (one-way ANOVA) was followed by Tukey's Post-Hoc tests. The p-value <0.05 ($p < 0.05$) was considered as statistically significant. All data

were expressed as mean \pm SEM.

RESULTS

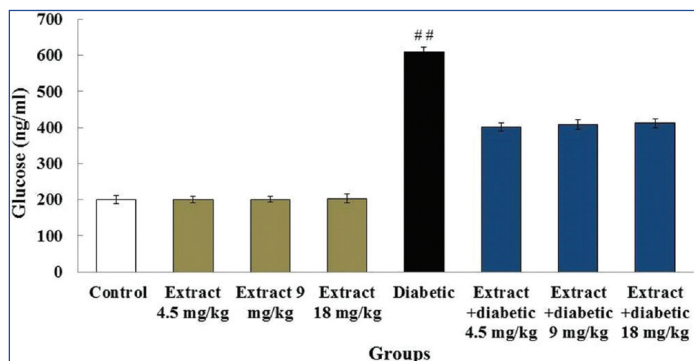
At the beginning of this study, there were 64 animals (8 groups), 48 (6 groups) of which were treated with the extract. Animals of diabetic group which died following diabetes induction were replaced again with new animals.

Serum level of VEGF: The level of serum VEGF in diabetic group (50.5 ± 3.1 pg/mL) reduced significantly compared to the control group (107.87 ± 0.6 pg/mL) ($p < 0.05$). Extract administration with doses of 4.5, 9 and 18 mg/kg showed no significant effects on VEGF expression in the extract groups (106.62 ± 2.4 pg/mL, 107.25 ± 2.2 pg/mL and 108.07 ± 3.5 pg/mL) in comparison to the control group (107.87 ± 0.6 pg/mL). Also, no significant alteration in VEGF serum concentration were detected among the diabetic group (50.5 ± 3.1 pg/mL) and the diabetic + extract groups (76.8 ± 1.4 pg/mL, 77.87 ± 3.6 pg/mL and 77.33 ± 2.3 pg/mL) ($p > 0.05$) [Table/Fig-1].



[Table/Fig-1]: The mean concentrations of serum VEGF in experimental groups. ##Significant difference in the diabetic group compared to the control group ($p < 0.05$)

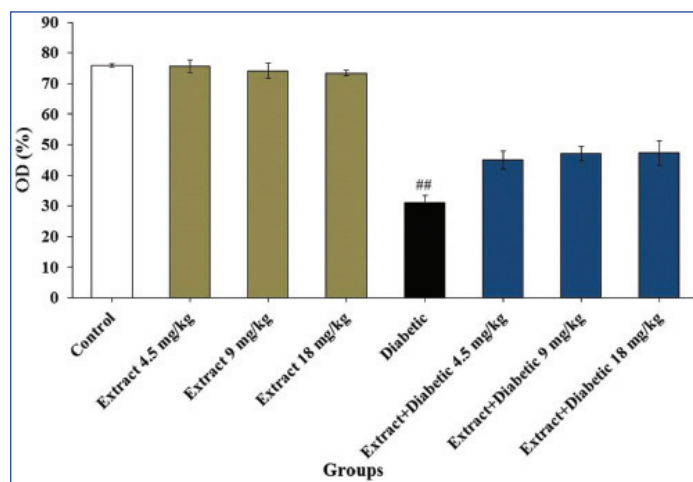
Levels of blood glucose: The comparison of mean blood glucose in different experimental groups were shown at various stages; the beginning of the experiment and first to fourth weeks of treatment. The blood glucose levels in diabetic (609.5 ± 13.1 ng/mL) and diabetic + extract groups (402.5 ± 11.4 ng/mL, 408.12 ± 14.2 ng/mL and 411.87 ± 11.5 ng/mL) were significantly higher than the control (200.37 ± 12.4 ng/mL) and extract groups (200.75 ± 9.5 ng/mL, 198.37 ± 8.6 ng/mL and 203.23 ± 12.7 ng/mL) ($p < 0.05$). However, there were no significant differences in blood glucose levels in control group (99.37 ± 12.4 ng/mL) in comparison with the extract groups (99.75 ± 9.5 ng/mL, 99.37 ± 8.6 ng/mL and 99.23 ± 12.7 ng/mL) as well as the diabetic group (509.5 ± 13.1 ng/mL) compared to the diabetic + extract groups (492.5 ± 11.4 ng/mL, 482.12 ± 14.2 ng/mL and 480.87 ± 11.5 ng/mL) ($p > 0.05$) [Table/Fig-2].



[Table/Fig-2]: Effects of diabetes and *T. vulgaris* on the mean blood glucose levels in rats ($n=8$ for each group). ##Significant difference in the diabetic group compared to the control group ($p < 0.05$)

Nitrite oxide: Biochemical analysis of serum nitrite oxide showed a significant decrease in diabetic group (31.13 ± 2.4) compared to the control group (75.94 ± 0.6) ($p < 0.05$). The mean nitrite oxide level was not significant in all extract groups (75.63 ± 2.1 , 74.19 ± 2.4 and 73.4 ± 0.8) compared to the control group (75.94 ± 0.6) ($p > 0.05$).

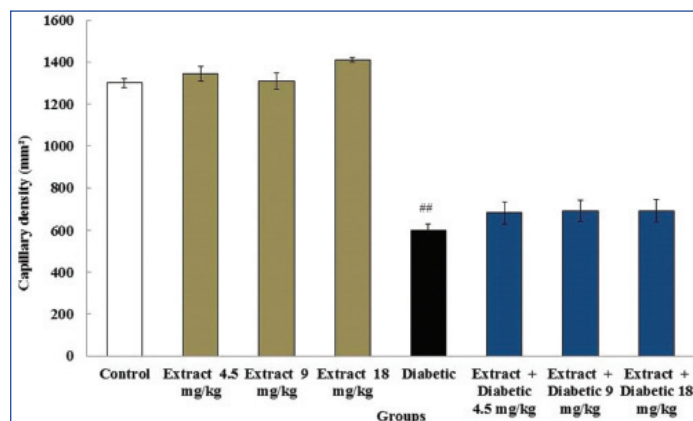
Also, this value was not significant in diabetic + extract groups (45.03±3.2, 47.18±2.4 and 47.38±4.1) in all doses compared to the diabetic group (31.13±2.4) ($p>0.05$) [Table/Fig-3].



[Table/Fig-3]: Effects of *T. vulgaris* and diabetes on the mean nitrite oxide levels.

##Significant difference compared to the control group ($p<0.05$). OD: Optic Density

Capillary density in heart: Results showed a significant alteration of capillary density in diabetic group (1050±30 mm³) compared to the control group (2100±22 mm³) ($p<0.05$). Findings showed no significant differences in the field of capillary density between the extract groups (2110±40 mm³, 2111±11 mm³ and 2104±34 mm³) and control group (2100±22 mm³) as well as the diabetic + extract groups (1480±51 mm³, 1490±48 mm³ and 1491±52 mm³)



[Table/Fig-4]: Effect of diabetes and *T. vulgaris* on the myocardial capillary density.

##Significant difference in diabetic group compared to the control group ($p<0.05$)

compared to diabetic rats ($p>0.05$) [Table/Fig-4].

DISCUSSION

As the obtained results in this study showed that diabetes reduces the cardiac capillary density, serum levels of VEGF and nitrite oxide compared to the control group. *T. vulgaris* at various concentrations of 4.5, 9, and 18 mg/kg increased the cardiac capillary density in diabetic groups receiving extract, but it was not statistically significant. Also, the capillary density rate in the extract groups was not significantly different compared to the control group. The findings of the present research showed no significant alteration in serum VEGF of diabetic group compared to the control group, which is consistent with some other studies [18-20].

According to the study of Hazarika S et al., the VEGF signalling in diabetic animals was lower expressed than the control group in the absence of ischaemia, which is in line with the results of the present study [19]. The results of Gao L and Yu DM, were in agreement with the findings of the present research indicating that the capillary density and revascularisation in the limbs of diabetic rats were decreased, with reduced mRNA and protein expression of eNOS, VEGF, and bFGF [20]. *T. vulgaris* has various biochemical

compounds like terpenes, glycosides, and flavonoids (including kaempferol and quercetin) [21,22]. Flavonoids inhibit angiogenesis process by reduction in VEGF expression [23,24]. In a study, the quercetin presented anti-angiogenesis effects with less proliferation and migration of vascular endothelial cells by reduced expression and activity of 2-metalloproteinase of matrix [25]. The results of this study also detected that the i.p prescription of *T. vulgaris* hydroalcoholic extract increases cardiac capillary density in diabetic rats, while it slightly reduces VEGF in experimental groups compared to the control group, but it is not statistically significant. VEGF is an effective inducer of angiogenesis in various models [26]. The findings of Li Y et al., confirmed the results of the present study indicating that the diabetes could decrease the plasma VEGF level [27]. As it was stated by previous studies, this consequence returns to VEGF tolerance of diabetes-induced vascular complications [16]. The probable reason for this phenomenon is the type of diabetes (Type I) induced for animal studies, which caused more severe complications and disabilities and less tolerance against diabetes. Thus, the herb extract exerts no therapeutic effects for alleviation of diabetes complications.

Also, this study revealed that the extract of *T. vulgaris* did not increase blood glucose levels significantly in extract groups and also had no decreasing effects on blood glucose levels in diabetic rats. However, its administration in diabetic+extract groups with specific doses reduced the blood glucose level at the end of the experiment (4th week) but not significantly. Science has revealed that the polysaccharides, flavonoids, polypeptides, and alkaloids existing in medicinal plants can justify the nature of hypoglycaemic and hypolipidemic [28,29]. Also, some studies have reported the positive-effects of medicinal plants with antioxidant properties [30].

The results of a study by Su HC et al., did not confirm the results of the present study indicating that the administration of flavonoids may increase the glucose uptake by hepatocytes, adipocytes and muscle cells [31]. A part of the hypoglycaemic effects of flavonoids administration seems to be related to the increased activity of hepatic hexokinase and glucokinase enzymes, as well as their insulin-like effect which reduces the symptoms of diabetes mellitus [32]. In this study, it was concluded that the serum nitrite oxide level in diabetic rats was lower than the control group. The statistical analysis demonstrated that the capillary density in myocardial tissue was correlated with the high serum angiogenic markers. Angiogenesis is controlled by a number of pro-angiogenic and anti-angiogenic factors released in extracellular matrix. Both nitrite oxide and VEGF have crucial roles in cellular process angiogenesis [33]. Nitrite oxide not only has a direct effect on angiogenesis but also affects other angiogenic growth factors by producing excessive nitrite oxide [34]. In endothelial nitrite oxide synthase-deficient rats, a decrease in nitrite oxide production can disrupt coronary vessels development and angiogenesis process [35]. The results of Silva AM et al., were in parallel with the findings of the present research, indicating that the serum nitrite oxide concentration in diabetic rats was lower [36]. Salahshoor MR et al., reported that the *T. vulgaris* administrated for male Wistar rats attenuated the nitrite oxide level which did not confirm the results of the current research [11].

The lack of therapeutic effect of *T. vulgaris* extract on blood glucose reduction may return to the non-toxic i.p doses, the amount of Flavonol active ingredient and the duration of the extract administration. It is also suspected that the active ingredients of *T. vulgaris* do not enter into the blood circulation due to low dose of active ingredient or not being absorbed through the i.p administration, or they have been metabolised into inactive hepatic metabolites. Another possible reason is the slight reduction in blood glucose that is neutralised by the absorbable carbohydrates which are normally found in the *T. vulgaris* extract. It is also possible that the blood glucose reducing substances along with their additives maybe existed in *T. vulgaris* extract to prevent blood glucose levels in the experimental rats.

Limitation(s)

Some mechanisms of action of this extract on coronary angiogenesis process were unclear. Animal death after diabetes induction; the number of animals in each group, ultimately remained unchanged, and in each group, 8 animals were tested. Dead animals following induction of diabetes were replaced again. Nitrite oxide measurement is strongly due to its very high volatility.

CONCLUSION(S)

T. vulgaris extract at all the dosages did not show significant effects on blood glucose level, nitrite oxide, serum capillary density, and VEGF in control and diabetic groups. This study showed that there are several factors leading to non-therapeutic effects of *T. vulgaris* to prevent the incidence of cardiovascular disorders in male Wistar rats. These factors are the type of diabetes, the dose of drug, and duration of drug administration. In future studies must be taken for complete association of the molecular interaction between *T. vulgaris*, diabetes and coronary angiogenesis.

REFERENCES

- Tahir E, Scherz B, Starekova J, Muellerleile K, Fischer R, Schoennagel B, et al. Acute impact of an endurance race on cardiac function and biomarkers of myocardial injury in triathletes with and without myocardial fibrosis. *Eur J Prev Cardio*. 2020;27(1):94-104.
- Melly L, Grosso A, Pop CS, Nolleveaux M, Di Maggio N, Banfi A, et al. Stimulation of cardiac angiogenesis through growth factors with a fibrin gel delivery platform. *J Heart Lung Transplant*. 2019;38(4):S246-51.
- Karunakaran U, Park KG. A systematic review of oxidative stress and safety of antioxidants in diabetes: focus on islets and their defense. *Diabetes Metab J*. 2013;37(2):106-12.
- 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(1):43-51.
- Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature*. 2000;407(6801):249-457.
- Zarei M, Khazaei M, Sharifi MR, Pourshanzari AA. Coronary angiogenesis during experimental hypertension: Is it reversible? *J Res Med Sci*. 2011;16(3):269-75.
- Martin A, Komada MR, Sane DC. Abnormal angiogenesis in diabetes mellitus. *Med Res Rev*. 2003;23(2):117-45.
- 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. *Nat Commun*. 2019;10(1):871-77.
- Silva NCC, Fernandes Júnior A. Biological properties of medicinal plants: A review of their antimicrobial activity. *J Venom Anim Toxins Incl Trop Dis*. 2010;16(3):402-13.
- Bistgani ZE, Hashemi M, DaCosta M, Craker L, Maggi F, Morshedloo MR. Effect of salinity stress on the physiological characteristics, phenolic compounds and antioxidant activity of *Thymus vulgaris* L. and *Thymus daenensis* Celak. *Ind Crop Prod*. 2019;135:311-20.
- Salahshoor MR, Roshankhah S, Jalili C. Antioxidative properties of *Thymus vulgaris* on liver rats induced by paclitaxel. *Pharmacol Res*. 2019;11(3):315-19.
- Gediköglü A, Sökmen M, Çivit A. Evaluation of *Thymus vulgaris* and *Thymra spicata* essential oils and plant extracts for chemical composition, antioxidant, and antimicrobial properties. *Food Sci Nutr*. 2019;7(5):1704-14.
- Kim JD, 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(3):165-76.
- Ghorbani R, Mokhtari T, Khazaei M, Salahshoor MR, Jalili C, Bakhtari M. The effect of walnut on the weight, blood glucose and sex hormones of diabetic male rats. *Int J Morphol*. 2014;32(3):833-38.
- 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(1):15-23.
- 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 (Sao Paulo)*. 2011;66(8):1419-24.
- 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(2):142-50.
- 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(3):254-61.
- 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(9):948-56.
- Gao L, Yu DM. Molecular mechanism of limbs' postischemic revascularization improved by perindopril in diabetic rats. *Chin Med J (Engl)*. 2008;121(21):2129-33.
- Roby MH, Sarhan MA, Selim KA, Khalel KI. Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and marjoram (*Origanum majorana* L.) extracts. *Ind Crop Prod*. 2013;43(1):827-31.
- Rota MC, Herrera A, Martínez RM, Sotomayor JA, Jordán MJ. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. *Food Control*. 2008;19(7):681-87.
- Mojzis J, Varinska L, Mojzisova G, Kostova I, Mirossay L. Antiangiogenic effects of flavonoids and chalcones. *Pharmacol Res*. 2008;57(4):259-65.
- 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(11):1600-09.
- 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(2-3):255-62.
- Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling - In control of vascular function. *Nat Rev Mol Cell Biol*. 2006;7(5):359-71.
- Li Y, Hazarika S, Xie D, Phippen 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(3):656-65.
- Murray-Lyon I, Eddleston A, Williams R, Brown M, Hogbin BM, Bennett A, et al. Treatment of multiple-hormone-producing malignant islet-cell tumour with streptozotocin. *Lancet*. 1968;292(7574):895-98.
- 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 manciit on streptozotocin induced. *Caring: Indonesian J Nurs Sci*. 2019;1(1):49-55.
- 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 (London)*. 2016;11(19):2581-602.
- Su HC, Hung LM, Chen JK. Resveratrol, a red wine antioxidant, possesses an insulin-like effect in streptozotocin-induced diabetic rats. *Am J Physiol Endocrinol Metab*. 2006;290(6):E1339-46.
- Vessal M, Hemmati M, Vasei M. Antidiabetic effects of quercetin in streptozotocin-induced diabetic rats. *Comp Biochem Physiol Toxicol Pharmacol*. 2003;135C(3):357-64.
- 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.
- 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(3):324-34.
- Martínez-Ruiz M, Vargas-Robles H, Ríos 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(5):377-85.
- 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(11):1139-42.

PARTICULARS OF CONTRIBUTORS:

- PhD, Department of Anatomical Sciences, Medical School, Kermanshah University of Medical Sciences, Kerman, Kermanshah, Iran.
- PhD, Department of Immunology, Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran.
- PhD, Department of Anatomical Sciences, Medical School, Kermanshah University of Medical Sciences, Kerman, Kermanshah, Iran.
- PhD, Student, Department of Health Education and Health Promotion, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran.
- PhD, Department of Anatomical Sciences, Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Mohammad Reza Salahshoor,
PhD, Department of Anatomical Sciences, Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran.
E-mail: reza.salahshoor@yahoo.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Dec 28, 2019
- Manual Googling: Jan 20, 2020
- iThenticate Software: Jan 30, 2020 (16%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: No
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: Dec 24, 2019

Date of Peer Review: Jan 13, 2020

Date of Acceptance: Jan 24, 2020

Date of Publishing: Feb 01, 2020