

Protective effects of a standardized extract of *Iris germanica* on pancreas and liver in streptozotocin-induced diabetic rats

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Abstract

Background and purpose: Previous studies have shown the antioxidant, anti-inflammatory, immunomodulatory, and hypolipidemic activities of *Iris germanica*. The aim of the present study was to evaluate the protective effects of hydroalcoholic extract of *Iris germanica* rhizomes on streptozotocin-induced diabetic rats.

Experimental approach: Twenty-four male Wistar rats were randomly assigned into four groups including a normal control group, diabetic control group, diabetic groups treated for 4 weeks with 100 and 200 mg/kg/day of the *Iris germanica* extract (IGE).

Findings/Results: Induction of diabetes significantly decreased the body weight gain and considerably increased the serum levels of glucose, triglyceride, blood urea nitrogen (BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). Diabetes also diminished the antioxidant capacity of the liver (decrease of thiol groups) and significantly degenerated pancreatic islands. The IGE at both doses of 100 and 200 mg/kg significantly reduced the levels of glucose, triglyceride, AST, ALT, and ALP. Moreover, IGE increased the total antioxidant capacity of the liver and ameliorated pancreatic island morphology. The extract had no significant effect on body weight and BUN level.

Conclusion and implication: These findings suggest that *Iris germanica* rhizomes inhibits the progression of hyperglycemia and hypertriglyceridemia and has protective effects against diabetes-induced injury of the liver and pancreas. Therefore, this plant has the potential to be used as a natural product for controlling diabetes.

Keywords: Diabetes; Glucose; *Iris germanica*; Lipids; Oxidative stress.

INTRODUCTION

Diabetes mellitus is a complex metabolic disorder, with a global prevalence of approximately 425 million people in 2017 (1). Despite current advances in the management of diabetic patients, a large number of them show severe diabetes complications particularly microvascular problems in the eyes, kidneys, and nerves (2,3). In addition, available glucose-lowering drugs have the potential to induce several side effects such as hypoglycemia, myalgia, body weight change, and gastrointestinal problems (4,5).

An increasing number of patients with diabetes use complementary and alternative therapies for controlling blood glucose (6). Experimental and clinical studies have confirmed the antidiabetic effects of herbal extracts and compounds isolated from medicinal plants (7). These antidiabetic effects include reducing blood glucose, improving dyslipidemia, modulating inflammatory responses, and inhibiting oxidative stress (8,9).

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Iris germanica Linn., a species of Iridaceae family, is widely distributed in temperate regions of the world. This plant has some health benefits, including antioxidant, anti-inflammatory, immunomodulatory, antimutagenic, and antimicrobial activities (10-13). It has been reported that ethanolic extract of *Iris germanica* rhizomes reduces the serum level of cholesterol and triglycerides in rats fed on a high-fat diet (14). Also, flavonoids isolated from rhizomes of *Iris germanica* were shown to inhibit α -amylase, the major digestive enzyme that catalyzes the hydrolysis of starch into glucose and maltose (15). However, so far, no *in vivo* study has been conducted on the antidiabetic effects of this plant. The aim of the present work was to evaluate the effects of *Iris germanica* rhizomes on fasting blood glucose (FBG), serum lipids, and diabetes-related changes in the liver of streptozotocin (STZ)-induced hyperglycemic rats.

MATERIALS AND METHODS

Plant material and extraction

Iris germanica was collected in spring from Mashhad (Northeast Iran) and identified by Mrs. M. Souzani at the herbarium of Faculty of Pharmacy (Mashhad University of Medical Sciences), where a voucher specimen was deposited (No. 13254). The rhizomes were washed, freshly cut into small pieces, and crushed in a juicer. The plant material (100 g) was suspended in 1 L of 70% ethanol (%v/v in distilled water) for 48 h at 40 °C under gentle shaking. The extract was filtered using filter paper (Whatman No. 1) and centrifuged for 5 min at 500 g to remove insoluble particles. The supernatant was dried at 40 °C and the solvent-free extract was kept at -20 °C until use.

Standardization of the extract

The *Iris germanica* extract (IGE) was standardized based on its content of phenolic compounds. A sample of 20 μ L of IGE (10 mg/mL) or gallic acid as a standard for phenolic compounds (0, 50, 100, 150, 250, and 500 mg/L) was added to 150 μ L of deionized water, 100 μ L of Folin-Ciocalteu reagent (Merck, Germany), and 300 μ L of sodium carbonate solution (1 mol/L). After 2 h, the

absorbance was measured by a spectrophotometer (Unico S-2100, USA) at 765 nm. The standard curve of gallic acid was plotted and the content of phenolic compounds of IGE was reported as milligram of gallic acid equivalents (16).

Animals and study groups

A total of 24 male Wistar rats (200-260 g) were randomly assigned into four groups (n = 6) including non-diabetic control group; diabetic control group; diabetic groups treated with IGE at the dose of 100 and 200 mg/kg/day. Diabetes was induced by a single intraperitoneal injection of STZ (Sigma, St. Louis, USA) at a dose of 65 mg/kg. After one week, the level of FBG was determined using an Accu-check active glucometer (Roche, Mannheim, Germany) and the rats with FBG \geq 200 mg/dL were enrolled in diabetic groups (16,17). The extract was administered by oral gavage once daily for 4 weeks. The animals in the control groups received saline in the same manner. Considering the body surface area of rat and human for dose translation, a dose of 200 mg/kg of IGE in the rat is approximately equivalent to 30 mg/kg in human (i.e., 2.2 g per day for a 70 kg adult), which is clinically relevant (18).

The study was approved by the animal ethical committee of Mashhad University of Medical Sciences, Iran (Ethical approval ID. IR.MUMS.REC.1396.53), and all experiments were carried out in accordance with the internationally accepted principles for laboratory animal use.

Serum analysis

At the end of the treatment period, the animals fasted for 12 h and blood samples were obtained by cardiac puncture. The samples were centrifuged at 500 g for 10 min and the obtained serums were kept at -20 °C until biochemical analysis. An automated biochemistry analyzer (BT 3000 plus, Biotechnica Instruments, Italy) was used for measuring the levels of glucose, creatinine, blood urea nitrogen (BUN), total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP).

Evaluation of thiol content

The content of thiol groups in the liver tissue was determined using the 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB; Sigma, St. Louis, USA). Briefly, 50 μ L of homogenized liver tissue was mixed with 1 mL of tris-EDTA buffer (pH 8.2). The absorbance of this mixture was measured at 412 nm (A1) against the buffer as blank. Then, 20 μ L of DTNB reagent (10 mmol/L) was added to the mixture and the absorbance was determined again (A2). The absorbance of DTNB (B) was also measured and the level of total thiol groups was calculated using the following equation:

$$\text{Thiol concentration (mmol/L)} = \frac{(A2 - A1 - B) \times 1.07}{0.05 \times 13.6}$$

Evaluation of superoxide dismutase activity

The superoxide dismutase (SOD) activity in the liver tissue was evaluated by a microplate colorimetric method based on its capability to inhibit the pyrogallol autoxidation. Pyrogallol generates superoxide ions through autoxidation and these ions convert 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan which is quantified by measuring the absorbance at 570 nm. The SOD activity was expressed as unit/mg of tissue protein.

Histological examination

At the end of the study, the pancreas and liver of the control and diabetic rats were removed and fixed in 10% formalin. The tissue specimens were embedded in paraffin blocks

and sectioned for staining with hematoxylin and eosin. The observation was performed using DMRB microscope (Leica, India), and the photographs were taken using a digital camera (Canon PowerShot S70, Japan).

Statistical analysis

Statistical comparison of biochemical data among different groups was performed by one-way analysis of variance (ANOVA) followed by Dunnett post hoc test. A paired *t*-test was used to compare the FBG before and after the study. The change in the body weight during different days of the study was compared by the repeated measures two-way ANOVA (day as the within-subjects factor and group as between-subjects factor). A *P*-value of less than 0.05 was considered statistically significant. Results are presented as mean \pm SEM.

RESULTS

Content of phenolic compounds in *Iris germanica*

The content of total phenols in IGE was 71 mg gallic acid equivalent per gram of the extract. Since the solid residue obtained from the plant extract was 15%, each gram of *Iris germanica* rhizome contains 10.6 mg phenolic compounds equivalent to gallic acid.

The body weight and blood glucose

Before the injection of STZ, the body weight was not statistically different between the animal groups (Table 1).

Table 1. Effects of the rhizomes extract of *Iris germanica* on the body weight of diabetic rats. Values are expressed as mean \pm SEM, n = 6. **P* < 0.05 and ****P* < 0.001 indicate significant differences in comparison with the diabetic control; #*P* < 0.05, ##*P* < 0.01, and ###*P* < 0.001 versus basal group in the corresponding group.

Time grouping	Body weight (g) in different groups			
	Normal control	Diabetic control	Diabetic IGE 100 (mg/kg)	Diabetic IGE 200 (mg/kg)
Before streptozotocin (Basal)	219.3 \pm 7.5	222.7 \pm 7.5	239.0 \pm 7.8	230.8 \pm 9.6
After streptozotocin (Day 1)	232.3 \pm 7.1###	205.6 \pm 6.3#	224.4 \pm 10.1#	223.5 \pm 9.8#
Day 7	258.4 \pm 6.5*,###	203.8 \pm 6.2##	222.5 \pm 19.1	218.2 \pm 6.3#
Day 14	284.5 \pm 5.9***,###	201.4 \pm 7.2##	228.0 \pm 19.3	207.9 \pm 10.8##
Day 21	294.3 \pm 5.6***,###	198.4 \pm 8.1##	224.2 \pm 19.9	205.5 \pm 9.3##
Day 28	300.2 \pm 5.8***,###	192.4 \pm 7.4###	210.8 \pm 19.5	201.7 \pm 10.6##

IGE, *Iris germanica* extract.

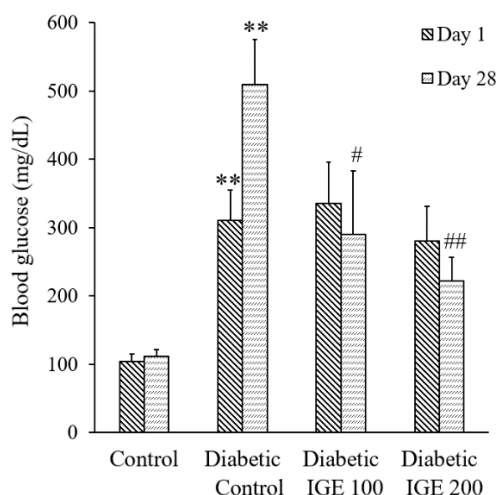


Fig. 1. The effects of *Iris germanica* rhizomes extract (100 and 200 mg/kg) on the level of fasting blood glucose in streptozotocin-induced diabetic rats. Data are shown as mean \pm SEM, n = 6. ** $P < 0.01$ Indicates significant differences versus the control group; # $P < 0.05$ and ## $P < 0.01$ compared to the diabetic control group. IGE, *Iris germanica* extract.

The repeated measures ANOVA showed that the weight of animals significantly altered with time, as a within-subjects factor ($F_{(5, 100)} = 3, P < 0.05$). Also, a significant effect of group, as a between-subjects factor, was found on the body weight ($F_{(3, 20)} = 8.5, P < 0.001$). At the end of the study, the non-diabetic group showed an increased body weight compared to the basal

level ($P < 0.001$). On the other hand, a significant weight loss was observed in the diabetic control group ($P < 0.001$). Treatment with the IGE had no significant effect on body weight.

Before starting the treatment (day 1), the level of FBG was significantly higher ($P < 0.01$) in diabetic groups compared to the normal control rats (Fig. 1). The diabetic control group showed a further increase in FBG level at day 28. Administration of both 100 and 200 mg/kg of IGE significantly inhibited the progression of hyperglycemia ($P < 0.05$).

Serum biochemical changes

While the level of total cholesterol did not change during the study period, the serum concentration of triglycerides was significantly higher ($P < 0.05$) in diabetic control rats compared to the non-diabetic group (Fig. 2A and B). Administration of both doses of IGE to diabetic animals significantly ($P < 0.05$) decreased the level of triglycerides. Also, while the level of creatinine did not alter during the study, a significant increase in the level of BUN ($P < 0.01$) was observed in diabetic groups when compared to the non-diabetic group (Fig. 2C and D).

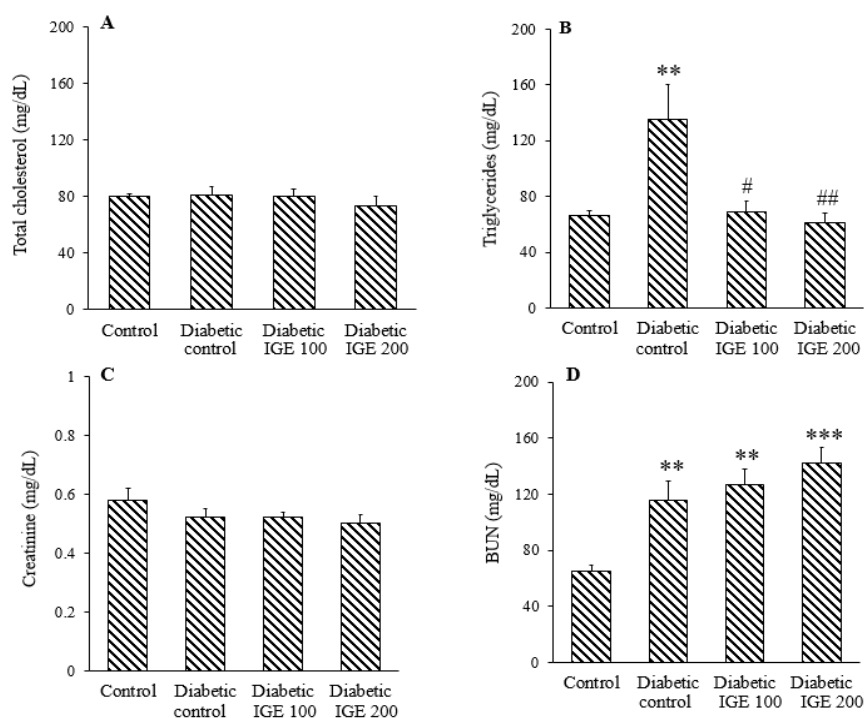


Fig. 2. The effects of *Iris germanica* rhizomes extract (100 and 200 mg/kg) on (A and B) the serum lipids and (C and D) levels of creatinine and BUN in streptozotocin-induced diabetic rats. Data are shown as mean \pm SEM, n = 6. ** $P < 0.01$ and *** $P < 0.001$ Indicate significant differences versus the control group; # $P < 0.05$ and ## $P < 0.01$ compared to the diabetic group. IGE, *Iris germanica* extract; BUN, blood urea nitrogen.

Treatment with IGE had no effect on the serum BUN with respect to the diabetic control group.

Induction of diabetes significantly increased ($P < 0.01$) the serum levels of liver enzymes ALT, AST, and ALP (Fig. 3). Administration of both doses of IGE could reduce the levels of liver enzymes in diabetic rats ($P < 0.05$).

Effects of *Iris germanica* on tissue oxidative stress

The content of thiol groups in the liver of diabetic control rats was lower than those in normal control rats (Fig. 4A). IGE at 200 mg/kg could increase the content of thiol groups in

diabetic animals ($P < 0.05$). There was no significant difference in the liver SOD activity between the study groups (Fig. 4B).

Histopathological changes

As shown in Fig. 5, the pancreas of nondiabetic rats had normal lobular architecture composed of exocrine and endocrine components. On the other hand, the pancreas of diabetic rats showed severe degeneration of the islands of Langerhans. The treatment with IGE ameliorated pancreatic island morphology. Except for a few residual cytoplasmic vacuoles, no significant histopathological lesion was observed in the liver of either group on day 28 (images not shown).

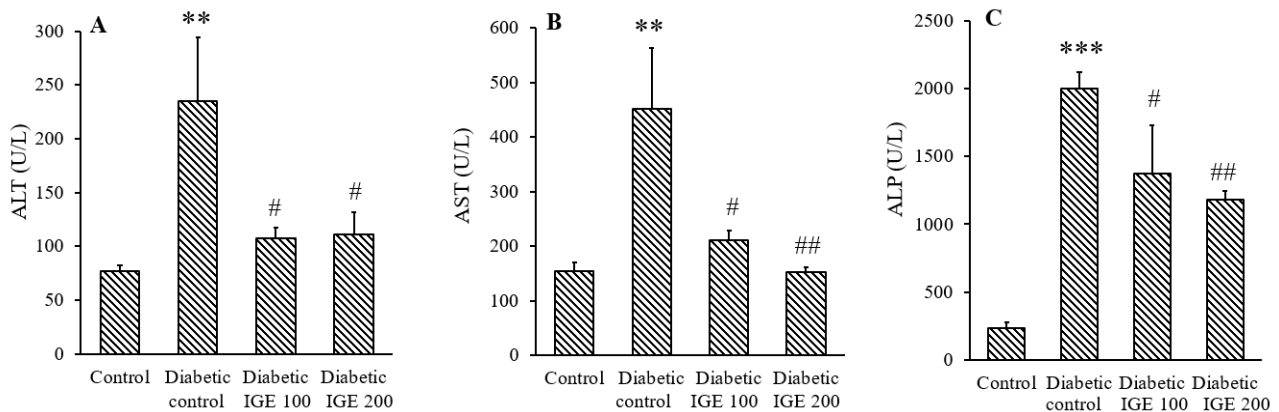


Fig. 3. The effects of *Iris germanica* rhizomes extract (100 and 200 mg/kg) on the serum levels of (A) ALT, (B) AST, and (C) ALP in streptozotocin-induced diabetic rats. Data are shown as mean \pm SEM, $n = 6$. ** $P < 0.01$ and *** $P < 0.001$ Indicate significant differences versus the control group; # $P < 0.05$ and ## $P < 0.01$ compared to the diabetic group. IGE, *Iris germanica* extract; ALT, alanine aminotransferase; AST, aspartate aminotransferase; and ALP; alkaline phosphatase.

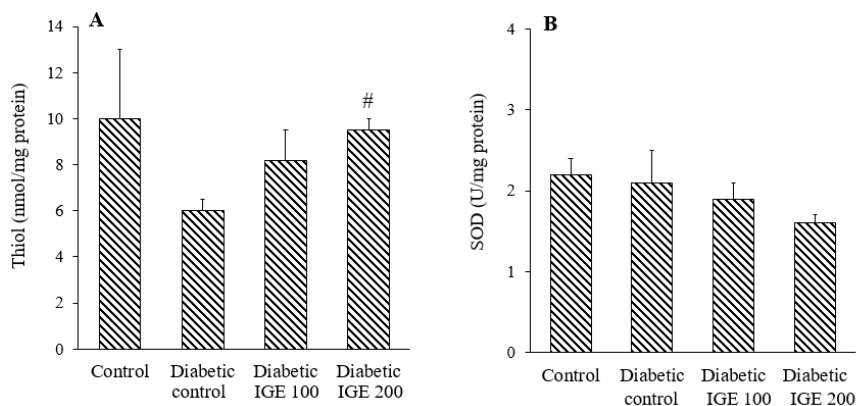


Fig. 4. The effects of *Iris germanica* rhizomes extract (100 and 200 mg/kg) on (A) the content of thiol groups and (B) activity of SOD in streptozotocin-induced diabetic rats. Data are shown as mean \pm SEM, $n = 6$. # $P < 0.05$ Indicate significant difference in comparison with the diabetic group. IGE, *Iris germanica* extract; SOD, superoxide dismutase.

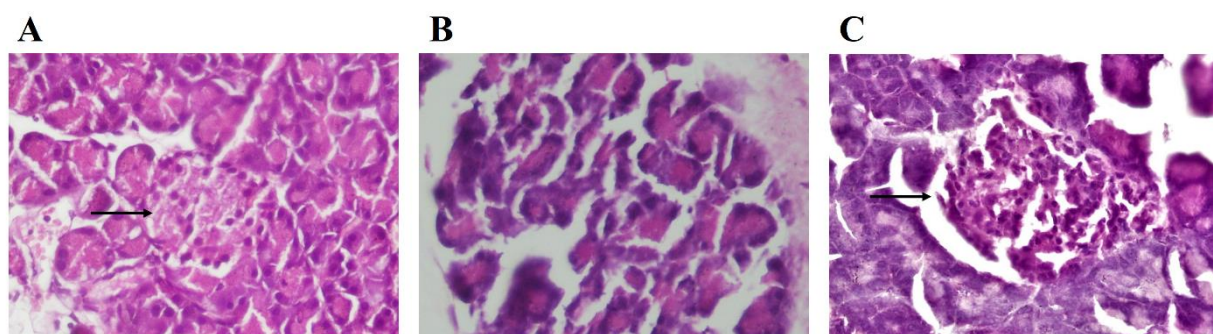


Fig. 5. Effect of *Iris germanica* rhizomes extract on the histopathological changes in the pancreas of streptozotocin-induced diabetic rats. (A) Normal pancreatic tissue with lobular architecture composed of exocrine and endocrine components (40×); (B) section of diabetic pancreas reveals no Langerhans island, the exocrine components are intact (40×); and (C) section of treated diabetic pancreas shows normal looking histology with mildly enlarged Langerhans island (40×). The black arrow shows a Langerhans island.

DISCUSSION

Previous studies have indicated that *Iris germanica* has antioxidant, anti-inflammatory, immunomodulatory, and hypolipidemic activities. Because the pathology of diabetes and its complications are associated with oxidative stress, inflammation, immune dysfunction, and dyslipidemia, the aim of this study was to evaluate the effects of *Iris germanica* on STZ-induced hyperglycemia.

The STZ is an alkylating agent that destroys pancreatic beta cells by modifying biological macromolecules and fragmenting DNA (19). Therefore, it develops symptoms of insulin-dependent diabetes including hyperglycemia and weight loss (16). In the present work, as expected, the pancreas of diabetic rats showed severe degeneration of the islands of Langerhans. Also, the diabetic control group began to show hyperglycemia and weight loss from day 1, which became worse on day 28. Although in the groups that received IGE the level of FBG at day 28 was not statistically different from the pretreatment level, both doses of IGE were able to prevent FBG from the further increase. This increases the possibility that IGE might have protected beta cells from further degeneration by STZ. Histopathological examination of the pancreas supports this possibility since treatment with IGE protected the islands of Langerhans against severe degeneration activity of STZ. Studies on diabetic animal models, isolated pancreatic islets, and insulin-releasing cell lines have shown that flavonoids can preserve the survival

and function of beta cells (20). Previous studies have shown that *Iris germanica* contains flavonoid compounds, which further support our histopathological results (15,21).

One of the limitations of our study is that we did not test glucose tolerance after oral administration of IGE. It is possible that the extract is able to diminish postprandial hyperglycemia, which should be evaluated by future experiments. This possibility is supported by the previous findings that flavonoids isolated from rhizomes of *Iris germanica* inhibit α -amylase and thereby reduce the hydrolysis of starch into glucose (15). Although our work is the first to study the effect of *Iris germanica* on blood glucose, one previous work showed that *Iris ensata*, another plant in the genus of *Iris*, had an antihyperglycemic effect in diabetic rabbits (22).

Hyperlipidemia is a risk factor for worsening the complications particularly cardiovascular diseases in diabetes (23). In our study, the level of serum triglycerides was increased in diabetic rats, and treatment with IGE could restore its level to that of the control group. Similarly, it has been shown that the ethanolic extract of *Iris germanica* rhizomes lowered the serum lipids of rats fed on a high-fat diet (13). This effect of *Iris germanica* can be helpful in preventing cardiovascular complications in diabetic patients.

Many studies have shown that STZ-induced hyperglycemia is associated with an increased level of serum ALT, AST, and ALP (16,24). An increase in the levels of these enzymes may be

a result of liver injury (e.g., reduced hepatic insulin sensitivity) and is observed more frequently among diabetic than nondiabetic cases (25,26). Administration of IGE significantly reduced the elevated levels of these liver enzymes and this effect was induced with both doses of the extract. In addition, IGE could restore the reduced level of thiol groups in the liver tissue of diabetic animals. Therefore, it is rational to suggest that *Iris germanica* induces hepatoprotective effect in diabetes through increasing the total antioxidant capacity of hepatocytes. The antioxidant effect of *Iris germanica* has been confirmed by several antioxidant assays, including free radical scavenging, reducing power, superoxide anion radical scavenging, H₂O₂ scavenging, and metal chelating activities (10,12,15,21). The antioxidant effect of herbal extracts including IGE is primarily attributed to their phenolic constituents particularly flavonoids and anthocyanins (21). In the present study, the content of phenolic compounds in IGE was 71 mg gallic acid equivalent per gram of the extract, which is close to the level of 68.8 g reported by Nadaroglu *et al.* (12).

Twenty-eight days after induction of diabetes, there was no significant difference in the liver SOD activity between the study groups. In STZ-induced diabetes, the activity of SOD has been reported to increase, decrease, or remain unchanged by different investigators. For example, Sedlak *et al.* reported an increase in the activity of this enzyme in the eye lens of STZ-diabetic rats (27). However, it has been indicated that STZ significantly decreased SOD activity in the liver and serum of diabetic rats (28,29). On the other hand, Sadi *et al.* observed no alterations in SOD activity in the kidneys of diabetic rats (30). Therefore, further studies should be carried out to elucidate the relationship between tissue SOD and diabetes.

CONCLUSION

The present study suggested that *Iris germanica* rhizomes inhibits the progression of hyperglycemia and hypertriglyceridemia and has protective effects against diabetes-induced injury of the liver and pancreas. Therefore, this

plant has the potential to be used as a natural product for the management of diabetes.

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Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contribution

A. Ghorbani proposed and designed the study. S. Hooshmand monitored and treated the animals under the supervision of A. Ghorbani. M.R. Mahdinezhad and S. Ehtiati performed the biochemical tests under the supervision of M. Soukhtanloo. S. Taraz Jamshidi performed histological examinations. A. Ghorbani analyzed the results with the contribution of M.R. Mahdinezhad. A. Ghorbani wrote the manuscript for publication with help of other authors.

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