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The Effects of Saffron *(Crocus sativus)* Aqueous Extract on TNF-α Levels in Liver, Kidney, and Lens Tissues of Diabetic Rats

Safran *(Crocus sativus)* Sulu Ekstresinin Diyabetik Sıçanların Karaciğer, Böbrek ve Lens Dokularındaki TNF-α Düzeylerine Etkileri

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Abstract

Objective: Diabetes mellitus is a serious health problem that can result in several complications like nephropathy, retinopathy, cataract, and liver injury. Recently, it has been demonstrated that the pro-inflammatory cytokines, especially TNF-α, play a determinant part in the progression of microvascular diabetic complications. On the other hand, medicinal plants such as saffron reportedly possess anti-inflammatory activity, which may reduce inflammation and improve the functioning of various organs affected by hyperglycemic conditions. Therefore, the aim of the present study was to evaluate the effects of saffron aqueous extract in diabetic rats by measurement of TNF-α levels in liver, kidney, and lens tissues.

Material and Methods: Thirty-four healthy albino Wistar adult rats were randomly divided into four groups namely, normal control (I), saffron control (II), diabetic control (III, and saffron treated (IV). Diabetes was induced in diabetic control (III) and saffron treated (IV) group with the help of a single i.p. injection of streptozotocin (60 mg/kg body weight). Whereas, equal volumes of citrate buffer by an i.p. injection were administered to rats in normal (Group I) and saffron control (Group II) groups. The treatment of the groups (II and IV) with saffron aqueous extract was commenced, seven days after injections, by administration of the extract (200 mg/kg body weight), in five doses per week. At the end of the experimental period, fasting blood glucose levels and TNF-α levels, from the indicated tissues, were determined and compared.

Results: Administration of saffron aqueous extract to diabetic rats lowered fasting blood glucose level significantly and prevented weight loss in treated diabetic rats (IV group). The results indicated that there was an elevation in levels of TNF-α in kidney, liver, and lens tissues of the diabetic group (III) compared to control groups (I and II) and saffron aqueous extract adjusted and normalized their levels in liver and kidney tissues.

Conclusion: The study demonstrates that saffron aqueous extract can protect the kidney and liver of diabetic rats against damage caused by hyperglycemia-induced inflammation, due to its anti-inflammatory potential.

Keywords: Saffron aqueous extract; diabetes; TNF-α; liver; kidney; lens

Özet

Amaç: Diabetes mellitus; nefropati, retinopati, katarakt ve karaciğer hasarı gibi çeşitli komplikasyonlara neden olabilen ciddi bir sağlık sorunudur. Son zamanlarda, proinflamatuar sitokinlerin, özellikle de TNF-α'nın, mikrovasküler diyabetik komplikasyonların progresyonunda belirleyici bir rol oynadığı gösterilmiştir. Öte yandan, safran gibi şifalı bitkilerin antiinflamatuar aktiviteye sahip olduğu ve inflamasyonu azaltarak hiperglisemik koşullardan etkilenen çeşitli organların işleyişini iyileştirebileceği bildirilmiştir. Bu çalışmada, diyabetik sıçanlarda safran sulu ekstresinin etkilerinin karaciğer, böbrek ve lens dokularındaki TNF-α düzeylerinin ölçülmesi ile değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Otuz dört sağlıklı albino Wistar erişkin sıçan; normal kontrol (I), safran kontrol (II), diyabetik kontrol (III) ve safran tedavisi (IV) olmak üzere dört gruba randomize edildi. Diyabetik kontrol (III) ve safran tedavisi (IV) gruplarında, tek bir i.p. streptozotosin enjeksiyonu (60 mg/kg vücut ağırlığı) ile diyabet indüklendi. Normal kontrol (I) ve safran kontrol (II) gruplarındaki sıçanlara ise i.p. enjeksiyon ile eşit volümde sitrat tamponu uygulandı. Grupların (II ve IV) safran sulu ekstre ile tedavisi, enjeksiyondan yedi gün sonra, ekstrenin haftada beş doz hâlinde uygulanmasıyla başlatıldı (200 mg/kg vücut ağırlığı). Deney süresinin sonunda, belirtilen dokularda açlık kan şekeri düzeyleri ve TNF-α düzeyleri saptandı ve karşılaştırıldı.

Bulgular: Diyabetik sıçanlara safran sulu ekstre uygulanması, tedavi alan diyabetik sıçanlarda (grup IV) açlık kan şekeri düzeyini anlamlı derecede düşürdü ve kilo kaybını önledi. Sonuçlar, kontrol gruplarına (I ve II) kıyasla diyabetik grubun (III) böbrek, karaciğer ve lens dokularında TNF-α düzeylerinde bir yükselme olduğunu ve safran sulu ekstrenin karaciğer ve böbrek dokularında bu düzeyleri düzelttiğini ve normalleştirdiğini göstermektedir.

Sonuç: Çalışma, safran sulu ekstrenin diyabetik sıçanların böbrek ve karaciğerini, antiinflamatuar potansiyeline bağlı olarak hiperglisemi kaynaklı inflamasyonun neden olduğu hasara karşı koruyabildiğini göstermektedir.

Anahtar kelimeler: Safran sulu ekstresi; diyabet; TNF-α; karaciğer; böbrek; lens

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Introduction

Saffron *(Crocus sativus* L.) belongs to the Iridaceae family and possesses significant pharmacological effects (1). It has been used as a food additive for several centuries and this establishes its safety for human consumption (2). Studies have reported the protective functions of the saffron against inflammation, tumors, depression, and oxidative stress (3-5). Most of the therapeutic properties of saffron are due to the presence of different constituents that have strong free radical scavenging activity (6). The major constituents that are involved in rendering biological function to saffron include safranal, picrocrocin, crocetin, crocin(s), and apocarotenoids (7). Based on the studies conducted on saffron, it may be suggested that this plant can be an appropriate alternative for the management of diabetic complications (8). While hypoglycemic effects of saffron have been studied in diabetic rats, it remains obscure as to how it affects different organs (8).

Since long, certain foods and herbs have been used in the prevention, management, and treatment of diseases and related health conditions (9). Diabetes mellitus (DM) is a chronic health problem, which has undesirable impacts on the quality of life in the affected patients. This life-threatening disease is characterized by chronic hyperglycemia, which causes dysfunction of different organs such as the eyes, kidneys, nervous system, and liver. Pathogenesis of diabetes is a complex interaction between various factors such as genetics, environment, metabolic disorders, and inflammatory mediators (10). Generally, inflammatory processes contribute majorly in both types of diabetes and the blood levels of interleukin (IL)-1, IL-16, IL-18, and tumor necrosis factor (TNF)-α are seen to increase significantly in diabetic individuals (11-13). Among the different pro-inflammatory cytokines that are released during hyperglycemia, TNF-α has the potential to promote NFκB activation, thus leading to apoptosis or programmed cell death (14). Reports have shown that the regulation of TNF-α may improve diabetic complications associated with chronic hyperglycemia.

Even though exogenous insulin and other medicines can regulate blood glucose level in diabetes, they cannot treat the different diabetic complications that affect the vascular system, kidney, retina, lens, peripheral nerves, and skin. In this regard, herbs with potential anti-inflammatory activities can be promising therapeutic agents against diabetes complications (15).

Therefore, the present investigation was undertaken in order to evaluate the effects of saffron on TNF-α levels in liver, kidney, and lens tissues of diabetic rats induced with streptozotocin (STZ).

Material and Methods

Experimental animals

Thirty-four healthy albino Wistar adult rats (males, 7-8 weeks), each weighing 215 g (mean weight), were obtained from Shiraz Institute for Stem Cell and Regenerative Medicine (Shiraz, Iran). All the animals were housed in standard cages at the room temperature (23 ±1°C) with a natural light-dark cycle and given free access to water and balanced diet (*ad libitum*). All animals were kept in this condition one week before the study, for adaptation purpose.

Animal ethics

The state committee on animal ethics, Shiraz University, Shiraz, Iran (IACUC no: 4687/63) approved this study. In addition, the recommendations of the European Council Directive (86/609/EC) of November 24, 1986, regarding the protection of animals used for experimental purposes, were considered.

Preparation of plant extract

The saffron aqueous extract (SAE) was prepared by adding one gram of dried and powdered saffron threads to 25 mL distilled water and keeping the mixture on an orbital shaker in a dark room for three days. The solution was then filtrated with Whatman No. 1 filter paper and freeze-dried to powder to be stored at -18 ºC till further studies (16-18).

STZ-diabetic Induction

For induction of diabetes in Group III and Group IV, STZ (Sigma, St. Louis, MO, USA)

solution was freshly prepared using 0.1 M cold citrate buffer (pH 4.5) and injected intraperitoneally (i.p) to the rats at the concentration of 60 mg/kg body weight (BW) (19, 20). Rats in normal (Group I) and saffron control (Group II) groups received equal volume of citrate buffer by an i.p injection.

About 72 h after STZ injection, the development of diabetes was confirmed by measuring the level of fasting blood glucose (FBG) with the help of glucometer (Accua Check, Germany). Animals displaying FBG above 250 mg/dL were included in the study.

Experimental design

After the induction of diabetes, all the groups were given the following treatments:

Group I: Served as the normal control group (healthy rats) and was given i.p. injection of normal saline, once a week for five weeks (n=7).

Group II: Served as the saffron control group (healthy rats) and was given i.p. injection of SAE (200 mg/kg BW), once a week for five weeks (n=7).

Group III: Served as the diabetic control group and was given i.p. injection of normal saline, once a week for five weeks (n=10).

Group IV: Served as saffron treated group and was given i.p. injection of SAE (200 mg/kg BW), once a week for five weeks (n=10).

During the study, the BW of animals was measured weekly. All rats were sacrificed after 42 days under standard anesthesia and their livers, kidneys, and lens were harvested.

Phosphate buffer (0.1 M, pH 7.4) solution was added to each harvested tissue and sonication was performed at 4°C using a Cole Farmer 4710 series ultrasonic homogenizer (Cole Farmer). The homogenized tissues were stored at -80 °C.

TNF-α measurement

The measurement of TNF-α levels in liver, kidney, and lens tissues was done with the help of a commercial kit based on enzyme-linked immunoassay (ELISA) (Cusabio Biotech Co., LTD., China). TNFα levels were expressed per gram of protein.

Total protein measurement

The total protein content of the homogenized tissues was estimated by Lowry method (21).

Statistical analysis

Data were reported as mean±SD and analyzed with One-Way ANOVA followed by Tukey's post hoc test. In addition, a paired t-test was used to compare the FBG and body weights (BWs) in groups before and after the intervention period. Comparison between the groups was performed using SPSS version 17 software and the significance level of $p<0.05$ was considered and included in the study.

Results

At the beginning of the study, there was no significant difference in the weight of the rats from different groups ($P>0.05$), as shown in Figure 1A and Table 1. A 10% decrease in BW of diabetic adult rats was seen during the final measurements when compared with the measurements at the beginning of the study. Moreover, SAE was able to prevent weight loss in diabetic animals and increased the BW of the diabetic group by 2%. However, the observed body weight changes were not found to be statistically significant ($p>0.05$).

On the last day of the experiment, the FBG level was seen to be significantly ($p<0.05$) higher in the diabetic group. Conversely, Group IV that was treated with SAE showed a significant decrease ($p <0.05$) in the FBG in comparison with the diabetic control rats (Figure 1B).

The changes in TNF-α in different groups are shown in Table 2. Initially, the level of TNFα was observed to be higher in kidney and lower in lens tissue. A significant increase ($p<0.05$) was observed in the TNF-α levels of lens, liver, and kidney tissues of diabetic control group when compared to control groups I and II (p values were ≤0.001). Furthermore, SAE was able to significantly lower TNF-α levels in liver and kidney tissues in comparison with an untreated diabetic group (p values were 0.002 and <0.001, respectively). Hence, SAE administration normalizes TNF-α level in both mentioned tissues of diabetic rats.

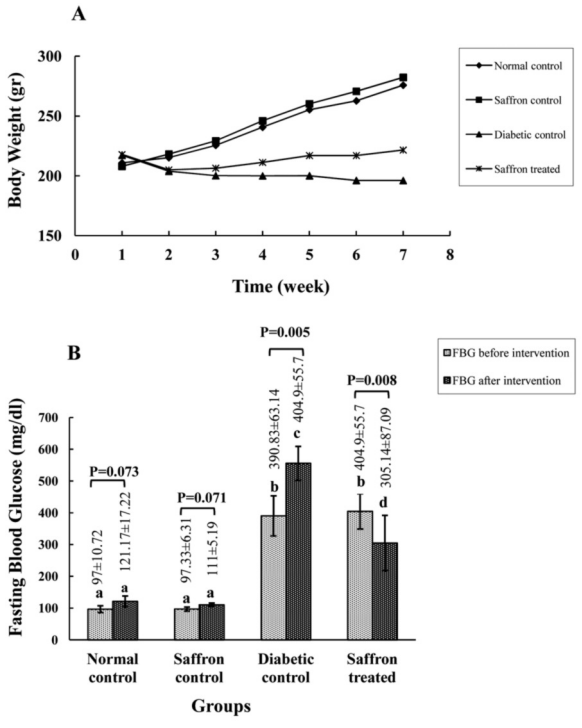

Figure 1: A) The effect of SAE on body weight in rats; **B)** The effect of SAE on fasting blood glucose level in rats. The different letters indicate the significance changes of the data that compares with another ($p<0.05$).

Discussion

The major cause of various microvascular damages in the liver, kidney, and lens of diabetic patients is chronic hyperglycemia. Nevertheless, recent studies have established that TNF-α is another underlying factor that influences the complications of this life-threatening disease (11). The regulation of TNF-α level in affected organs could prove beneficial by improving or delaying the complication of diabetes including cataracts, hepatitis, and nephropathy. Blood sugar and BW are the primary factors that need to be monitored accurately in diabetes. Numerous researchers have reported the reduction in BW of STZ diabetic rats (14, 22, 23). During diabetes, due to defect in insulin secretion or function, the excessive glucose production during gluconeogenesis is not useful for the body; therefore, diabetic individuals will lose weight and muscle wasting occurs (24). The results of the present study proved that SAE is able to prevent diabetes-related weight loss and reverse gluconeogenesis. Hypoglycemic effect of saffron is a principal mechanism known to

Table 1. Body weight (gr) of experimental rats during seven weeks of treatment.

	Groups			
Time	**Normal Control**	**Saffron Control**	**Diabetic control**	**Saffron treated**
Week 1	210.83±20.04	208±27.31	217.17±10.65	217.86±4.41
Week 2	215.2±17.59	218.17±30.8	203.83±16.12	205±8.29
Week 3	225.5±27.84	229.25±35.39	200.17±14.27	206.43±8.77
Week 4	240.67±19.7	246.00±40.56	200.00±14.44	211.29±17.62
Week 5	255.33±13.99	260.20±33.12	200.10±26.51	217.00±26.62
Week 6	262.67±7.26	270.67±33.12	196.17±26.51	217.00±26.68
Week 7	275.83±5.85	282.33±32.73	196.17±24.83	221.57±24.41

Data are expressed as mean + SD (n=7).

Table 2. The level of TNF-α in lens, Liver, and kidney of experimental rats.

		Tissues		
Groups	**Treatment regimen**	**Lens (pg/g protein)**	**Liver (ng/g protein)**	**Kidney (ng/g protein)**
I	Normal Control	914.67±92.94[a]	326.919±131.54[a]	446.52±196.57[a]
II	Saffron Control	901.5±168.26[a]	301.878±111.102[a]	419.98±122.63[a]
III	Diabetic Control	1266.67±84.13[b]	803.49±161.53[b]	1672.96±197.76[b]
IV	Saffron Treated	1143.33±117.96[b]	457.40±137.25[a]	520.037±171.09[a]

Data are expressed as mean + SD (n=7). Different letters in each column indicates statistically significant of changes between groups ($p<0.05$). Values that have a different superscript small letter (a, b, c, d, e) differ significantly from each other among groups.

prevent weight loss in diabetes (25). The diabetic control group had a constant high blood glucose level, which suggested that the beta cells of the pancreas were destroyed by STZ (Figure1B). However, a significant decrease in the level of blood glucose was seen in group IV that was treated with SAE.

Different studies have also demonstrated hypoglycemic as well as weight loss reversal effects of saffron. Kianbakht and Hajiaghaee (2011) stated that saffron has an ameliorating effect on FBG in diabetic rats (26). A study by Mohajeri et al. determined that the saffron ethanolic extract was able to reduce FBG and cause regenerative modification against damages in the endocrine cells of the pancreas in alloxan diabetic rats (27). Improvement of BW and blood glucose and regulation of insulin in diabetic rats treated with saffron has been described earlier by Elgnzar et al. (15) and Arasteh et al. (28). Furthermore, the study by Samarghandian et al. (2014) reported that saffron was able to decrease hyperglycemia and ameliorate the status of oxidative stress in rats with diabetic encephalopathy (29). In this context, the suggested hypoglycemic mechanism of saffron involves reduction of insulin resistance and prevention of intestinal glucose absorption (30). Some reports have also shown that saffron can stimulate glucose uptake in skeletal muscles and adipose tissues (31, 32).

Inflammation is a pathophysiological condition, which contributes to local tissue damage in diabetes. Chronic hyperglycemia, which is the major characteristic of a diabetic patient, can directly endorse an inflammatory process following cytokine production and destruction of the pancreatic β cell (33).

Findings from the present study demonstrated that STZ increased the TNF-α levels in the liver, kidney, and lens of rats. These results agree with those from the studies by Hasegawa et al. (34), Lee et al. (35) and Moller (36), indicating the role of TNF-α in the pathogenesis of both diabetes types. Ingaramo et al. investigated the role of TNF-α intracellular pathway in the development of apoptosis in the liver of STZ diabetic rats and found that the significant increase in the liver TNF-α level induced apoptotic cell death via the activation of caspase-8, NFB and JNK pathways (37). On the other hand, it is well established that IL-1, IL-6, IL-18, and TNF-α are major inflammatory cytokines that participate in the pathogenesis of diabetic nephropathy (34, 38). Increased TNF secretion is a key factor that can help in the detection of diabetic nephropathy in the early stages of diabetes (39).

Moreover, Joussen et al. (2004) showed that in diabetes, a strong connection occurred between plasma level of TNF-α and retinopathy severity (40). Later, Joussen et al. (2009) reported that TNF-α was an important molecule in the pathogenesis of diabetes complications, which characterize diabetic retinopathy. The etanercept TNF-α inhibitor was found to significantly reduce retinal cell apoptosis in the eye (41).

Since the progression of diabetic retinopathy, nephropathy and liver apoptosis have been linked to TNF-α levels, so the agents having the ability to lower the levels of this inflammatory cytokine are proposed to be promising preventatives for diabetes complications. The findings of the present study demonstrated that SAE possesses the ability to lower TNF-α in lens, liver, and kidney of diabetic rats. A study on neuronally differentiated PC-12 cells showed that crocin, a major component of saffron, was able to suppress TNF-α and prevent ethanol-induced impairment of learning and memory in mice (42), which is in agreement with the results of this study. Furthermore, in a study by Moallem et al. (43), the saffron extract was shown to inhibit the effect of diazinon by decreasing the serum levels of TNF-α, direct 8-iso-prostaglandin $F_{2\alpha}$ (oxidative stress marker) and soluble protein-100 β (S100β, neuronal damage marker). Hader et al. (44) suggested that crocin's ameliorative effect on diabetic nephropathy might be attributed to its ability to scavenge free radicals, enhance host antioxidant defense system and inhibit inflammatory and fibrotic cascade activation. A study by Christodoulou et al. (45) reports that improvement of glucose levels in diabetic atherosclerotic rats is probably mediated by a favorable modification of inflammatory

mechanisms. Therefore, the value of saffron in preventing of diabetic complications should be considered while plant-derived compounds have been used instead of medicines derived from chemical substances (46).

Conclusion

In general, saffron is considered to be a natural alternative remedy for prevention of hyperglycemia and weight loss in STZ-diabetic rats. Moreover, in this study, SAE was seen to exhibit protective ability against diabetic complications by preventing TNF-α production in liver, kidney, and lens.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Authorship Contributions

Idea/Concept: Mahboobeh Ashrafi; Design: M. Ashrafi; Control/Supervision: Mahboobeh Ashrafi; Data Collection and/or Processing: M. Ashrafi, Zahra Afsar, Saeed Nazifi; Analysis and/or Interpretation: Mahboobeh Ashrafi, Hoda Erjaee; Literature Review: Hoda Erjaee; Writing the Article: Mahboobeh Ashrafi, Hoda Erjaee; Critical Review: Saeed Nazifi, Mahboobeh Ashrafi; References and Fundings: Mahboobeh Ashrafi, Saeed Nazifi; Materials: Mahboobeh Ashrafi, Saeed Nazifi.

References

1. Abdullaev FI. Biological effects of saffron. Biofactors. 1993;4:83-86.
2. Moshiri M, Vahabzadeh M, Hosseinzadeh H. Clinical applications of saffron (Crocus sativus) and its constituents: a review. Drug Res (Stuttg). 2014;64: 287-295.
3. Hosseinzadeh H, Karimi G, Niapoor M. Antidepressant effect of Crocus sativus L. stigma extracts and their constituents, crocin and safranal, in mice. Paper read at I International Symposium on Saffron Biology and Biotechnology. 2003; 650.
4. Hosseinzadeh H, Khosravan V. Anticonvulsant effect of aqueous and ethanolic extreacts of Crocus sativus L stigmas in mice. Arch Iran Med. 2002;5:44-47.
5. Hosseinzadeh H, Younesi HM. Antinociceptive and anti-inflammatory effects of Crocus sativus L. stigma and petal extracts in mice. BMC Pharmacol. 2002;2:7.
6. Bukhari SI, Manzoor M, Dhar MK. A comprehensive review of the pharmacological potential of Crocus sativus and its bioactive apocarotenoids. Biomed Pharmacother. 2018;98:733-745.
7. Rios JL, Recio MC, Giner RM, Mañez S. An update review of saffron and its active constituents. Phytother Res. 1996;10:189-193.
8. Mohajeri D, Mousavi G, Doustar Y. Antihyperglycemic and pancreas-protective effects of Crocus sativus L. (Saffron) stigma ethanolic extract on rats with alloxan-induced diabetes. J Biol Sci. 2009;9:302-310.
9. Kermani T, Kazemi T, Molki S, Ilkhani K, Sharifzadeh G, Rajabi O. The efficacy of Crocin of Saffron (Crocus sativus L.) on the components of metabolic syndrome: a randomized controlled clinical trial. J Res Pharm Pract 2017;6:228-232.
10. Francés DE, Ingaramo PI, Ronco MT, Carnovale CE. Diabetes, an inflammatory process: oxidative stress and TNF-alpha involved in hepatic complication. J Biomed Sci Eng. 2013;6:645-653.
11. Alexandraki KI, Piperi C, Ziakas PD, Apostolopoulos NV, Makrilakis K, Syriou V, Diamanti-Kandarakis E, Kaltsas G, Kalofoutis A. Cytokine secretion in longstanding diabetes mellitus type 1 and 2: associations with low-grade systemic inflammation. J Clin Immunol. 2008;28:314-321.
12. Esposito K, Nappo F, Marfella R, Giugliano G, Giugliano F, Ciotola M, Quagliaro L, Ceriello A, Giugliano D. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans. Circulation. 2002;106:2067-2072.
13. Foss NT, Foss-Freitas MC, Ferreira MA, Cardili RN, Barbosa CM, Foss MC. Impaired cytokine production by peripheral blood mononuclear cells in type 1 diabetic patients. Diabetes Metab. 2007;33:439-443.
14. Srinivasan K, Ramarao P. Animal models in type 2 diabetes research: an overview. Indian J Med Res. 2007;125:451-472.

15. Elgazar AF, Rezq AA, Bukhari HM. Anti-hyperglycemic effect of saffron extract in alloxan-induced diabetic rats. Eur J Biol Sci. 2013;5:14-22.
16. Bathaie Z, Miri H, Mohagheghi MA, Mokhtari-Dizaji M, Shahbazfar AA, Hasanzadeh H. Saffron aqueous extract inhibits the chemically-induced gastric cancer progression in the wistar albino rat. Iran J Basic Med Sci. 2013;16:27-38.
17. Hosseinzadeh H, Sadeghnia HR, Ziaee T, Danaee A. Protective effect of aqueous saffron extract (Crocus sativus L.) and crocin, its active constituent, on renal ischemia-reperfusion- induced oxidative damage in rats. J Pharm Pharm Sci 2005;8:387-393.
18. Shirali S, Bathaei SZ, Nakhjavani M, Ashoori MR. Effects of saffron (Crocus Sativus L.) aqueous extract on serum biochemical factors in streptozotocin-induced diabetic rats S. Iran J Med Aromatic Plants. 2012;28:293-308.
19. Brøndum E, Nilsson H, Aalkjaer C. Functional abnormalities in isolated arteries from goto-kakizaki and streptozotocin-treated diabetic rat models. Horm Metab Res. 2005;37:56-60.
20. Gobbo MG, Costa CF, Silva DG, de Almeida EA, Góes RM. Effect of melatonin intake on oxidative stress biomarkers in male reproductive organs of rats under experimental diabetes. Oxid Med Cell Longev. 2015;2015:614579.
21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265-275.
22. Erjaee H, Rajaian H, Nazifi S, Chahardahcherik M. The effect of caraway (Carum carvi L.) on the blood antioxidant enzymes and lipid peroxidation in streptozotocin-induced diabetic rats. Comp Clin Path. 2015;24:1197-1203.
23. Muruganandan S, Srinivasan K, Gupta S, Gupta PK, Lal J. Effect of mangiferin on hyperglycemia and atherogenicity in streptozotocin diabetic rats. J Ethnopharmacol. 2005;97:497-501.
24. Pushparaj P, Tan CH, Tan BK. Effects of Averrhoa bilimbi leaf extract on blood glucose and lipids in streptozotocin-diabetic rats. J Ethnopharmacol. 2000;72:69-76.
25. Shirwaikar A, Rajendran K, Dinesh Kumar C, Bodla R. Antidiabetic activity of aqueous leaf extract of Annona squamosa in streptozotocin-nicotinamide type 2 diabetic rats. J Ethnopharmacol. 2004;91:171-175.
26. Mohajeri D, Amouoghli Tabrizi B, Mousavi G, Mesgari M. Anti-diabetic activity of Crocus sativus L (Saffron) stigma ethanolic extract in alloxan-induced diabetic rats. Res J Biol Sci. 2008;3:1102-1108.
27. Kianbakht S, Hajiaghaee R. Anti-hyperglycemic effects of saffron and its active constituents, crocin and safranal, in alloxan-induced diabetic rats. J Med Plants. 2011;3:82-89.
28. Arasteh A, Aliyev A, Khamnei S, Delazar A, Mesgari M, Mehmannavaz Y. Effects of hydromethanolic extract on saffron (Crocus sativus) on serum glucose, insulin and cholesterol levels in healthy male rats. Planta Medica. 2010;4:397-402.
29. Samarghandian S, Azimi-Nezhad M, Samini F. Ameliorative effect of saffron aqueous extract on hyperglycemia, hyperlipidemia, and oxidative stress on diabetic encephalopathy in streptozotocin induced experimental diabetes mellitus. Biomed Res Int. 2014;2014:920857.
30. Xi L, Qian Z, Xu G, Zhou C, Sun S. Crocetin attenuates palmitate-induced insulin insensitivity and disordered tumor necrosis factor-alpha and adiponectin expression in rat adipocytes. Br J Pharmacol. 2007;151:610-617.
31. Yang YC, Hsu HK, Hwang JH, Hong SJ. Enhancement of glucose uptake in 3T3-L1 adipocytes by Toona sinensis leaf extract. Kaohsiung J Med Sci. 2003;19:327-333.
32. Youn JY, Park HY, Cho KH. Anti-hyperglycemic activity of Commelinacommunis L.: inhibition of alpha-glucosidase. Diabetes Res Clin Pract. 2004;1: S149-S155.
33. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001;414: 813-820.
34. Hasegawa G, Nakano K, Sawada M, Uno K, Shibayama Y, Ienaga K, Kondo M. Possible role of tumor necrosis factor and interleukin-1 in the development of diabetic nephropathy. Kidney Int. 1991;40:1007-1012.
35. Lee IA, Lee JH, Baek NI, Kim DH. Antihyperlipidemic effect of crocin isolated from the fructus of Gardenia jasminoides and its metabolite crocetin. Biol Pharm Bull. 2005;28:2106-2110.
36. Moller DE. Potential role of TNF-alpha in the pathogenesis of insulin resistance and type 2 diabetes. Trends Endocrinol Metab. 2000;11:212-217.
37. Ingaramo PI, Ronco MT, Francés DE, Monti JA, Pisani GB, Ceballos MP, Galleano M, Carrillo MC, Carnovale CE. Tumor necrosis factor alpha pathways developes liver apoptosis in type 1 diabetes mellitus. Mol Immunol. 2011;48:1397-1407.
38. Hasegawa G, Nakano K, Kondo M. Role of TNF and IL-1 in the development of diabetic nephropathy. Nefrologia. 1995;15:1-4.
39. DiPetrillo K, Gesek FA. Pentoxifylline ameliorates renal tumor necrosis factor expression, sodium retention, and renal hypertrophy in diabetic rats. Am J Nephrol. 2004; 24:352-359.
40. Joussen AM, Poulaki V, Le ML, Koizumi K, Esser C, Janicki H, Schraermeyer U, Kociok N, Fauser S, Kirchhof B, Kern TS, Adamis AP. A central role for inflammation in the pathogenesis of diabetic retinopathy. FASEB J. 2004;18:1450-1452.
41. Joussen AM, Doehmen S, Le ML, Koizumi K, Radetzky S, Krohne TU, Poulaki V, Semkova I, Kociok N. TNF-alpha mediated apoptosis plays an important role in the development of early diabetic retinopathy and long-term histopathological alterations. Mol Vis. 2009;15:1418-1428.
42. Soeda S, Ochiai T, Paopong L, Tanaka H, Shoyama Y, Shimeno H. Crocin suppresses tumor necrosis factor-alpha-induced cell death of neuronally differentiated PC-12 cells. Life Sci. 2001;69:2887-2898.

43. Moallem SA, Hariri AT, Mahmoudi M, Hosseinzadeh H. Effect of aqueous extract of Crocus sativus L. (saffron) stigma against subacute effect of diazinon on specific biomarkers in rats. Toxicol Ind Health. 2014;30:141-146.
44. Abou-Hany HO, Atef H, Said E, Elkashef HA, Salem HA. Crocin mediated amelioration of oxidative burden and inflammatory cascade suppresses diabetic nephropathy progression in diabetic rats. Chem Biol Interact. 2018;284:90-100.
45. Christodoulou E, Kadoglou NP, Stasinopoulou M, Konstandi OA, Kenoutis C, Kakazanis ZI, Rizakou A, Kostomitsopoulos N, Valsami G. Crocus sativus L. aqueous extract reduces atherogenesis, increases atherosclerotic plaque stability and improves glucose control in diabetic atherosclerotic animals. Atherosclerosis. 2018;268:207-214.
46. Rameshrad M, Razavi BM, Hosseinzadeh H. Saffron and its derivatives, crocin, crocetin and safranal: a patent review. Expert Opin Ther Pat. 2018;28:147-165.