

Hypoglycemic, hepatoprotective, and hypolipidemic effects of hydroalcoholic extract of *Eryngium billardieri* root on nicotinamide/streptozotocin-induced type II diabetic rats

Samira Khani¹, Maasoume Abdollahi², Zeynab Asadi³, Mohamad Nazeri⁴,
 Mohammad Amin Nasiri⁵, Hossein Yusefi⁶, Abbas Moghadam⁷, and Hamid Heidari^{8,9,*}

¹Neuroscience Research Center, Qom University of Medical Sciences, Qom, I.R. Iran.

²Department of Anatomical Sciences, Medical Sciences Faculty, Tarbiat Modares University, Tehran, I.R. Iran.

³Student Research Committee, Faculty of Nursing, Qom University of Medical Sciences, Qom, I.R. Iran.

⁴Department of Exercise Physiology, Science and Research Branch, Islamic Azad University, Ashtian, I.R. Iran.

⁵Student Research Committee, Kashan University of Medical Sciences, Kashan, I.R. Iran.

⁶Student Research Committee, Qom University of Medical Sciences, Qom, I.R. Iran.

⁷Cellular and Molecular Research Center, Faculty of Medicine, Qom University of Medical Sciences, Qom, I.R. Iran.

⁸Cellular and Molecular Research Center, Qom University of Medical Sciences, Qom, I.R. Iran

⁹Department of Physiology and Pharmacology, Faculty of Medicine, Qom University of Medical Sciences, Qom, I.R. Iran.

Abstract

Background and purpose: *Eryngium billardieri* has been used to control diabetes in traditional medicine. This research was performed to study the antidiabetic, hepatoprotective, and hypolipidemic effects of *E. billardieri* root extract (EBRE) on streptozotocin/nicotinamide-induced type 2 diabetic male rats.

Experimental approach: Type two diabetic animals were treated by three different doses of EBRE (100, 200, and 400 mg/kg), orally administered for 4 weeks. Ultimately, after anesthesia, the glucose, insulin, lipid profiles, hepatic enzyme levels in the blood and liver, and pancreas tissues of the animals were analyzed.

Findings/Results: Induction of diabetes caused a diminution in insulin level, high-density lipoprotein (HDL), and significantly enhanced the level of other lipid profiles, glucose, and liver enzymes ($P < 0.05$). Administration of the EBRE to diabetic-male rats significantly reduced glucose level, lipid profiles, and liver enzymes, and increased the level of HDL to near normal.

Conclusion and implications: The results of the present study showed that *E. billardieri* had a positive effect on diminishing the lipid profiles, liver enzymes, and controlling diabetes. The most effective dose was found to be 100 mg/kg.

Keywords: Antidiabetic; *Eryngium billardieri*; Nicotinamide; Streptozotocin.

INTRODUCTION

Diabetes syndrome is a widespread metabolic disturbance in the world, which is marked by an abnormal lipid profile, inadequate secretion, or insulin dysfunction (1,2). This disease is the most common chronic disorder in the whole world. It is estimated that by 2030, the number of people suffering from the disease will reach more than 370 million (3). Increased generation of reactive oxygen species and the occurrence of oxidative stress

led to the destruction of insulin-secreting beta cells of the islets of Langerhans. All of these play key roles in the induction and progression of diabetes syndrome (4). Free radicals are produced uncontrollably in diabetic patients by glucose oxidation, non-enzymatic glycation of proteins, followed by oxidative damage of glycosylated proteins (5).

Access this article online



Website: <http://rps.mui.ac.ir>

DOI: 10.4103/1735-5362.310526

*Corresponding author: H. Heidari
 Tel: +98-9354041243, Fax: +98-2533209127
 Email: physiology_86@yahoo.com

Increased levels of free radicals and the simultaneous reduction of defense mechanisms can lead to tissue and enzyme damage, followed by increased lipid peroxidation and insulin resistance in diabetic patients (6). Although anti-diabetic drugs such as biguanides and sulphonylureas are available today, they lead to many complications such as lactic acidosis, gastrointestinal disturbance, blood dyscrasia, and hypersensitivity reactions (7). Hypoglycemic traditional herbs have been of great interest in recent years given their low cost, several varieties of effective combinations, lower side effects in comparison to synthetic drugs, and the recommendations of the World Health Organization (8). *E. billardieri* is among the many herbs, which have been recommended for the cure of diabetes. Also known as Shkay and Shocked Al Bayda, *E. billardieri* has been reported for its antidiabetic effects (9). *E. billardieri* is a clear herbaceous plant with a stem height of 1 to 2 m covered with white cotton cords. Some of its properties include low branch stem, wing-like side, lobe and thorn in the length of the main stem, and its branched and long oval leaves with edges of the lobe and serrated end to the strong horn and sharp-pointed. Its fruits are hazelnut painted gray to black scales and end to a bunch of dark brown color. This plant grows in the north of Iran in the provinces of Gorgan, Mazandaran, Azerbayejan, and Hamedan (10). The plant contains compounds such as alkaloids, saponins, flavonoids, terpenoids, coumarone, chlorogenic acid, caffeic acid, and beta carotene. Results of previous studies show that this plant has biological effects including anti-inflammatory, antioxidant, cytotoxic, anti-apoptotic, antibacterial and fungal, and antimalaria. The antiradical and antioxidant compounds found in the plant can improve the function of the liver in hypercholesterolemia mice without adverse side effects on renal function. In addition, the alkaloid compounds in the plant help to reduce fat absorption from the intestine (9,11). In spite of the active ingredients in this herb, which have probably important effects on the control of blood glucose and fat, few works have been carried out in this field. Because of *Eryngium* species roots effects against various inflammatory conditions and a wide range of ailments are well-known in the traditional medicines

worldwide (12), the aim of this study was to evaluate the antidiabetic and hypolipidemic effects of the *E. billardieri* root extract (EBRE) on streptozotocin/ nicotinamide-induced diabetic rats. In this study, we applied the streptozotocin/ nicotinamide in order to mild impairment in the secretion of insulin hormone that is more closely resembled in the induction of type 2 diabetes (13).

MATERIALS AND METHODS

Plant preparation and extract

Fresh roots of *E. billardieri* were bought from medicinal plants bazaar in Qom province in Iran and authenticated scientifically by the Department of Medicinal Plant of Qom University of Medical Sciences. The roots were then dried and turned into a powder by an electric mill. To prepare the extract, 50 g of the powdered plants were soaked in 200 mL of hydroalcoholic solvent (distilled water:ethanol, 96% mixture; 60:40) and kept for 72 h in a closed dish at room temperature. The roots were then filtered (No. 1 Whatman filter paper) at the end. After removing the solvent and drying at room temperature, about 7 g (with a purity of 15%) of a semisolid mass were obtained, which were kept at 4 °C prior to use (14). A voucher specimen of (No. TMRC. 2291) *E. billardieri* is deposited in the herbarium of Traditional Medicine Research Center (TMRC) of Shahid Beheshti University of Medical Sciences, Tehran, I.R. Iran.

Animals

Thirty-six Wistar male rats weighting 200-240 g were used. During the research, all animals in groups had free availability for feeding and were kept in a house with controlled conditions (temperature: 20-24 °C, humidity: 40-45%, and luminosity: 12/12-h light/dark). Ethical principles (Ethic No. IR.MUQ.REC.1395.10) of work on animals were taken in accordance with the agenda of the Ethics Committee of the Medical Sciences University, Qom, I.R. Iran.

Study groups and interventions

Male rats were randomly distributed into six groups (n = 6). The groups included the control (group 1), streptozotocin/nicotinamide-induced

diabetic with no treatment (group 2) who received normal saline orally, diabetic rats with oral administration of glibenclamide (GLI; 0.25 mg/kg BW, Sigma Aldrich Co., USA) (group 3), and diabetic rats orally fed with EBRE at 100, 200, and 400 mg/kg daily (9) for 28 days (15) (groups 4-6). The experiments were carried out over a period of four weeks.

Induction of type two model of diabetes

For inducing diabetes, the male rats fasted overnight and received intraperitoneally an injection of nicotinamide (Merck Co., Germany) dissolved in normal saline at a dose of 110 mg/kg. After 15 min, streptozotocin (Sigma Co, USA) dissolved in citrate buffer, pH 4.5 (Sigma Aldrich Co., USA) was injected intraperitoneally at 60 mg/kg. The fasting blood glucose levels of the rats were measured after 72 h of streptozotocin administration and the rats with blood glucose levels of above 200 mg/dL were regarded as diabetic (15).

Biochemical assessment

The blood glucose levels were determined using an Elegance glucometer (CT-X10, Convergent Technologies, Germany). In order to evaluate biochemical factors, serum was separated by blood centrifugation for 10 min at 2000 g, 4 °C and stored at -20 °C until analysis (16). To evaluate the serum insulin levels, a commercial ELISA kit (Monobind, California, USA) was used according to the manufacturer's instructions. The homeostatic model assessment function of pancreatic β -cell (HOMA- β) and insulin resistance (HOMA-IR) were then obtained through the following equations (17):

$$\text{HOMA} - \beta = \frac{20 \times \text{insulin } (\mu\text{IU/mL})}{\text{Fasting blood glucose } \left(\frac{\text{mmol}}{\text{L}}\right) - 3.5} \quad (1)$$

$$\text{HOMA} - \text{IR} = \frac{\text{Fasting blood glucose } (\text{mg/dL}) \times \text{insulin } (\mu\text{IU/mL})}{405} \quad (2)$$

The low-density lipoprotein cholesterol (LDL-c), serum level of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c), the level of alkaline phosphatase (ALP), serum glutamic pyruvic transaminase (SGPT), and glutamic oxaloacetic transaminase (SGOT) were assessed using commercial kits (Pars Azmoon, Iran).

In addition, the levels of very low-density lipoprotein cholesterol (VLDL-c), which equals one-fifth of TG content, were determined using the Norbert formula (18). As the zone of atherogenic risk, the atherogenic index of plasma (AIP = Log (TG/HDL-C)) was assessed (19).

Histopathological evaluation

For the histological examination, the samples (liver and endocrine pancreas) were fixed in 10% formalin (20) for at least 24 h, then tissues were dehydrated with a sequence of ethanol solutions, embedded in paraffin, cut into 5 μm sections and stained with hematoxylin and eosin dye (H&E stain). Slides were observed under a light microscope (Olympus, Tokyo, Japan) connected to a camera (Digital Microscope BMZ-04-DZ) in a blind manner (21). Six microscope slides per animal were examined for assessment of histological changes. The fat deposit and nuclear pyknosis indices were assessed for the liver. For semi-quantitative evaluation of these indices following scoring system was used: 0 = normal, 1 = mild, 2 = moderate, and 3 = severe, and the total score was calculated by averaging all scores of each group (22). Morphological changes related to degenerated cells were counted in five fields of each tissue section of the endocrine pancreas. The degree of injuries was expressed as the percentage mean of lesions in five different fields (zigzag manner) in each section (23).

Statistical analysis

Acquired data were demonstrated as mean \pm SEM. Statistical assessment by analysis of variance (one-way ANOVA) followed by Tukey's test was performed by SPSS Statistics, V. 17.01 (SPSS Inc, Chicago, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Effect of EBRE on blood glucose

As indicated in Table 1, the induction of diabetes resulted in elevated blood glucose and the insulin concentration decreased, remarkably compared with the control group ($P < 0.001$).

Table 1. Effect of EBRE on insulin, fasting plasma glucose levels, HOMA-IR, and HOMA-β in streptozotocin-induced diabetic rat. Results are expressed as mean ± SEM, n = 6.

Groups	Glucose (mg/dL)	Insulin (μIU/mL)	HOMA-IR	HOMA-β
Control	99.00 ± 6.30	2.70 ± 0.25	0.69 ± 0.09	156.06 ± 21.1
Diabetic	211.57 ± 10.7 ^{###}	1.63 ± 0.13 ^{###}	0.85 ± 0.08	20.42 ± 2.16 ^{###}
Diabetic+ glibenclamide	132.16 ± 9.38 [*]	2.53 ± 0.14 ^{***}	0.82 ± 0.06	72.26 ± 10.3 ^{###,*}
Diabetic + EBRE (100 mg/kg)	118.80 ± 9.48 ^{***}	2.84 ± 0.14 ^{***}	0.84 ± 0.08	98.96 ± 11.2 ^{#,***}
Diabetic + EBRE (200 mg/kg)	197.40 ± 16.5 ^{###}	2.40 ± 0.17	1.19 ± 0.15 ^{##}	34.66 ± 4.84 ^{###}
Diabetic + EBRE (400 mg/kg)	178.28 ± 12.1 ^{###}	1.91 ± 0.17 [#]	0.83 ± 0.07	34.31 ± 8.42 ^{###}

EBRE, *Eryngium billardieri* root extract; HOMA-β, homeostatic model assessments of pancreatic β-cell function; HOMA-IR, homeostatic model assessment of insulin resistance. **P* < 0.05 and ****P* < 0.001 indicate significant differences compared to the diabetic group; #*P* < 0.05, ##*P* < 0.01, and ###*P* < 0.001 versus control group.

Table 2. Effect of EBRE on lipid profile (mg/dL) and atherogenic index in streptozotocin-induced diabetic rat. Results are expressed as mean ± SEM, n = 6.

Groups	Cholesterol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	Atherogenic index
Control	46.71 ± 5.59	40.57 ± 4.5	35.42 ± 3.50	2.81 ± 0.32	8.11 ± 0.90	0.34 ± 0.04
Diabetic	68.57 ± 5.8 [#]	68.14 ± 4.91 ^{##}	16.78 ± 1.61 ^{###}	4.26 ± 0.30 [#]	13.62 ± 0.98 ^{##}	0.74 ± 0.07 ^{###}
Diabetic + GLI	55.33 ± 3.92	58.16 ± 7.92	23.33 ± 1.42	3.64 ± 0.31	11.63 ± 1.58	0.55 ± 0.04 [*]
Diabetic + EBRE (100 mg/kg)	43.8 ± 3.35 [*]	38.4 ± 4.92 ^{**}	20.4 ± 3.15	2.33 ± 0.36 ^{**}	7.68 ± 0.98 ^{**}	0.49 ± 0.09 ^{**}
Diabetic + EBRE (200 mg/kg)	48.0 ± 3.19	42.4 ± 4.53 [*]	35.6 ± 4.74 ^{**}	3.66 ± 0.21	8.48 ± 0.90 [*]	0.37 ± 0.05 ^{***}
Diabetic + EBRE (400 mg/kg)	47.42 ± 5.16 [*]	46.14 ± 3.88 [*]	36.42 ± 4.69 ^{**}	2.69 ± 0.25 ^{**}	9.22 ± 0.77 [*]	0.31 ± 0.06 ^{***}

EBRE, *Eryngium billardieri* root extract; GLI, glibenclamide; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides; VLDL, very low-density lipoprotein. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 indicate significant differences in comparison with the diabetic group; #*P* < 0.05, ##*P* < 0.01, and ###*P* < 0.001 versus control group.

Daily administration of EBRE for 4 weeks to diabetic rats at the dose of 100 mg/kg significantly reversed these changes (*P* < 0.001). In group 3, taking GLI, the blood glucose level reduced and the level of insulin increased, remarkably (*P* < 0.05 and *P* < 0.001, respectively) in comparison to the diabetic group. Moreover, according to the HOMA-IR evaluation criteria, the insulin resistance in the diabetic group was enhanced in comparison to the control group, but it was not significant. Based on HOMA-β criteria, the beta cells function diminished significantly in group 2 compared with the control intact group (*P* < 0.001). In addition, 28-day treatment of diabetic animals with GLI and EBRE at 100 mg/kg, significantly increased HOMA-β compared to the diabetic group (*P* < 0.05 and *P* < 0.001, respectively).

Effect of EBRE on body weight

As indicated in Fig. 1, after induction of diabetes, the rats' body weight was significantly decreased compared with the control group

(*P* < 0.05). Although the body weight increased in other groups than the diabetic group, the changes were not significant.

Effect of EBRE on lipid profile level

As shown in Table 2, after induction of diabetes in rats, the HDL level diminished (*P* < 0.01) and the levels of VLDL, TG, LDL and cholesterol increased (*P* < 0.01 and *P* < 0.05, respectively) significantly compared with the control group. According to the experimental results listed in Table 2, 28-day treatment of diabetic rats with EBRE at 100 and 400 mg/kg, significantly decreased the levels of LDL (*P* < 0.01) and cholesterol (*P* < 0.05) relative to the diabetic group. The concentration of HDL increased in the diabetic groups, which received 200 and 400 mg/kg of EBRE (*P* < 0.01). Treatment of diabetic rats with EBRE, remarkably reduced the VLDL and TG levels in doses of 100 mg/kg (*P* < 0.01), 200 and 400 mg/kg (*P* < 0.05) compared with the diabetic group.

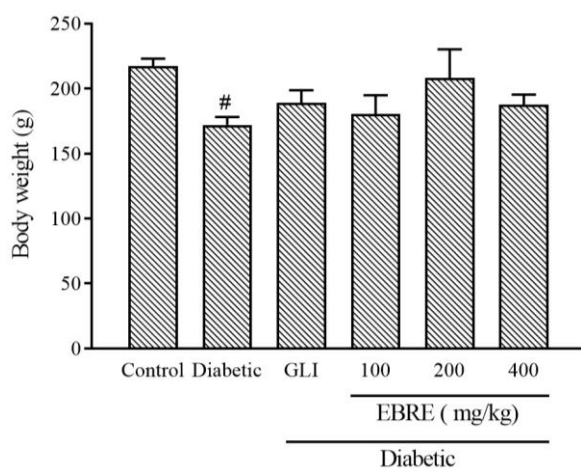


Fig. 1. Effect of EBRE on the body weight after 4 weeks in streptozotocin-induced diabetic rat. Results are expressed as mean \pm SEM, $n = 6$. $^{\#}P < 0.05$ Indicates significant differences versus the control group. GLI, Glibenclamide; EBRE, *Eryngium billardieri* root extract.

Effect of EBRE on level of liver enzymes

Induction of diabetes in rats significantly increased their serum level of liver enzymes compared to the intact rats ($P < 0.001$). According to the acquired data shown in Table 3, the treatment of diabetic rats with EBRE, significantly reduced the liver enzymes

at 100 and 200 mg/kg ($P < 0.01$ and $P < 0.05$, respectively). Furthermore, the serum level of SGPT diminished in diabetic rats after four-week administration of GLI and a dose of 400 mg/kg EBRE ($P < 0.05$).

Effect of EBRE on histopathological changes of liver tissue

In order to evaluate the effect of EBRE on histopathology of the liver, H&E staining was performed. The intact control group displayed a normal liver cytoarchitecture (Fig. 2A and Table 4). Microscopic examination of liver sections of the untreated diabetic group revealed significant injuries represented by fat deposit and nuclear pyknosis ($P < 0.001$; Fig. 2B and Table 4). Liver sections from animals treated with GLI (0.25 mg/kg) showed remarkable improvement in liver fat deposit and nuclear pyknosis ($P < 0.001$ and $P < 0.05$; Fig. 2C and Table 4). Treatment of animals with EBRE extract, reduced nuclear pyknosis at dose 400 mg/kg ($P < 0.01$) and fat deposit at doses 200 and 400 mg/kg ($P < 0.01$ and $P < 0.05$; Fig. 2D, E, F, and Table 4).

Table 3. Effect of EBRE on SGOT and SGPT (U/L) levels in streptozotocin-induced diabetic rat. Results are expressed as mean \pm SEM; $n = 3$.

Groups	SGPT (U/L)	SGOT (U/L)
Control	167.7 \pm 12.6	289.71 \pm 20.9
Diabetic	251.3 \pm 6.62 ^{###}	494.71 \pm 28.3 ^{###}
Diabetic + glibenclamide	193.3 \pm 5.56 [*]	386.50 \pm 32.8
Diabetic + EBRE (100 mg/kg)	170.8 \pm 8.97 ^{**}	331.60 \pm 21.6 ^{**}
Diabetic + EBRE (200 mg/kg)	187.6 \pm 11.9 [*]	354.00 \pm 32.5 [*]
Diabetic + EBRE (400 mg/kg)	193.1 \pm 9.90 [*]	399.42 \pm 26.8

EBRE, *Eryngium billardieri* root extract; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase. $^*P < 0.05$ and $^{**}P < 0.01$ indicate significant differences compared to the diabetic group; $^{###}P < 0.001$ versus control group.

Table 4. The quantified histopathological changes of rat livers in hematoxylin and eosin stained sections. Results are expressed as mean \pm SEM; $n = 3$.

Groups	Nuclear pyknosis	Fat deposit
Control	0.08 \pm 0.02	0.12 \pm 0.01
Diabetic	1.64 \pm 0.21 ^{###}	2.06 \pm 0.15 ^{###}
Diabetic + glibenclamide	1.10 \pm 0.07 ^{###,*}	1.40 \pm 0.09 ^{###,***}
Diabetic + EBRE (100 mg/kg)	1.20 \pm 0.14 ^{###}	1.82 \pm 0.11 ^{###}
Diabetic + EBRE (200 mg/kg)	1.14 \pm 0.10 ^{###}	1.64 \pm 0.08 ^{###,*}
Diabetic + EBRE (400 mg/kg)	1.02 \pm 0.07 ^{###,**}	1.50 \pm 0.06 ^{###,**}

EBRE, *Eryngium billardieri* root extract. $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ indicate significant differences compared to the diabetic group; $^{###}P < 0.001$ versus control group.

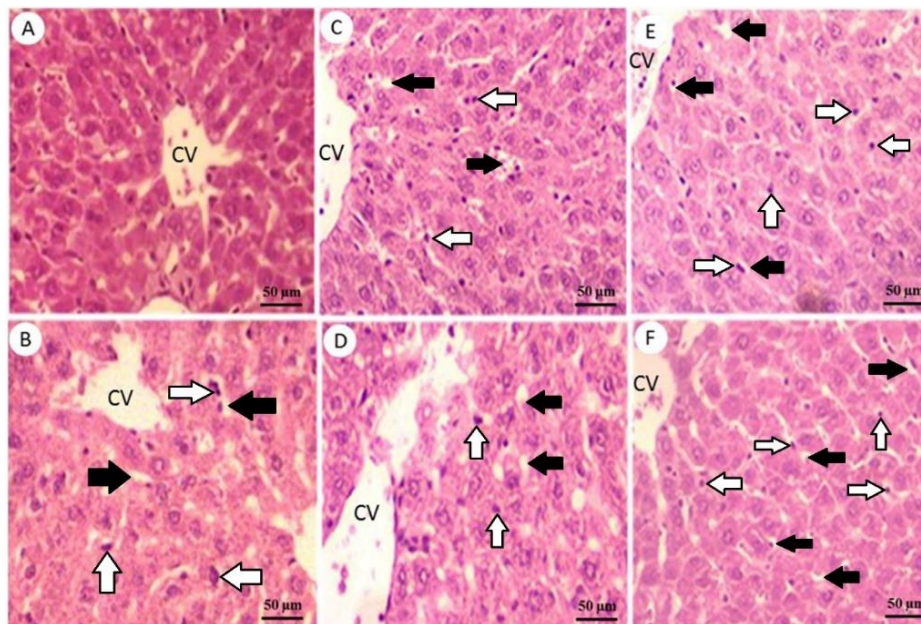


Fig. 2. Liver sections stained with hematoxylin and eosin. (A) Normal histological view of the liver in control group rats; (B) microscopic examination of liver tissue of untreated diabetic group showing some pathological changes and injury including fat deposit and nuclear pyknosis, which displaying degenerative changes compared to the control normal rats; (C) liver section from an animal treated with glibenclamide (0.25 mg/kg) showing a significant reduction in nuclear pyknosis and fat deposit; (D-F) liver of animals treated with 100, 200, and 400 mg/kg doses of *Eryngium billardieri* root extract, respectively which indicating the improvement of pathological injury. Magnification: 200×. Black arrows, fat deposit; white arrows, nuclear pyknosis.

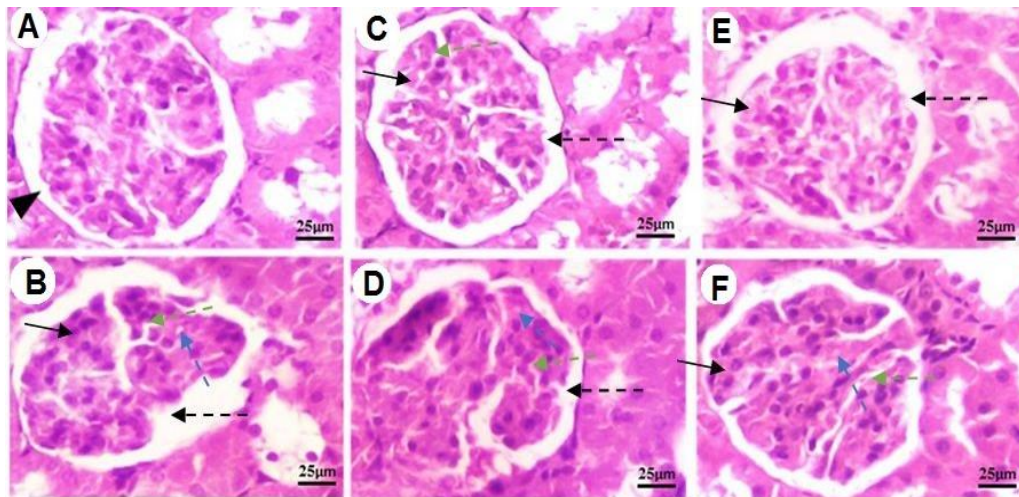


Fig. 3. Pancreas sections stained with hematoxylin and eosin. (A) The histological study of pancreas sections shows the normal appearance of pancreatic islets (black triangle) in the control group; (B) microscopic examination of pancreatic tissue of untreated diabetic group shows some pathological changes and injury including degeneration and shrinkage (irregular space in the islets of Langerhans, dashed-green arrows), disruption of normal endocrine architecture (dashed-black arrows), and necrotic cells with pyknotic nuclei (black arrows), swelling (spaces in the islets of Langerhans, dashed-blue arrows) which all of them displaying degenerative changes compared to the control normal rats; (C) pancreas section from an animal treated with glibenclamide (0.25 mg/kg) showing necrosis reduction (black arrows) and cellular degeneration (dashed-green arrows), significant endocrine structure restoration (dashed-black arrows); (D-F) pancreas of animals treated with EBRE at 100, 200, and 400 mg/kg, respectively. Treatment of animals whit EBRE at 100 mg/kg shows a slight decrease in endocrine structure disruption (dashed-black arrows), cellular degeneration (dashed-green arrows), and swelling (dashed-blue arrows). Treatment of rats whit EBRE at 200 and 400 mg/kg indicates a remarkable reduction in degeneration and shrinkage (dashed-green arrows), disruption of normal endocrine structure (dashed-black arrows), minimum vacuolar swelling (dashed-blue arrows), and necrosis (black arrows). Magnification: 200×. EBRE, *Eryngium billardieri* root extract

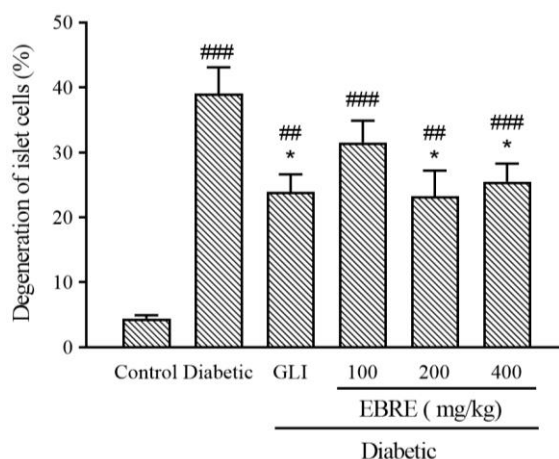


Fig. 4. Effect of EBRE on percentage of total damaged cells (degeneration of islet cells) in streptozotocin-induced diabetic rat. Results are expressed as mean \pm SEM, $n = 6$. * $P < 0.05$ indicates significant differences in comparison with the diabetic group; ## $P < 0.01$, and ### $P < 0.001$ versus control group. GLI, Glibenclamide; EBRE, *Eryngium billardieri* root extract.

Effect of EBRE on histopathological changes of pancreas tissue

The histological appearance of the pancreatic islets of the control group showed normal endocrine and acinar architecture (Fig. 3A and Fig. 4). Microscopic examination of pancreatic sections of the untreated-diabetic group revealed significant injuries and pathological changes, represented by vacuolar swelling, necrotic cells, disruption of normal endocrine architecture, shrinkage and degeneration of cells ($P < 0.001$; Fig. 3B; Fig. 4). Pancreas sections from animals treated with GLI (0.25 mg/kg) showed remarkable improvement in pancreatic islets endocrine structure, cellular degeneration, and necrosis reduction ($P < 0.05$; Fig. 3C; Fig. 4). Treatment of animals with 200 and 400 mg/kg doses of EBRE, significantly decreased the percentage of degenerative and necrotic cells and vacuolar changes and restored endocrine structure ($P < 0.05$; Fig. 2D, E, F; Fig. 4).

DISCUSSION

In our research remarkable diminish in insulin level and increase in blood glucose level was observed in streptozotocin/nicotinamide-induced diabetic animals in comparison to the control group rats. The level of insulin in type II diabetes mellitus rats in this research implied

that some insulin secretory β cells are healthy and can still produce and secrete insulin. One of the anti-hyperglycemic mechanisms is elevating insulin production and decreasing glucose absorption from the intestine surface (24). The result of our study showed that treatment with EBRE can significantly reduce blood glucose levels and elevate serum levels of insulin in diabetic rats. Some antioxidant ingredients of the herb such as flavonoids and carotene can probably reduce the level of oxidative stress in beta cells and improve their function to produce insulin and subsequently decrease blood glucose level (9).

Some insulin-related biomarkers such as HOMA-IR and HOMA- β assay are used to reveal the health of insulin generating cells and insulin function in T2DM (25). In this study, the induction of diabetes elevated the level of HOMA-IR and HOMA- β in diabetic rats. However, only HOMA- β , significantly increased. After treatment with EBRE, the level of HOMA-B reduced remarkably compared with the diabetic group. This can be due to the effect of the plant extract on B-cell and improved insulin production. The results of the hormonal analysis were also supported by histopathological findings of the pancreas as histopathological changes reached up in the diabetic group relative to the control group.

Metabolic disorders related to insulin insensitivity, insulin secretion, and hyperglycemia is reflected by changes in the islet structure, size, or function. This subject in our research demonstrated severe injuries represented by the islets shrinkage (atrophy), irregular islets cellular swelling and degeneration, and the presence of necrotic cells in the diabetic animals (23). The severity of these pathological changes in diabetic rats was remarkably decreased after treatment of extracts and GLI (Fig. 4).

Our findings indicated that the average body weight was significantly diminished in the rats after induction of diabetes by streptozotocin. Our results match those of other researches, demonstrating that streptozotocin-induced diabetes leads to severe loss in body weight (26). Diabetes is concomitant with the decrease of body weight due to excessive lysis of tissue proteins and wasting muscle mass because of

insulin shortage (27). Administration of EBRE for diabetic rats led to increased body weight in comparison to diabetic control rats which the change was not significant. In our study, the diabetic control group showed an elevation in the levels of LDL, VLDL, TG, and a decrease in the levels of HDL, which are in agreement with the findings of Farsani et al. (28). After treatment with EBRE, remarkable decreases were observed in the levels of LDL, VLDL, and TG and an elevation was observed in the level of HDL. The mechanisms involved in this process are probably the inhibition of cholesterol synthesis, increased consumption of it, decreased fat absorption in the intestines, increase bowel movements and reduce fat absorption. The hypoglycemic and hypolipidemic properties of some compounds present in *E. billardieri* extract such as flavonoids and tannins have also been reported (29). In addition, saponin as one of the active components of *E. billardieri* can modulate lipid profiles by suppressing cholesterol luminal absorption and increasing cholesterol secretion through biliary excretion (30). Alkaloids as another component of extract that also inhibit cholesterol synthesis (31). In addition to the mechanisms mentioned above, a significant reduction in serum lipid levels in the diabetic rats treated with the extract was probably caused as a consequence of increased insulin levels. It is well recognized that insulin shortage reduces lipoprotein lipase activity and leads to hypertriglyceridemia and an increase in plasma free fatty acid concentration (32).

One of the important insulin-dependent tissues is the liver, which is damaged during type II diabetes mellitus (33).

In our study in streptozotocin/nicotinamide-induced diabetic rats, serum levels of SGOT and SGPT elevated indicating liver dysfunction. EBRE led to a remarkable reduction in SGPT and SGOT in the treated groups, which indicated this herb could have a preservative effect on the liver. In addition to serum assessment, also in this research evaluation of the histological appearance of the liver indicated a striking increase in fat deposit and nuclear pyknosis in liver tissue of the diabetic group compared to the intact group which this subject represents the liver injury (22). Treatment of diabetic rats with EBRE, by

lessening the severity of injuries, improved liver structure.

Previous studies have proven that flavonoids, one of the components of *E. billardieri*, are able to quench nitric oxide synthase and those ingredients, which are able to diminish the production of nitric oxide in the liver have hepatoprotective effects (34). On the other hand, the hepatoprotective effect of EBRE on the liver may be related to the antidiabetic properties of the plant. Investigations demonstrated that insulin acts as a hormone-sensitive lipase inhibitor (35). Thus, the activation of hormone-sensitive lipase during insulin shortage is accompanied by the increase of the lipolysis cycle, the liberation of free fatty acids, and stimulation of the conversion of fatty acids into phospholipids and cholesterol in the plasma and liver (36). Furthermore, flavonoids, tannins, and terpenoids in *E. billardieri* have anti-inflammatory and antioxidant properties and help to protect the liver against oxidative stress-induced factors, which cause hepatotoxicity (9).

CONCLUSION

The results of this study indicated that *E. billardieri* root extract has hypoglycemic and hepatoprotective effects and can modulate lipid profiles. Therefore, this plant can be useful in type II diabetes mellitus treatment. However, more research is needed to clarify the exact mechanisms of the antidiabetic effects of *E. billardieri*.

Acknowledgements

This work has been financially supported by the Vice-Chancellor of Research Affairs of Qom University of Medical Sciences under Grant No. IR.MUQ.REC.1395.10. The authors are also deeply thankful for the technical collaboration of the Qom Danesh Laboratory.

Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contribution

H. Heidari supervised and conducted the study and reviewed the manuscript. S. Khani and M. Abdollahi analyzed the hormonal and

tissue data. Z. Asadi and M. Nazeri helped with the literature search prepared the proposal and carried out the laboratory studies. M.A. Nasiri drafted the manuscript and helped in laboratory studies. H. Yusefi prepared the extract and blood sample collecting and data acquisition. A. Moghadam also has interpreted the tissue data. All authors have read and approved the final manuscript.

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