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Original Article

The effect of saffron aqueous extract on oxidative stress parameters and important biochemical enzymes in the testis of streptozotocin-induced diabetic rats

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Abstract

Introduction: Sexual dysfunction and infertility are frequently associated with diabetes in men and experimental animals. Oxidative stress and alteration in testis are responsible for complication in diabetes. Saffron has antidiabetic and antioxidant properties that improves the functions of various organs. Therefore, the aim of the present study was to investigate the effects of administration of saffron aqueous extract in testis tissues of diabetic rats.

Methods: The fasted rats were injected by a single intraperitoneal (ip) injection of a freshly prepared solution of streptozocin (STZ, 65mg/kg) in 0.1 M cold citrate buffer (pH=4.5). Three days after STZ administration, the animals with fasting blood glucose concentrations of over 250mg/dl were considered to be diabetic and were used in the experimental groups as follows: normal control (1), diabetic control (2), saffron control (3) and saffron treated (4). The treatment was started on the 7th day after STZ injection with ip injection of saffron (200mg/kg), five doses and weekly to groups (3 and 4). At the end of the experimental period, fasting blood glucose levels and the activity of ALT, AST, ALP, LDH, SOD, CAT, GPx and MDA content were determined in testis tissues.

Results: Results showed saffron administration decreased elevated biochemical enzymes levels in testis of diabetic rats. Also, saffron significantly increased CAT and GPx activities in testis of diabetic rats. MDA levels had no significant changes in all experimental groups.

Conclusion: The results demonstrated that saffron administration improved antioxidant enzymes function against oxidative stress.

Keywords:
Saffron aqueous extract;
Diabetes mellitus;
Testis;
Biochemical and antioxidant enzymes

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Introduction

Diabetes mellitus is a heterogenic metabolic disorder affecting nearly over 124 million people worldwide (Laakso et al., 2001). Defects in insulin secretion and insulin function are characterization of diabetes which causes hyperglycemia. If this life threatening disease

remains untreated, it will lead to serious damages in different vital organs including male reproductive system. A large number of studies in men with diabetes, and in animal models show that diabetes causes male infertility due to sexual dysfunction and hypogonadism. Diabetes affects male reproductive function at different levels including changes in the quality of sperm, spermatogenesis, testicular morphology, sertoli glucose metabolism, ejaculation disorder, reduction in testosterone and libido (Jain and Jangir, 2014). Testis are considered as the primary reproductive organ and are one of the major organs affected by diabetes (Baccetti et al., 2002; Koh, 2007). Diabetic rats exhibit decreased testicular weight, sperm count, sperm motility and testosterone levels, and increased frequency of abnormal spermatogenesis (Scarano et al., 2006). Changes in testicular tissue including testis apoptosis may be the major component of the infertility in diabetes (Roy et al, 2014). Therefore, consideration the abnormalities in testis could be really beneficial to treat or reduce serious damages and infertility. Over production of reactive oxygen species (ROS) and impaired antioxidant defenses are accompanied with diabetes. Evidence indicates that oxidative stress in diabetes people is the trigger for many alterations on sexual function. In this regard, reducing oxidative stress in patients suffering from diabetes could be really valuable (Amaral et al., 2008). There are number of medications used to treat people with diabetes; however, common medications for diabetes does not always prevent the complications of the disease. In recent years, attention has been focus on herbs with high antioxidant activity to prevent and protect oxidative damage caused by free radical species in diabetes (Stavic, 1994). *Crocus sativus* L. commonly known as saffron is a perennial stem less herb of the Iridaceae family. Saffron has been used in traditional medicine as an antispasmodic, eupeptic, gingival sedative, anti-catarrhal, nerve sedative, carminative, diaphoteric, expectorant, stimulant, stomachic and aphrodisiac agent. The pharmacological activities of saffron are attributed to many of its active constituents including safranal, crocetin, crocin and quercetin (Rios et al., 1996). Theses constituents of saffron exhibits high antioxidant activity which makes it an ideal candidate for treatment of disease associated with oxidative stress (Khajuria et al., 2010). Testicular dysfunction in diabetes is well established, whereas the impact of oxidative stress associated with diabetes on the testis remains elusive. Herein, the aim of the present study was to evaluate the effect of saffron aqueous extract on oxidative stress parameters including superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and malondialdehyde (MDA) in testis of diabetes rats and to investigate the performance of saffron extract on the improvement of testicular tissue by determining the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH).

Materials and methods

Experimental animals

Thirty-two adult healthy male albino Wistar rats (7-8 weeks) weighting 200–225 g were purchased from Shiraz University of Medical Science (Shiraz, Iran). The animals were housed in standard cages at room temperature (23±1 °C) with a 12:12-h light-dark cycle with free access to tap water and balanced diet (*ad libitum*). For adaptation, all animals were kept in this condition one week prior to the study.

Animal ethics

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

Preparation of saffron aqueous extract

Sterile distilled water (25 ml) was added to 1 g of the dry saffron threads powder and was placed in orbital shaker for three days. The mixture was then filtered using number one Whatman filter paper. The filtered extract was freeze dried and the final powdered extract was stored at -18 °C for further studies.

Induction of experimental diabetes

The fasted rats were injected by a single intraperitoneal (ip) injection of a freshly prepared solution of streptozocin (STZ, 65 mg/kg) in 0.1 M cold citrate buffer (pH=4.5) (Brondum et al., 2005; Gobbo et al., 2015). For normal control group, rats were

injected with citrate buffer. After 72 h of diabetes induction, the level of fasting blood glucose (FBS) were measured by glucometer (Accu Chek, Germany). Animals with the FBS above 250 mg/dl were included in the experiment.

Experimental design

The rats were randomly divided into four groups of eight rats in each group as follows: group 1: served as normal control group, injected with normal saline once a week (ip for 5 weeks); group 2: served as diabetic control group, injected with normal saline once a week (ip for 5 weeks); group 3: served as saffron control group, injected once a week with saffron aqueous extract (200 mg/kg, ip, for 5 weeks) and group 4: served as saffron treated group, injected once a week with saffron aqueous extract (200 mg/kg, ip, for 5 weeks).

Body weights of animals were measured on days 0, 7, 14, 21, 28, 35 and 42 of the experiment. At the end of the experiment, rats in all groups were sacrificed under anesthesia and testis of rats were harvested. For each animal, 100 mg of testicular tissue were weighted and a solution of phosphate buffer (0.1 M, pH=7.4) was added and homogenized by sonication at 4 °C using a Cole Farmer 4710 series ultrasonic homogenizer (Cole Farmer). Soft tissues homogenate of each animal were divided in aliquots and were kept at –80 °C until analysis.

Biochemical analysis

Clinical biochemical analyses included AST, ALT, ALP and LDH activities which were determined with standard methods and commercial kits and biochemical auto analyzer (Alpha Classic AT^{++}, Iran).

Antioxidant enzymes

The SOD and GPX activity were measured by commercial kits (RANSOD kit, Randox Com, UK). Also, the activity of CAT was measured using the commercial catalase assay kit (Oxford Biomedical Research, Inc., USA) based on the colorimetric method.

Measurement of MDA

The modified HPLC method was employed to measure MDA. Final product was analysed by UV spectrophotometer at 532 nm and values were finally expressed as mmol/l.

Statistical analysis

The values are reported as mean±SD. Data were analyzed by one way ANOVA and for comparison between groups post hoc (Tukey) were performed using SPSS version 17 software. The significance level was considered (P<0.05) and included in the study.

Results

The effect of administration of saffron on body weight of the rats during the treatment period is shown in Figure 1A. The results showed 10.13% reduction in the body weight of diabetic rats in control group during this period; while in the saffron treated group, there was no reduction in weight during the treatment period. In addition, the body weight of treated group with saffron showed 2 % increase in compared to the beginning of the experiment; however this finding was not statistically significant.

FBS measurements were performed 72 hours after administration of STZ. The results showed a significant increase in FBS of diabetic rats (>250 mg/dl) compared to the control group and saffron control group (P<0.05). At the end of the experiment and after treatment with aqueous extract of saffron, FBS levels were measured in all groups. The results showed that in diabetic control group there was a significant increase in the FBS compared to the beginning of the experiment (P<0.05); however in treated group with saffron the FBS levels showed a significant decrease (P<0.05) compared with the beginning of the period (Fig. 1B).

The activity of the AST enzyme in the testis of rats in the diabetic group showed a significant increase (P<0.05) compared to normal control group. Moreover, the activity of this enzyme in saffron treated group showed a significant decrease (P>0.05) compared to the diabetic control group (Fig. 2A).

ALT activity in the testis of diabetic control group showed 14 % increase compared to normal control group which is not statistically significant (P>0.05). Moreover, the level of ALT in saffron control group showed no significant difference compared to control group. On the other hand, the activity of this enzyme in the saffron treated group showed no significant difference compared to saffron control group and normal control group (Fig. 2B). The activity of ALP enzyme in the testis of diabetic control group

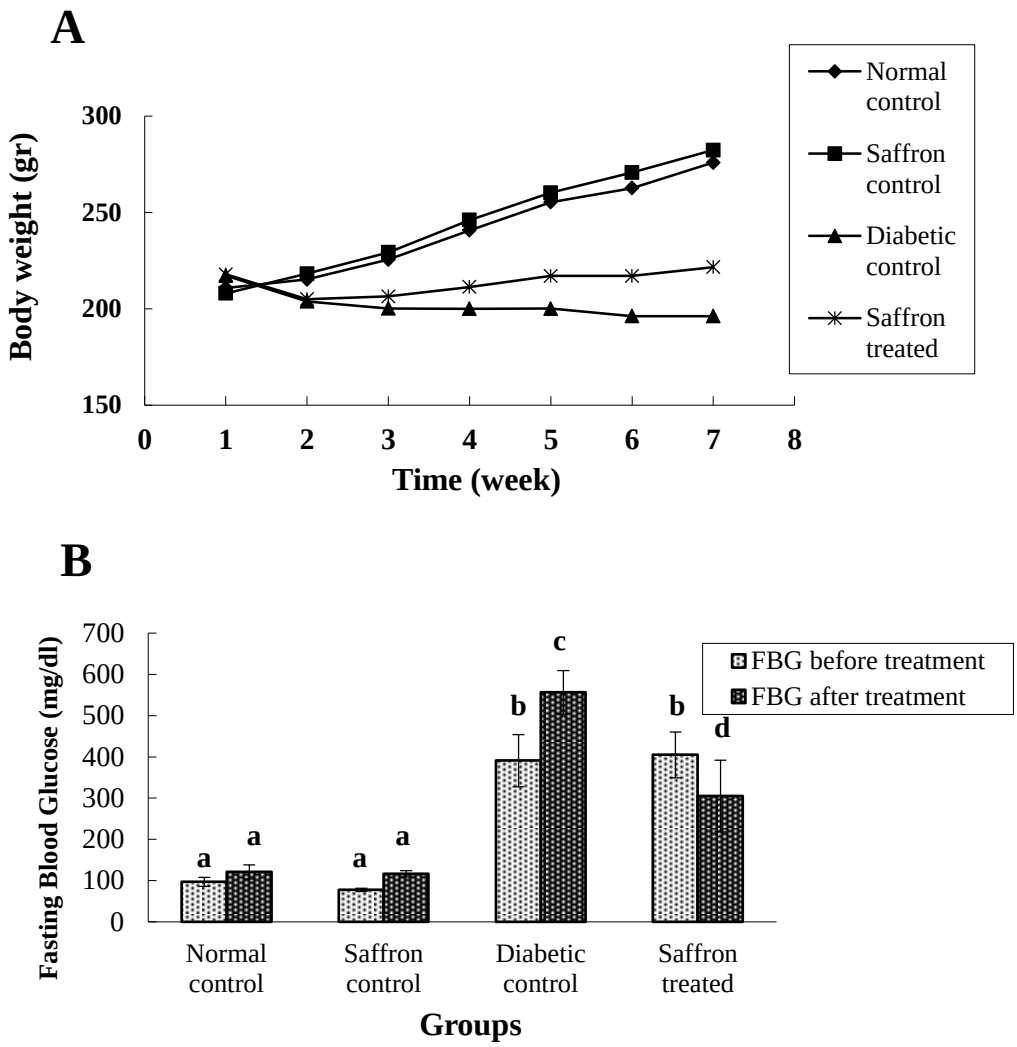

Fig.1. Effect of saffron extract on body weight (A) and fasting blood glucose level (B) in rats.

significantly increased compared to normal control group and saffron control group (P<0.05). Administration of saffron in the diabetic rats ameliorated the activity of ALP by 15% compared to diabetic control group but, this finding was not statistically significant. Additionally, the activity of this enzyme in saffron control group showed no significant difference compared to normal control group (Fig. 2C). LDH activity in the testis of diabetic rats showed a significant increase compared to other three groups (P<0.05). Furthermore, administration of saffron normalized activity of LDH (Fig. 2D).

The activity of antioxidant enzymes (GPx, CAT and SOD) and the level of MDA are shown in Figure 3. GPx activity showed no significant difference compared to normal control group and saffron control group. The activity of GPx was significantly increased in saffron treated group (Fig. 3A). Measurement of CAT shows that there is a reduction in the level of this enzyme in diabetic group, although this finding was not significant. Moreover, the level of CAT in the saffron treated group showed a significant increase compared to diabetic control group. On the other hand, the activity of CAT in saffron control group showed no significant difference compare to normal control group. The level of SOD and MDA in testis of rats showed no significant difference among four groups included in the experiment.

Discussion

Diabetes mellitus is a degenerative disease that has deleterious effects on male reproductive function, possibly through an increase in oxidative stress (Amaral et al., 2008). Testis is the primary organ responsible for production of sperm. Abnormalities in spermatogenesis due to increased apoptosis cause by oxidative stress during diabetes have been studied

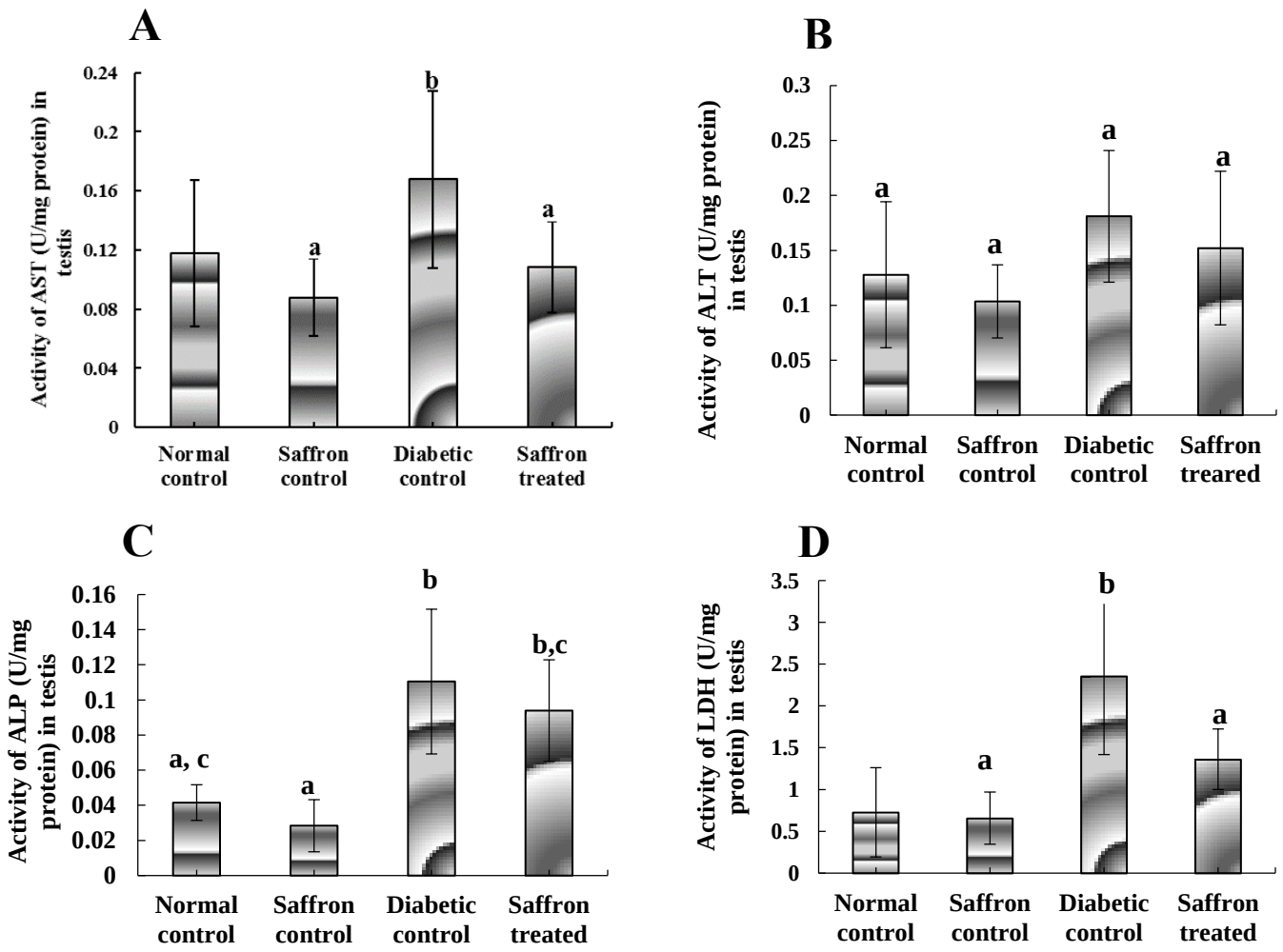

Fig.2. Effect of saffron aqueous extract on the activity of A) AST (U/mg); B) ALT (U/mg); C) ALP (U/mg) and D) LDH (U/mg) in testis tissue.

by many researchers (Cai et al., 2000; El-Demerdash et al., 2005; Mansour et al., 2002; Mohasseb et al., 2011; Roy et al, 2014). Alteration in testicular tissue as well as testis apoptosis is the most possible cause of infertility in diabetic individuals (Roy et al, 2014). Therefore, investigating the changes in enzymes and tissue components of testis is critical in order to minimize the complications of diabetes in this organ.

Evocation of free radical production and reduction of antioxidant defense system are results of the persistent and chronic hyperglycemia in diabetics (Baynes and Thorpe, 1996; Ihara et al., 1999). Recently agents with potential antioxidant activity have attracted considerable attention to prevent and protect oxidative damage (Stavic, 1994).

Several researchers have been shown that induction of diabetes with STZ decreases the body weight in rats (Ene et al., 2007; Erjaee et al., 2015; Haidari et al., 2011). Diabetic patients have insulin deficiency therefore they are not able to use the excessive glucose which is produced by gluconeogenesis and the result will be muscle wasting and weight loss (Shirwaikar et al., 2004). Outcome of the present study showed that administration of saffron is able to prevent the weight loss in diabetic rats. On the other hand, saffron has hypoglycemic effect which can reverse the gluconeogenesis thus, inhibits muscle wasting (Mohajeri et al., 2008). The persistent elevated blood glucose in diabetic control group proves that STZ destroys beta cells in pancreas. A significant decrease in blood glucose was observed in treated group with saffron (P<0.05). The hypoglycemic effect of saffron along with reversing weight loss has been reported by many researches. Kianbakht and Hajiaghaee (2011) reported that saffron reduces blood glucose in diabetic rats. Mohajeri et al. (2008) showed that saffron ethanolic extract decreases fasting blood sugar in alloxan

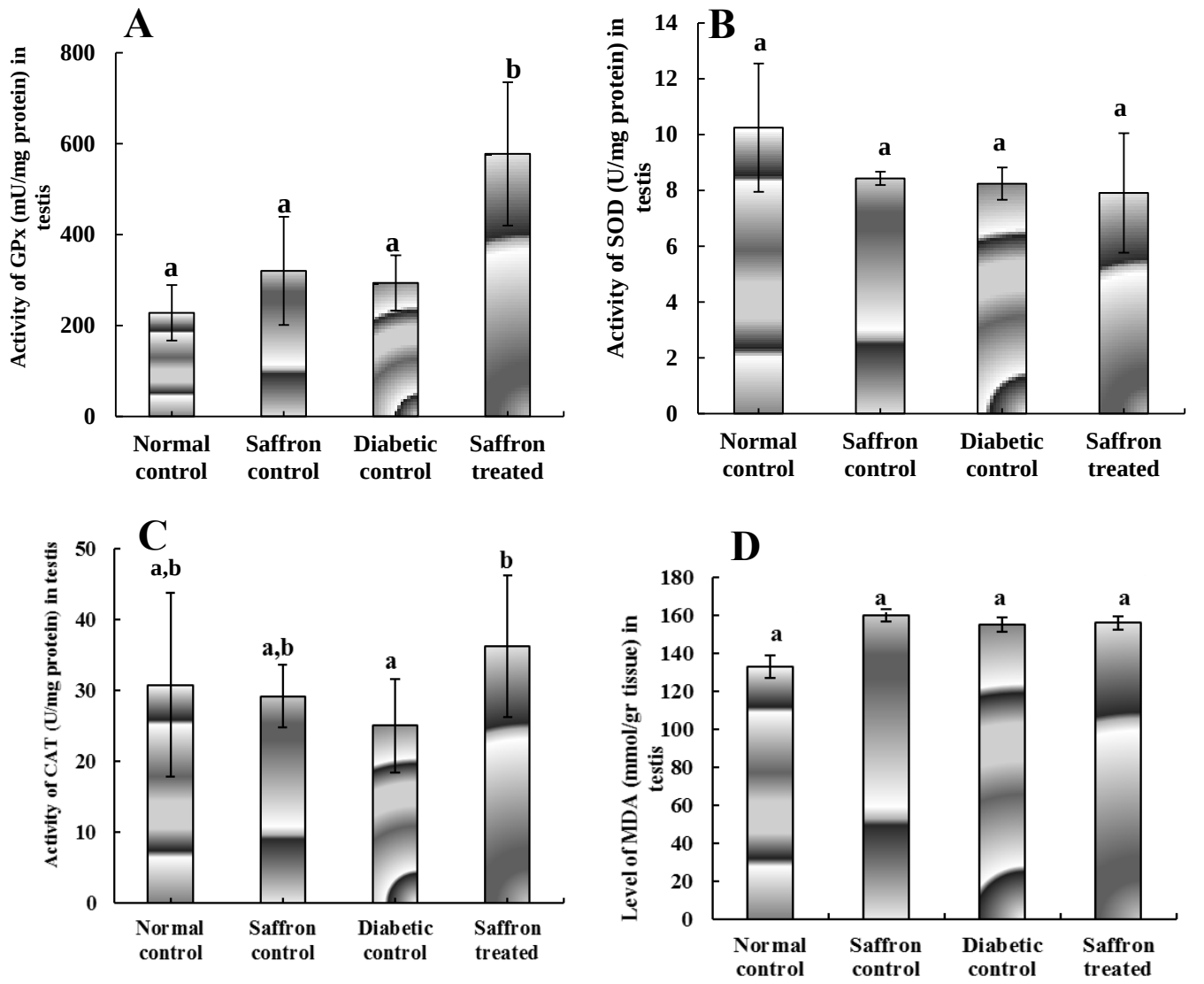

Fig.3. Effect of saffron aqueous extract on the activity of A) GPx (U/mg); B) SOD (U/mg); C) CAT (U/mg) and D) MDA (mmol/gr tissue) level in testis tissue.

diabetic rats by providing a regenerative modification against damage in endocrine cells of pancreas. In the study of Elgazar et al. (2013), saffaron extract improved body weight of diabetic rats and caused a significant change in serum level of blood glucose and insulin profile. Arasteh et al. (2010) reported that saffron extract has significant hypoglycemic effect, increases insulin level and it able to regenerate pancreatic cells. The exact hypoglycemic mechanism of saffron has not been proved yet; however, investigates of Kang et al. (2012) showed that saffron plays a beneficial role in glucose metabolism of differentiated C2C12 skeletal muscle cells. They suggested that activation of AMPK/ACC and MAPKs pathways is directly associated with saffron induced glucose uptake. Moreover, they have reported that saffron increases insulin sensitivity which is coupled to basal glucose translocation of GLUT4 through both of insulin independent (AMPK/ACC and MAPKs) and insulin-dependent (PI 3-kinase/Akt and mTOR) pathways. On the other hand, decreasing insulin resistance, motivating glucose uptake in peripheral tissues and inhibition of intestinal glucose absorption are other mechanisms involve in hypoglycemic effect of saffron (Xi et al., 2005; Yang et al., 2003; Youn et al., 2004).

Oxidative stress is now thought to make a significant contribution in pathogenies of diabetes. Both enzymatic and nonenzymatic antioxidants are responsible for detoxification of ROS in the body (Frei et al., 1988). Due to persistent oxidative stress, lipids and proteins will undergo oxidation, which is associated with changes in their structure and functions (Lobo et al., 2010). In the last decade,

research has demonstrated that plants with high antioxidant capacity play a crucial role in the prevention of diabetes. Saffron is one of these plants which exhibited significant radical scavenging and antioxidant (Khajuria et al., 2010). The results of the present study showed that changes in activity of antioxidant enzymes (SOD, GPx and CAT) and MDA level in the testis of diabetic control rats are not significant compared to the normal control group (P<0.05); however, administration of saffron in diabetic rats significantly increased the activity of antioxidant enzymes CAT and GPx compared to diabetic control group. One of the reasons may be given in this regard is that sexual dysfunction and reproductive problems is a late complications of diabetes. Besides, according to the results presented in this study, the progress of oxidative stress is started in the testis of diabetic rats during 5 weeks experiment. Previous studies reported that diabetes increases the level of oxidative stress in the testis (El-Demerdash et al., 2005; Mansour et al., 2002; Mohasseb et al., 2011). In most of these studies in order to observe the effect of diabetes on testicular tissue, a longer testing period was selected. Therefore, the results of the present study provide new information about the effect of time on the pathogenesis of diabetes on the male reproductive function. Gobbo et al. (2015) investigated the antioxidant system response of male reproductive organs during early and late phases of diabetes and the influence of melatonin treatment. They have showed that the enzymes activities and lipid peroxidation is not affected in testis of diabetic rats after one week or two months. In the study of Amaral et al. (2006), major changes in the testis has been achieved after 3 months of STZ administration. Moreover, they have reported that no differences in carbonyl groups (protein oxidation) are found during these 3 months experiment. In another research by Amaral et al. (2009), several aspects of mitochondrial function were measured including mitochondrial calcium loading capacity, as well as respiratory and electric potential function. Additionally, oxidative stress production, antioxidant levels and possible apoptotic alterations were also evaluated in the study of Amaral et al. (2009). They suggested that in animal models that mimic untreated type 1 diabetes the severe effects of the condition on spermatogenesis are not directly mitochondrial mediated. According to the Amaral et al. (2006) and Amaral et al. (2009) findings which stated that oxidation of proteins remains unchanged within one month after induction of diabetes and the function of mitochondria is normal in diabetes; the lack of variation in the activity of antioxidant enzymes in diabetic group in the present study could be well explained.

Following treatment with saffron, the activity of CAT and GPx significantly increased in the testis compared to diabetic control group (P<0.05). This is the first report regarding the effect of saffron extract on oxidative stress indicators in testis of diabetic rats. There are several studies demonstrated the antioxidant and antidiabetic properties of saffron active ingredients including safranal, crocin, crocetin and quercetin. Crocin, a powerful antioxidant found in saffron extract have been shown significant hypoglycemic and hypolipidemic effects in STZ induced type 2 diabetic. Results of Shirali et al. (2013) showed that crocin is able to significantly decrease the levels of serum glucose and advanced glycation end products, triglyceride, total cholesterol, low-density lipoprotein and increases the high-density lipoprotein in the diabetic rats. Moreover, reports from Rajaei et al. (2013) study showed the anti-hyperglycemic effect of saffron in STZ diabetic rats. Researches from Jin et al. (2009) also demonstrated that crocin decreases the contents of fasting serum glucose and hyperlipemia and increases the sugar tolerance. On the other hand, the results of Asri-Rezaei et al. (2015) indicated that separate and combined treatments with crocin and zinc chloride is able to improve the blood levels of zinc, glucose, insulin, MDA and total antioxidant capacity in STZ diabetic rats. Farshid and Tammadonfard (2015) showed neuroprotective effects of crocin, safranal and insulin in a rat model of diabetic neuropathy. They have reported that crocin and safranal enhances the neuroprotective effect of insulin. They have also suggested that the neuroprotective effects of these chemical compounds are due to their anti-hyperglycemic and antioxidant properties.

In a study by Samarghandian et al. (2014), safranal showed preventive effect against oxidative damage in aged male rat brain. Both brain and testis are surrounded by barriers. The blood-testis barrier (BTB) is one of the tightest blood-tissue *barriers* in the mammalian body and the blood–brain barrier (BBB) is a highly selective semipermeable membrane

barrier. Therefore, when safranal is able to cross BBB is more probable to also cross BTB. As a result, it is suggested that safranal is one of the important constitute of saffron which probably have the ameliorating effect against oxidative stress in testis.

Crocetin, another phenolic component in saffron, have various pharmacological activity including antioxidant activity (Magesh et al., 2006). The antioxidant property of crocetin makes it a suitable insulin sanitizing compound. It has been reported that crocetin ameliorates the defects related to insulin resistance including impaired glucose tolerance, hyperinsulinemia, dyslipidemia and hypertension due to high-fructose diet and dexamethasone injection in rats (Xi et al., 2005). Moreover, the insulin resistance caused by high-fat diet in rats is enhanced by crocetin (Sheng et al., 2008). In addition, crocetin inhibited adhesion of leukocytes to the bovine aortic endothelial cells (BEC) induced by advanced glycation end products (AGEs) and AGEs-induced BEC apoptosis possibly through its antioxidant activity and thus it has been suggested that crocetin may prevent diabetes-associated vascular complications (Xiang et al., 2006a; Xiang et al. 2006b).

Kanter et al. (2012) studied the protective effects of quercetin, against apoptosis and oxidative stress in testis of diabetic rats. In this research they indicated that quercetin administration attenuated diabetes related testicular dysfunction by renewing the activities of the antioxidant enzymes and down regulation the levels of ROS. Quercetin is one of the flavonoids found in saffron which prevents oxidant injury and cell death by several mechanisms, such as scavenging oxygen radicals and protecting against lipid peroxidation (Bors et al., 1990; Inal et al., 2002).

AST, ALT, ALP and LDH are present in many mammalian tissues and have been studied for the last several years (Sharma et al. 2014). Oxidative stress followed by tissue damage causes an increase in various enzymes at tissues including testis (Iwai et al., 2001; Ohta et al., 2007). In the present study the effect of saffron aqueous extract on the biochemical activity of enzymes AST, ALT, ALP and LDH in testicular tissue were also evaluated. Results showed that saffron is able to normalize these enzymes in the testis of diabetic rats. Therefore, it could be suggested that the oxidative stress induced by STZ may mediate the disturbance in testis which is reflected by the present increase in AST, ALT, ALP and LDH. These results are in agreement with the findings of Mansour et al. (2002) and El-Demerdash (2005) who showed that the activity of AST, ALT and LDH is increased in the testis of rats with diabetes (Mansour et al., 2002). Saffron ameliorates the effect of oxidative stress on testis by alteration in AST, ALT, ALP as well as LDH and these enzymes could be used as a marker for showing impairment in male reproductive system.

Conclusion

Infertility in young men with type 1 or type 2 diabetes is showing a dramatic increase in recent years and testes have vital functions that are very important to the male reproductive system. The results showed that the induction of diabetes in experimental animals can affect on testicular tissue and administration of saffron extract is effective in alleviating the complication on this tissue due to its remarkable antioxidant effect. However, further researches are needed to illustrate its exact mechanism of action on diabetes.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflicts of interest.

References

Amaral S, Moreno AJ, Santos MS, Seiça R, Ramalho-Santos J. Effects of hyperglycemia on sperm and testicular cells of goto-kakizaki and streptozotocin-treated rat models for diabetes. Theriogenology 2006; 66: 2056-2067.

Amaral S, Mota PC, Lacerda B, Alves M, Pereira Mde L, Oliveira PJ, et al. Testicular mitochondrial alterations in untreated streptozotocin-induced diabetic rats. Mitochondrion 2009; 9: 41-50.

Amaral S, Oliveira PJ, Ramalho-Santos J. Diabetes and the impairment of reproductive function: Possible role of mitochondria and reactive oxygen species. Curr

Diabetes Rev 2008; 4: 46-54.

Arasteh A, Aliyev A, Khamnei S, Delazar A, Mesgari M, Mehmannavaz Y. Crocus sativus on serum glucose, insulin and cholesterol levels in healthy male rats. J Med Plants Res 2010; 4: 397-402.

Asri-Rezaei S, Tamaddonfard E, Ghasemsoltani-Momtaz B, Erfanparast A, Gholamalipour S. Effects of crocin and zinc chloride on blood levels of zinc and metabolic and oxidative parameters in streptozotocin-induced diabetic rats. Avicenna J Phytomed 2015; 5: 403-12.

Baccetti B, La Marca A, Piomboni P, Capitani S, Bruni E, Petraglia F, et al. Insulin-dependent diabetes in men is associated with hypothalamo-pituitary derangement and with impairment in semen quality. Hum Reprod 2002; 17: 2673-2677.

Baynes JW, Thorpe SR. The role of oxidative stress in diabetic complications. Curr Opin Endocrinol Diabetes Obes 1996; 3: 277-284.

Bors W, Heller W, Michel C, Saran M. Flavonoids as antioxidants: Determination of radical-scavenging efficiencies. Methods Enzymol 1990; 186: 343-355.

Brondum E, Nilsson H, Aalkjær C. Functional abnormalities in isolated arteries from goto-kakizaki and streptozotocin-treated diabetic rat models. Horm Metab Res 2005; 37: 56-60.

Cai L, Chen S, Evans T, Deng DX, Mukherjee K, Chakrabarti S. Apoptotic germ-cell death and testicular damage in experimental diabetes: Prevention by endothelin antagonism. Urol Res 2000; 28: 342-347.

El-Demerdash FM, Yousef MI, El-Naga NI. Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. Food Chem Toxicol 2005; 43: 57-63.

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.

Ene A, Nwankwo E, Samdi L. Alloxan-induced diabetes in rats and the effects of black caraway (carum carvi l.) oil on their body weight. Res J Med Med Sci 2007; 2: 48-52.

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.

Farshid AA, Tamaddonfard E. Histopathological and behavioral evaluations of the effects of crocin, safranal and insulin on diabetic peripheral neuropathy in rats. Avicenna J Phytomed 2015; 5: 469-78.

Frei B, Stocker R, Ames BN. Antioxidant defenses and lipid peroxidation in human blood plasma. Proc Natl Acad Sci U S A 1988; 85: 9748-9752.

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.

Haidari F, Seyed-Sadjadi N, Taha-Jalali M, Mohammed-Shahi M. The effect of oral administration of carum carvi on weight, serum glucose, and lipid profile in streptozotocin-induced diabetic rats. Saudi Med J 2011; 32: 695-700.

Ihara Y, Toyokuni S, Uchida K, Odaka H, Tanaka T, Ikeda H, et al. Hyperglycemia causes oxidative stress in pancreatic beta-cells of gk rats, a model of type 2 diabetes. Diabetes 1999; 48: 927-32.

Inal M, Akgün A, Kahraman A. Radioprotective effects of exogenous glutathione against wholebody g-ray irradiation: Age-and gender-related changes in malondialdehyde levels, superoxide dismutase and catalase activities in rat liver. Methods Find Exp Clin Pharmacol 2002; 24: 209-12.

Iwai K, Onodera A, Matsue H. Antioxidant activity and inhibitory effect of gamazumi (viburnum dilatatum thunb.) on oxidative damage induced by water immersion restraint stress in rats. Int J Food Sci Nutr 2001; 52: 443-451.

Jain GC, Jangir RN. Modulation of diabetes-mellitus-induced male reproductive dysfunctions in experimental animal models with medicinal plants. Pharmacogn Rev 2014; 8: 113-21.

Jin YZ, Xie WL, He RB. Effect of crocin on decreasing hyperglycaemia and hyperlipemia in experimental hyperlipemia and non-insulin dependent diabetes rats. Acta Acad Med CPAF 2009; 3.

Kang C, Lee H, Jung ES, Seyedian R, Jo M, Kim J, et al. Saffron (crocus sativus l.) increases glucose uptake and insulin sensitivity in muscle cells via multipathway mechanisms. Food Chem 2012; 135: 2350-2358.

Kanter M, Aktas C, Erboga M. Protective effects of quercetin against apoptosis and oxidative stress in streptozotocin-induced diabetic rat testis. Food Chem Toxicol 2012; 50: 719-725.

Khajuria DK, Asad M, Asdaq S, Kumar P. The potency of crocus sativus (saffron) and its constituent crocin as an immunomodulator in animals. Lat Am J Pharm 2010; 29.

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.

Koh PO. Streptozotocin-induced diabetes increases apoptosis through jnk phosphorylation and bax activation in rat testes. J Vet Med Sci 2007; 69: 969-971.

Laakso M. Cardiovascular disease in type 2 diabetes: Challenge for treatment and prevention. J Intern Med 2001; 249: 225-235.

Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 2010; 4: 118-26.

Magesh V, Singh JP, Selvendiran K, Ekambaram G, Sakthisekaran D. Antitumour activity of crocetin in accordance to tumor incidence, antioxidant status, drug metabolizing enzymes and histopathological studies. Mol Cell Biochem 2006; 287: 127-135.

Mansour HA, Newairy AS, Yousef MI, Sheweita SA. Biochemical study on the effects of some egyptian

herbs in alloxan-induced diabetic rats. Toxicology 2002; 170: 221-228.

Mohajeri D, Tabrizi BA, 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.

Mohasseb M, Ebied S, Yehia MA, Hussein N. Testicular oxidative damage and role of combined antioxidant supplementation in experimental diabetic rats. J Physiol Biochem 2011; 67: 185-194.

Ohta Y, Chiba S, Tada M, Imai Y, Kitagawa A. Development of oxidative stress and cell damage in the liver of rats with water-immersion restraint stress. Redox Rep 2007; 12: 139-147.

Rajaei Z, Hadjzadeh MAR, Nemati H, Hosseini M, Ahmadi M, Shafiee S. Antihyperglycemic and antioxidant activity of crocin in streptozotocin-induced diabetic rats. J Med Food 2013; 16: 206-210.

Rios JL, Recio MC, Giner RM, Manez S. An update review of saffron and its active constituents. Phytother Res 1996; 10: 189-193.

Roy S, Metya SK, Rahaman N, Sannigrahi S, Ahmed F. Ferulic acid in the treatment of post-diabetes testicular damage: Relevance to the down regulation of apoptosis correlates with antioxidant status via modulation of tgf-β1, il-1β and akt signalling. Cell Biochem Funct 2014; 32: 115-124.

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: 1-12.

Scarano WR, Messias AG, Oliva SU, Klinefelter GR, Kempinas WG. Sexual behaviour, sperm quantity and quality after short-term streptozotocin-induced hyperglycaemia in rats. Int J Androl 2006; 29: 482-488.

Sharma U, Pal D, Prasad R. Alkaline phosphatase: An overview. Indian J Clin Biochem 2014; 29: 269-278.

Sheng L, Qian Z, Shi Y, Yang L, Xi L, Zhao B, et al. Crocetin improves the insulin resistance induced by high-fat diet in rats. Br J Pharmacol 2008; 154: 1016-1024.

Shirali S, Zahra Bathaie S, Nakhjavani M. Effect of crocin on the insulin resistance and lipid profile of streptozotocin-induced diabetic rats. Phytother Res 2013; 27: 1042-1047.

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.

Stavic B. Role of chemopreventers in human diet. Clin Biochem 1994; 27: 319-332.

Xi L, Qian Z, Shen X, Wen N, Zhang Y. Crocetin prevents dexamethasone-induced insulin resistance in rats. Planta Med 2005; 71: 917-922.

Xiang M, Qian ZY, Zhou CH, Liu J, Li WN. Crocetin inhibits leukocyte adherence to vascular endothelial cells induced by ages. J Ethnopharmacol 2006a; 107: 25-31.

Xiang M, Yang M, Zhou C, Liu J, Li W, Qian Z. Crocetin prevents ages-induced vascular endothelial cell apoptosis. Pharmacol Res 2006b; 54: 268-274.

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.

Youn JY, Park HY, Cho KH. Anti-hyperglycemic activity of commelina communis l.: Inhibition of α-glucosidase. Diabetes Res Clin Pract 2004; 66: S149-S155.