http://www.cjmb.org

Open Access Original Article

Crescent Journal of Medical and Biological Sciences
Vol. 4, No. 3, July 2017, 108–114
eISSN 2148-9696

Protective Effect of *Galega officinalis* Extract on Streptozotocin-Induced Kidney Damage and Biochemical Factor in Diabetic Rats

Seyd-Hosein Abtahi-Evari[1], Majid Shokoohi[2*], Ali Abbasi[2], Asghar Rajabzade[3], Hamed Shoorei[4], Hosein Kalarestaghi[5]

Abstract

Objective: Diabetes mellitus (DM) is impairing secretion of insulin or resistance to insulin. Herbal medicine plays an important role in the management of DM. We aimed to test antidiabetic effects of *Galega officinalis* on diabetic rats.

Materials and Methods: Twenty-eight male Wistar rats were randomly divided into 4 groups (n=7). Diabetes was induced by streptozotocin (STZ) (50 mg/kg). Diabetic rats were calcified into a diabetic control group (DC), DHEG group (50 mg/kg hydroalcoholic extract of *G. officinalis*), DG group (5 mg/kg glibenclamide). After 20 days, rats' blood samples, kidney, liver, and pancreas were kept in -70°C to test blood levels of glucose, insulin, lipid profile, some oxidative stress markers and antioxidant enzymes.

Results: The fasting blood sugar (FBS) levels in the normal, DHEG, and DG groups were significantly lower than the DC group ($P<0.05$); The levels of insulin in the DC, DHEG, and DG groups were significantly lower than the normal group ($P<0.05$); The serum level of urea and creatinine was significantly increased in DC group and significantly decreased in other group ($P<0.05$). Diabetes causes degenerative damages in rats kidney and treatment with *G. officinalis* extract protected kidney tissue against diabetes-induced damages.

Conclusion: The results of the present study indicated that *G. officinalis* could be beneficial for the treatment of diabetes through improving tissue sensitivity to insulin and preventing tissue damages.

Keywords: Diabetes, Streptozotocin, *Galega officinalis*, Kidney damage, Rat

Introduction

Diabetes mellitus (DM) is one of the most public metabolic sicknesses result from the lack of insulin or the presence of insulin resistance in peripheral tissues or both that is accompanied by high blood glucose concentration (1,2). The complications of diabetes are major health problems in developed and developing countries (2). It affects approximately 4% of the population worldwide and is estimated to increase by 5.4% in 10 years later (3). In diabetes, chronic hyperglycemia is associated with long-term damages, dysfunction and eventually the failure of organs, especially the eyes, kidneys, cardiovascular system, and nerves (3). Besides hyperglycemia, other factors such as, dyslipidemia and hyperlipidemia are also involved in the development of cardiovascular complications which are the major cause of morbidity and mortality (4,5). Nowadays, the available therapies for diabetes include insulin and/or various oral chemical antidiabetic drugs, including sulfonylureas, thiazolidinediones, α-glucosidase inhibitors, etc. Each of the above-mentioned oral antidiabetic agents is associated with a number of serious adverse effects (6). Medicinal plants may play an important role in the management of blood glucose through different mechanisms. Some medicinal plants may contain insulin-like substances (7), inhibit insulin's activity or increase beta β-cells in the pancreas by activating the regeneration of this cells (8,9), or some may serve as antioxidants by reducing the oxidative stress due to free radicals in the pancreas (10,11). *Galega officinalis* (Papilionaceae) is a native plant from southeastern Europe and was used in the traditional medicinal system of Bulgaria, Italy, and India (12,13) in the treatment of DM (14). A biologically active alkaloid galegine, exhibiting a hypoglycemic effect in vivo was isolated from *G. officinalis* (15). The discovery of the plant's active hypoglycemic factor led to the expansion of metformin, a biguanide which has been used to treat type 2 DM (14,16). In fact, the only example of an approved antidiabetic drug that was extending from an herbal

Received 18 October 2016, **Accepted** 10 February 2017, **Available online** 2 March 2017

[1]Department of Basic Sciences, Faculty of Medicine, Gonabad University of Medical Sciences, Gonabad, Iran. [2]Student in nursing (BSc), Student Research Committee, Gonabad University of Medical Sciences, Gonabad, Iran. [3]Department of Anatomical Sciences, Faculty of Medicine, Lorestan University of Medical Sciences, Khorammabad, Iran. [4]Department of Anatomical Sciences, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran. [5]Department of Anatomy and Pathology, Research Laboratory for Embryology and Stem Cell, Faculty of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran.
*Corresponding Author: Majid Shokoohi, Tel: +989158009047, Email address: a.shokoohy@yahoo.com

source with a long history of use for diabetes is metformin from *G. officinalis* (17). According to the diabetes causes, damage to organs and tissues such as the kidneys, the purpose of this study was evaluated protective effects of *G. officinalis* on the kidney damage and biochemical factors associated with diabetes.

Materials and Methods

Animals

For this study was used 28 male Wistar rats weighing 200-250 g. Rats were maintained for 2 weeks on the same diet. They had free access to the rat chow and tap water and were kept at 25°C with a 12 h/12 h light/dark cycle. All experimental methods were administered in accordance with Gonabad University of Medical Science guidelines for cares and use of laboratory animals.

Animal Preparation

The rats randomly divided into a 7-rat healthy control group and 21-rat experimental groups. In the experimental groups, diabetes was induced by intraperitoneal injection of a single dose of 50 mg/kg streptozotocin (STZ) (Sigma) dissolved in 5 mM citrate buffer (pH = 4.5). After 72 hours of injection, the rats with blood glucose levels more than 250 mg/dL were confirmed to be diabetic. Diabetic rats were randomly grouped as follows, 7 rats each; the diabetic control (DC) received glycerol 20% in normal saline as vehicle, diabetic rats treated with 50 mg/kg hydroalcoholic extract of *G. officinalis* (DHEG), and diabetic rats treated with 5 mg/kg glibenclamide as standard drug (DG). All the groups were received the corresponding treatment once per day for 20 days, intraperitoneally. In the control group (normal), the same volume of citrate buffer was injected instead STZ. After the treatment period which lasts for 21 days, rats were anesthetized and their blood samples were drawn, and also their kidneys, livers, and pancreases were kept in -70°C. Blood samples were used for testing of glucose, insulin, lipid profile, some oxidative stress markers, and antioxidant enzymes their hearts serum samples were stored at -70°C.

Kidneys Weight

In order to measure the weight of kidneys, right and left removed kidneys of rat bodies were weighed by a digital scale, respectively.

Histopathological Studies

The species were fixed in 10% formalin, then dehydrated and embedded in paraffin to 5 µm cutting by a microtome. Finally, all of the species were stained by hematoxylin-eosin (H & E) staining. For this propose, some slides were used to evaluate the histological changes and other of slides were used to evaluate the glomeruli and urinary space diameters.

Morphological Parameters

The stained samples by H & E in the previous stage were carried out to measure diameters and number changes of glomeruli and urinary spaces. ImageJ software was used to measurement.

PAS Stain to Evaluate the Basic Membrane

In order to measure the thickness of the basement membrane, periodic acid–Schiff (PAS) staining was used.

Serum Glucose and HDL and LDL Assays and Cholesterol Levels

At the beginning of the study, blood glucose levels were determined using a portable glucometer on samples collected from the tip of the tail vein. At the end of the study, serum glucose levels were determined using commercial kits (Parsazmun, Iran). The value was expressed in the unit of mg/dL.

Assay of Serum Insulin Level

Serum insulin levels were measured by a method based on enzyme-linked immunosorbent assay (ELISA) using the commercial kit of Rat Insulin (Mercodia).

Assay of Serum Glutamate-Pyruvate Transaminase Level

The serum level of glutamate-pyruvate transaminase (GPT) was assayed using the method of Parsazmun kit.

Statistical Analysis

One-way analysis of variance (ANOVA) followed by a multiple two-tailed *t* test was used for the analysis of significant differences among the collected data. $P < 0.05$ was considered as significant difference.

Results

Kidneys Weight

The kidneys weight was significantly increased in the DC group as compared with the normal group ($P < 0.05$). Also, in the DG and DHEG groups, the kidneys weight was significantly reduced as compared with the DC group ($P < 0.05$). However, kidneys weight was significantly reduced in the DHEG group than the normal group ($P < 0.05$) (Figure 1).

Histological Parameters of Kidneys

The glomerular diameters were significantly increased in the DC group as compared with the normal group ($P < 0.05$). The glomerular diameters were significantly reduced in the treated groups of DG and DHEG ($P<0.05$). The diameters of urinary spaces (around the glomeruli) were significantly decreased in the DC group as compared with the normal group ($P < 0.05$). The urinary space diameters were increased in the DG and DHEG groups as compared with the DC group ($P < 0.05$). The number of glomeruli was significantly decreased in the DC group as compared with the normal group ($P < 0.05$). Also, it was increased in the DG and DHEG groups as comparing with the DC group ($P < 0.05$). But, in the therapeutic groups of DG and DHEG, the number of glomeruli was lower than the normal group ($P < 0.05$; Table 1) (Figure 2).

Table 1. Comparison the Glomerular Diameters and Diameters of Urinary Space and Number of Glomeruli in Normal, DC, DG and DHEG Groups

Group	Glomerular Diameters	Diameters of Urinary Space	Number of Glomeruli
Normal	258.94±2.085	35.5± 1.16	32137.5±331.84
DC	339.13 ±12.70†	21.88± .82†	21841.75±161.62†
DG	239.90± 10.68*	34.70± .65*	24399.75±280.18*†
DHEG	247.86±4.88*	32.08± .44*	25388.75±491.30*†

Normal; The healthy control group receiving normal saline. DC; diabetic control group, receiving normal saline. DG; Treated with 5 mg/kg glibenclamide. DHEG; A diabetic group that receiving 50 mg/kg hydroalcoholic extracts of *Galega officinalis*.
The asterisk * shows significant difference with the DC group and the symbol of † means the significant difference with the Normal group ($P \leq 0.05$).

Thickness of the Basement Membrane

Evaluation of the basement membrane in different groups showed that the thickness of the basement membrane was increased in the diabetic control group than the normal group. Moreover, in the DG and DHEG groups, the basement membrane thickness was lower than the DC group (Figure 3).

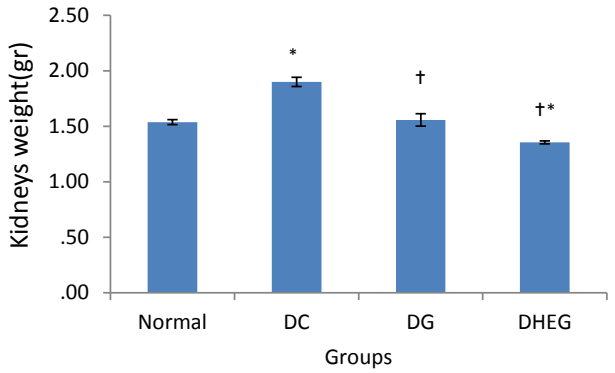

Figure 1. Comparison the Kidney Weight in Normal, DC, DG and DHEG groups. Normal; The healthy control group receiving normal saline. DC; diabetic control group, receiving normal saline. DG; Treated with 5 mg/kg glibenclamide. DHEG; A diabetic group that receiving 50 mg/kg hydroalcoholic extracts of *Galega officinalis*.The Asterisk sign shows significant difference with the Normal group and the symbol of † means the significant difference with the DC group ($P \leq 0.05$).

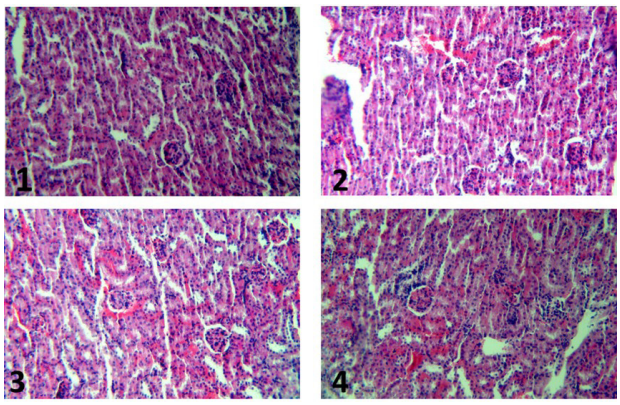

Figure 2. Histological parameters, H&E staining: (1) Normal; The healthy control group receiving normal saline. (2) DC; diabetic control group, receiving normal saline. (3) DG; Treated with 5 mg/kg glibenclamide. (4) DHEG; A diabetic group that receiving 50 mg/kg hydroalcoholic extracts of *Galega officinalis*.

Fasting Blood Level of Glucose

Fasting blood sugar (FBS) levels were significantly increased in the DC group as compared with the normal group ($P < 0.05$). Comparisons between the DC, DG, and DHEG groups were showed a significant decrease in the DG and DHEG groups as compared with the DC group ($P < 0.05$; Figure 4).

Serum Insulin Level

The serum insulin level was significantly decreased in all groups of DC, DG, and DHEG as compared with the normal group ($P < 0.05$), and also it was increased in the DG and DHEG groups as compared with the DC group. The increase in the DHEG group was significant ($P < 0.05$; Figure 5).

Serum Lipid Profile

Serum level of cholesterol: The serum level of cholesterol was significantly increased in the DC group as compared with the normal group ($P < 0.05$). Moreover, the cholesterol level was significantly decreased in all groups of the DG and DHEG as compared with the DC group ($P < 0.05$; Table 2).

Serum level of low-density lipoprotein (LDL): The serum level of LDL was showed a slight increase in the DC group as compared with the normal group ($P > 0.05$). The level of LDL was significantly reduced in the DG and DHEG groups as compared with the DC group ($P < 0.05$; Table 2).

Serum level of high-density lipoprotein (HDL): The serum level of HDL was significantly decreased in the DC group as compared with the Normal group ($P < 0.05$). Also, it was significantly increased in the DG and DHEG groups as compared with the DC group ($P < 0.05$). On the other hand, the level of HDL was showed an insignificantly increase in the DHEG group as compared with normal group ($P > 0.05$; Table 2)

Serum level of triglycerides (TG): The serum level of TG was significantly increased in the DC group as compared with the Normal group ($P < 0.05$). Moreover, the level of TG was significantly decreased in the DHEG group as compared with the DC group ($P < 0.05$; Table 2).

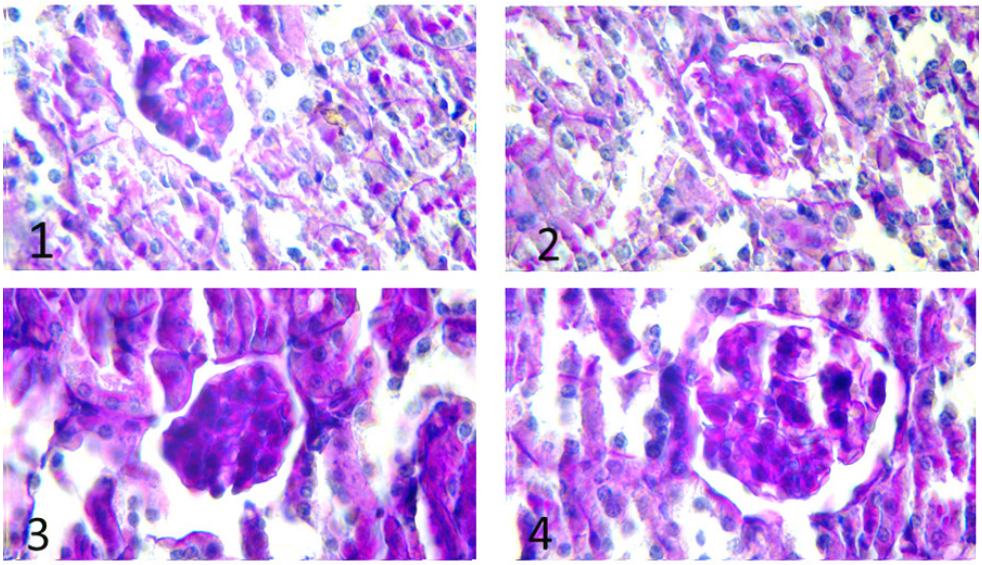

Figure 3. Histological Parameters, PAS staining: (1) Normal; The healthy control group receiving normal saline. (2) DC; diabetic control group, receiving normal saline. (3) DG; Treated with 5 mg/kg glibenclamide. (4) DHEG; A diabetic group that receiving 50 mg/kg hydroalcoholic extracts of *Galega officinalis*.

Serum level of creatinine (Cr): The serum level of Cr was significantly increased in the DC group as compared with the Normal group ($P<0.05$). Also, the level of Cr was significantly decreased in the DG and DHEG groups as compared with the DC group ($P<0.05$; Figure 6).

Serum level of urea: The serum level of urea was significantly increased in the DC group as compared with the Normal group ($P<0.05$). Furthermore, the level of urea was significantly decreased in the DG and DHEG groups as compared with the DC group ($P<0.05$; Figure 7).

Serum level of GPT: The serum level of GPT was significantly increased in the DC group as compared with the normal group ($P<0.05$). Moreover, the level of GPT was showed a significant difference in the DG and DHEG groups as compared with the DC group ($P<0.05$; Figure 8).

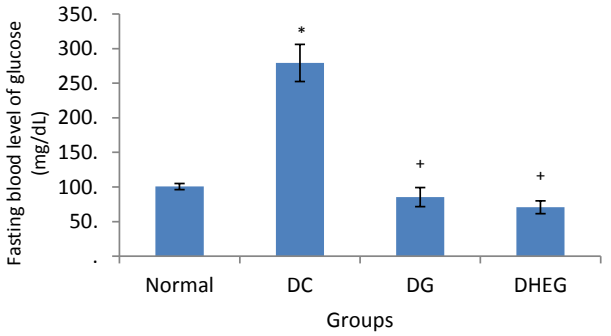

Figure 4. Comparison the Fasting Blood Level of Glucose in Normal, DC, DG and DHEG groups. Normal; The healthy control group receiving normal saline. DC; diabetic control group, receiving normal saline. DG; Treated with 5 mg/kg glibenclamide. DHEG; A diabetic group that receiving 50 mg/kg hydroalcoholic extracts of *Galega officinalis*. The asterisk sign shows significant difference with the Normal group and the symbol of + means the significant difference with the DC group ($P\leq0.05$).

Discussion

At the moment, extant drug regimens to control DM have some drawbacks, therefore, needs to find out safer and more efficient antidiabetic drugs are felt (18-20). DM causes a disorder in the absorbent of glucose as well as glucose metabolism. A single dose of STZ as low as 50 mg/kg produces an incomplete destruction of pancreatic beta cells even though the rats become permanently diabetic (21). The purpose of this study was to evaluate the potential of the effectiveness of *G. officinalis* on blood biochemical factors associated with diabetes in rats which

Table 2. Comparison the Serum Level of Cholesterol, HDL, LDL and TG in Normal, DC, DG and DHEG groups

Group	TG (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	Cholesterol (mg/dL)
Normal	38.5965±1.58	21.1474±1.51	43.6894±1.59	73.0150±2.32
DC	62.3747±4.58†	23.2278±3.68	30.9222±0.53†	79.9289±1.04†
DG	48.0503±2.59*	16.0891±2.99*	39.9676±2.61*	72.9426±2.30*
DHEG	35.7895±4.13*	11.9152±2.03*	45.2413±1.15*	68.5726±3.37*

Normal; The healthy control group receiving normal saline. DC; diabetic control group, receiving normal saline. DG; Treated with 5 mg/kg glibenclamide. DHEG; A diabetic group that receiving 50 mg/kg hydroalcoholic extracts of Galega officinalis.
The asterisk * shows significant difference with the DC group and the symbol of † means the significant difference with the Normal group ($P\leq0.05$).

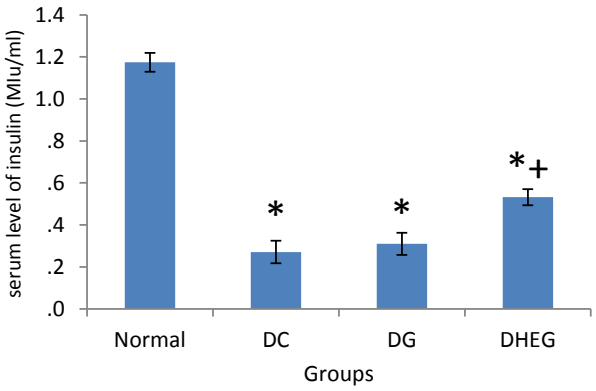

Figure 5. Comparison the Serum Level of Insulin in Normal, DC, DG and DHEG groups. Normal; The healthy control group receiving normal saline. DC; diabetic control group, receiving normal saline. DG; Treated with 5 mg/kg glibenclamide. DHEG; A diabetic group that receiving 50 mg/kg hydroalcoholic extracts of *Galega officinalis*. The asterisk sign shows significant difference with the Normal group and the symbol of + means the significant difference with the DC group ($P \leq 0.05$).

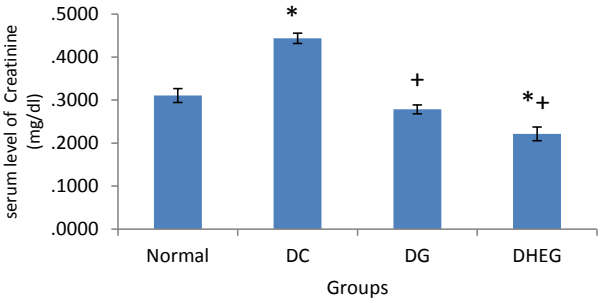

Figure 6. Comparison the Serum Level of Creatinine in Normal, DC, DG and DHEG groups. Normal; The healthy control group receiving normal saline. DC; diabetic control group, receiving normal saline. DG; Treated with 5 mg/kg glibenclamide. DHEG; A diabetic group that receiving 50 mg/kg hydroalcoholic extracts of *Galega officinalis*. The asterisk sign shows significant difference with the Normal group and the symbol of + means the significant difference with the DC group ($P \leq 0.05$).

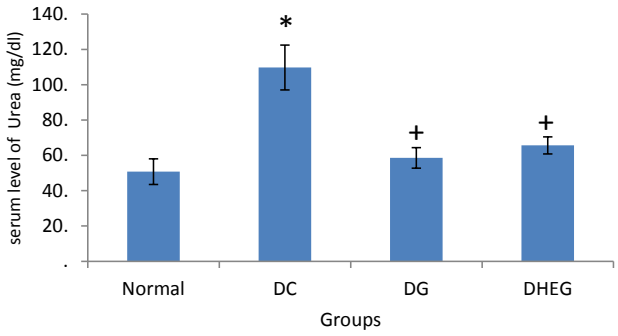

Figure 7. Comparison the Serum Level of Urea in Normal, DC, DG and DHEG groups. Normal; The healthy control group receiving normal saline. DC; diabetic control group, receiving normal saline. DG; Treated with 5 mg/kg glibenclamide. DHEG; A diabetic group that receiving 50 mg/kg hydroalcoholic extracts of *Galega officinalis*. The asterisk sign shows significant difference with the Normal group and the symbol of + means the significant difference with the DC group ($P \leq 0.05$).

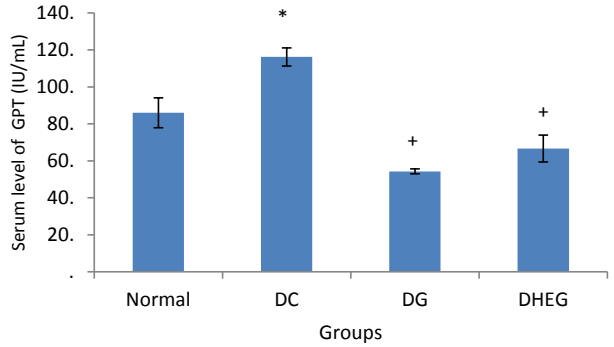

Figure 8. Comparison the Serum Level of GPT in Normal, DC, DG and DHEG groups. Normal; The healthy control group receiving normal saline. DC; diabetic control group, receiving normal saline. DG; Treated with 5 mg/kg glibenclamide. DHEG; A diabetic group that receiving 50 mg/kg hydroalcoholic extracts of *Galega officinalis*. The asterisk sign shows significant difference with the Normal group and the symbol of + means the significant difference with the DC group ($P \leq 0.05$).

were diabetic with the method of induced-STZ. The results of this study showed that intraperitoneal injections of 50 mg/kg of STZ Could be induced hyperglycemia and diabetes in rats during 72 hours. Nabi et al showed that 50 mg/kg of STZ could be induced hyperglycemia and diabetes during 72 hours (22). Also, Kumar et al induced diabetes type 1 by intraperitoneal injection of STZ that was solved in the citrate buffer (pH=4.7) (23). In the present study, after confirming hyperglycemia in rats, to control blood sugar was used *G. officinalis* extract that the treatment with the extract of this plant was able to greatly control the blood sugar in diabetic rats. The reasons of its anti-hyperglycemic are the compounds such as alkaloids, flavonoids, glycosides, resin, steroids, tannins, and phenols which according to the research conducted that these compounds have antidiabetic trait (24). Also, Shen stated that *Galega* due to having Bygvanydyn composition has similar function with metformin. Therefore, it can be supposed that *Galega* can be useful to reduce blood sugar (25). This reason can be assumed as the antidiabetic effects of *Galega*. Also, to compare the severity of glucose lowering used of glibenclamide that the severity of the same condition of glucose lowering in both groups was almost identical. As Rajasekaran et al for comparison the power of glucose lowering used of groups were treated with glibenclamide (26). The comparison of serum insulin level in the different groups showed that induced diabetes by STZ cause damage to pancreatic beta cells and reduce blood insulin levels (21,27). In a study, Ravi et al showed that STZ can cause damage to the beta cells of the pancreas and decreased serum levels of insulin (28). This entry also was confirmed by other studies (21,27). Treatment with *Galega* extract can be increased insulin levels that may be due to the presence of compounds such as flavonoids, tannins, saponins, glycosides, resin, and steroids in this plant. Therefore, it has high antioxidant property and is

caused protection of beta cells against oxidative stress (24). Coskun et al showed that flavonoids can lower blood sugar, protect the beta cell against oxidative stress and maintain pancreatic beta cell integrity (29). Moreover, other studies have suggested that antioxidants can protect the beta cells against oxidative stress and prevent beta cell destruction (30,31). As results showed, antioxidant property of *Galega* increases serum insulin levels relative to glibenclamide. DM is a disease associated with metabolism, can cause a disturbance in lipid metabolism (33). In the present study by comparing the lipid profiles in different groups, we found that diabetes leads to increase levels of cholesterol, LDL, and triglycerides and also decrease HDL levels. This results are in line with the study of Husain et al study (32). On the other hand, in our study, the level of LDL in the diabetic control group relative to the normal group insignificantly increased. Serum level of lipid profile could be controlled during treatment by *Galega* extract. The extract of this plant significantly reduced levels of TG, cholesterol, and LDL and also increased the level of HDL. One of the reasons that why this plant can control lipid profile may be due to have abundant antioxidant compounds such as flavonoids (24). A study has shown that flavonoids can control blood lipid levels (34). The kidney is an organ that diabetes impairs their function so that increased blood levels of urea and creatinine are indicative of impaired in glomerular filtration (35), which is due to oxidative stress. Also, Ravi et al showed that the oxidative stress in diabetic rats can damage the liver and kidney tissues and also disrupt their functions. In the present study in diabetic rats, kidneys weight, the diameter of glomeruli, and the thickness of the basement membrane increased, and also the number of glomeruli, and diameter of the urinary spaces decreased which indicate damage to the kidney tissue and reduction in glomerular filtration (28). In agreement with our results, Sharma et al demonstrated that serum level of urea and creatinine increased in diabetes (36). Results of this study showed that treatment with *G. officinalis* extract can protect the kidney tissue against tissue damage which induced by oxidative stress related to diabetes. Decreasing the kidneys weight, reducing the diameter of glomeruli, increasing the number of glomeruli, increasing the diameter of the urinary spaces, decreasing the thickness of the basement membrane, and also decreasing the level of urea and creatinine in diabetic rats may be due to the presence of abundant antioxidant compounds of the plant (24). In agreement with our results, Sharma et al showed that antioxidants can reduce the level of urea and creatinine (36).

The liver is one of the other organs that diabetes has side effects on it in which the levels of GPT and ALP increase. Marzouk et al showed that diabetes leads to impair the metabolism of liver and increase levels of its enzymes (37). Moreover, Maritim et al found same results (38). According to the results of the present study, we observed that *Galega* extract reduced the level of GPT and also by its antioxidant property prevent the liver damage in diabetic rats. Marzouk et al showed that herbal antioxidants inhibit the increasing levels of liver enzymes (37).

Conclusion

The results of the present study indicated that *G. officinalis* could be beneficial for the treatment of diabetes and reducing the FBS through improving the serum level of insulin and preventing kidney tissue damages by antioxidant feature.

Ethical Issues

This study was performed after approving by the ethics committee of Gonabad University of Medical Sciences

Conflicts of Interests

None.

Financial Support

Gonabad University of Medical Sciences supported the study.

Acknowledgments

Authors would like to thank from Deputy of research and technology and Student Research Committee of Gonabad University of Medical Sciences for financial support of this study.

Reference

1. Momin M, Momin S, Kurhade S, Mohebkhan, Butte K. *Nigella sativa:* Blessed Seed. Res Phyochem Pharmacho. 2013;3:78-84.
2. Hafez DA. Effects of *Nigella sativa* oil and thymoquinone on renal oxidative stress and apoptosis rate in streptozotocin-diabetic rats. J Am Sci. 2013;9(3):327-333.
3. Kim SH, Hyun SH, Choung SY. Anti-diabetic effect of cinnamon extract on blood glucose in db/db mice. J Ethnopharmacol. 2006;104(1-2):119-23. doi: 10.1016/j.jep.2005.08.059.
4. Sheetz MJ. Molecular understanding of hyperglycemias adverse effects for diabetic complications. J Am Med Assoc. 2002;288:2579-2588.
5. Goldstein J, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. J Clin Invest 1973;52:1544–1568. doi:10.1172/JCI107332.
6. Moller DE. New drug targets for type 2 diabetes and metabolic syndrome. Nature 2001;414(6865):821-827. doi: 10.1038/414821a.
7. Gray AM, Flatt PR. Insulin–releasing and insulin– like activity of the traditional antidiabetic plant *Coriandrum sativum* (Coriander). Br J Nutr. 1999;81(3):208-209.
8. Moneim AA, El-Feki M, Salah E. Effect of *Nigella sativa,* fish oil and gliclazide on alloxan diabetic rats. Biochemical and histopathological studies. Journal-Egyptian German Society of Zoology. 1997;23:237–265.
9. Shanmugasundaram ER, Gopith KI, Radha SK, Rajendram VM. Possible regeneration of the islets of Langerhans in streptozocin diabetic rats given *Gymnema sylvester* leaf extract. J Ethnopharmcol. 1990;30:265-269.

10. Adewole SO, Ojewole JA. Insulin-induced immunohistochemical and morphological changes in pancreatic beta-cells of streptozotocin-treated diabetic rats.Methods Find Exp Clin Pharmacol. 2007;29(7):447-455. doi:10.1358/mf.2007.29.7.1119168.
11. Singh SN, Vats P, Suri S, et al. Effect of an antidiabetic extract of *Catharanthus roseus* on enzymic activities in streptozotocin induced diabetic rats. J Ethnopharmcol. 2001;73(3):269-277.
12. Chopra RH, Nagar SL, Chopra IP. 1956. Glossary of Indian Medicinal Plants. New Delphi, India; 1956:20.
13. Petkov VD. Modern Phytotherapy Medizina I. Sofia: Fizkultura Publications House; 1982:307. [Bulgarian].
14. Oubré AY, Carlson TJ, King SR, Reaven GM. From plant to patient: an ethnomedical approach to the identification of new drugs for the treatment of NIDDM.Diabetologia. 1997;40(5):614-617. doi:10.1007/s001250050724.
15. Reuter G. Arginin als Vorstufe von Galegin in *Galega officinalis* L. Zur Biochemie und Physiologie von Galegin in Galega officinalis L., III. Mitt. Arch Pharm. 1963;296(8):516-522.
16. Palit P, Furman BL, Gray AI. Novel weight-reducing activity of Galega officinalis in mice. J Pharm Pharmacol. 1999;51(11):1313-9.
17. Vuksan V, Sievenpiper JL. Herbal remedies in the management of diabetes: lessons learned from the study of ginseng. Nutr Metab Cardiovasc Dis. 2005;15(3):149-160. doi:10.1016/j.numecd.2005.05.001.
18. Rang HP, Dale MM. The Endocrine System Pharmacology. 2nd ed. Harlow: Longman; 1991:504.
19. Grover JK, Yadav S, Vats V. Medicinal plants of india with antidiabetic potential. J Ethnopharmacol. 2002;1:81-100.
20. Shu YZ. Recent natural products based drug development: a pharmaceutical industry perspective. J Nat Prod. 1998;61:1053-1071.
21. Kumar S, Singh R, Vasudeva N, Sharma S. Acute and chronic animal models for the evaluation of anti-diabetic agents. Cardiovasc Diabetol. 2012;11:9
22. Nabi SA, Kasetti RB, Sirasanagandla S, Tilak TK, Kumar MV, Rao CA. Antidiabetic and antihyperlipidemic activity of Piper longum root aqueous extract in STZ induced diabetic rats. BMC Complement Altern Med. 2013;13:37. doi:10.1186/1472-6882-13-37.
23. Kumar S, Vasudeva N, Sharma S. GC-MS analysis and screening of antidiabetic, antioxidant and hypolipidemic potential of Cinnamomum tamala oil in streptozotocin induced diabetes mellitus in rats. Cardiovasc Diabetol. 2012;11:95. doi:10.1186/1475-2840-11-95.
24. Rasekh HR, Nazari P, Kamli-Nejad M, Hosseinzadeh L. Acute and subchronic oral toxicity of *Galega officinalis* in rats. J Ethnopharmacol. 2008;116(1):21-6. doi:10.1016/j.jep.2007.10.030.
25. Shen M. A second look at the ancient drug: new insights into metformin. Ann Transl Med. 2014;2(6):51.
26. Rajasekaran S, Sivagnanam K, Subramanian S. Antioxidant effect of Aloe vera gel extract in streptozotocin-induced diabetes in rats. J Pharm Rep. 2005;57:90-98.
27. Umamaheswari S, Mainzen Prince PS. Antihyperglycaemic effect of 'Ilogen-Excel', an ayurvedic herbal formulation in streptozotocin-induced diabetes mellitus. Acta Pol Pharm. 2007 Jan-Feb;64(1):53-61.
28. Ravi K, Ramachandran B, Subramanian S. Protective effect of *Eugenia jambolan*a seed kernel on tissue antioxidants in streptozotocin-induced diabetic rats. Biol Pharm Bull. 2004;27(8):1212-1217.
29. Coskun O, Kanter M, Korkmaz A, Oter S. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and beta-cell damage in rat pancreas. Pharmacol Res. 2005 Feb;51(2):117-23. doi:10.1016/j.phrs.2004.06.002.
30. Robertson RP. Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. J Biol Chem. 2004;279(41):42351-42354
31. Srivivasan A, Menon VP, Periaswamy V, Rajasekaran KN. Protection of pancreatic beta-cell by the potential antioxidant bis-ohydroxycinnamoyl methane, analogue of natural curcuminoid in experimental diabetes. J Pharm Pharm Sci. 2003;6:327-333.
32. Husain GM, Singh PN, Kumar V. Antidiabetic activity of standardized extract of Picrorhiza kurroa in rat model of NIDDM. Drug Discov Ther. 2009;3(3):88-92.
33. Veerapur VP, Prabhakar KR, Parihar VK, et al. Antidiabetic, hypolipidemic and antioxidant activity of *Dodonaea viscosa* aerial parts in streptozotocin-induced diabetic rats. Int J Phytomed. 2010;2:59-70.
34. Koshy AS, Vijayalakshmi NR. Impact of certain flavonoids on lipid profiles--potential action of Garcinia cambogia flavonoids. Phytother Res. 2001 Aug;15(5):395-400.
35. Meyerovitch J, Farfel Z, Sack J, Shechter Y. Oral administration of vanadate normalizes blood glucose levels in streptozotocin-treated rats. Characterization and mode of action. J Biol Chem. 1987;262(14):6658-6662.
36. Sharma S, Kulkarni SK, Chopra K. Curcumin, the active principle of turmeric (*Curcuma longa*), ameliorates diabetic nephropathy in rats. Clin Exp Pharmacol Physiol. 2006;33(10):940-945. doi:10.1111/j.1440-1681.2006.04468.x
37. Marzouk M, Soliman AM, Omar TY. Hypoglycemic and antioxidative effects of fenugreek and termis seeds powder in streptozotocin-diabetic rats. Eur Rev Med Pharmacol Sci. 2013;17(4):559-565.
38. Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol. 2003;17(1):24-38.