



Effects of Hydroalcoholic Extracts of Cloves (*Syzygium aromaticum*) on the Serum Biomarkers, Antioxidant Status, and Histopathological Changes of Kidneys in Diabetic Rats

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Abstract

Objectives: The purpose of this study was to assess the possible impact of hydroalcoholic extracts of cloves (*Syzygium aromaticum*) on glucose status, lipid profile, and histopathological changes in the kidneys of diabetic rats.

Materials and Methods: Thirty-two rats (male) were distributed into 4 groups (n=8), including one group as healthy control and three diabetic groups. Streptozotocin was used for inducing diabetes (50 mg/kg). Diabetic rats were grouped into a control group (DC), diabetic treated with 4 mg/kg hydroalcoholic extract of *S. aromaticum* (DSA), and the DG group receiving 5 mg/kg glibenclamide. After the treatment period, the blood samples of the rats were frozen in -70°C for measuring glycemic indices, insulin, lipid profile, some oxidative stress markers, and enzymes with antioxidant properties. Finally, the kidney was removed for the histological study.

Results: Control, DSA, and DG groups had significantly lower levels of fasting blood sugar compared to the DC group ($P<0.05$) while the levels of insulin were significantly lower in the DC, DSA, and DG groups compared to the control group ($P<0.05$). The serum levels of urea and creatinine statistically reduced in all groups other than the DC group ($P<0.05$). Conversely, the levels of superoxide dismutase, glutathione peroxidase, and significantly increased in the DSA and DG as compared to the DC group although the serum levels of malondialdehyde statistically decreased in the therapeutic groups. *S. aromaticum* showed antioxidant properties and protected the kidneys of the experimented rats from renal damages, resulting from diabetes.

Conclusions: This study demonstrated that *S. aromaticum* may have beneficial effects in diabetes through improving glycemic control and lipid profile and preventing diabetes-induced kidney damages.

Keywords: Cloves, Diabetes, Lipid profile, Antioxidant status, Kidney damages

Introduction

Diabetes mellitus (DM), as a chronic metabolic disease, is identified by hyperglycemia and the late development of vascular and neuropathic complications (1) and high blood glucose concentrations due to either impaired secretion of insulin or its peripheral resistance or both (2). It is expected that over 75% of all people with diabetes will be from developing countries by 2025 (3). DM affects 4% of the world's population, rising to 5.4% by 2025 (4), mainly causes hyperglycemia and hyperlipidemia (5), and some other complications (e.g., atherosclerosis, nephropathy, neuropathy, and microvascularization) are common in patients with DM (6). It is clear that hyperlipidemia is the main reason for developing atherosclerosis in this group of patients (7,8) with a 2-3 times higher risk of coronary artery disease and related lipid profile and lipoprotein abnormalities (9). Currently, the recommended treatments for diabetes are

lifestyle modifications, insulin therapy (10), and a variety of oral glucose-lowering drugs such as sulfonylureas, thiazolidinediones, and α -glucosidase inhibitors all with various complications (11,12). Different treatment modalities act through distinct mechanisms for controlling diabetes (13,14), including stimulating the secretion of insulin such as sulfonylurea and meglitinides drugs (15), improving the glucose peripheral absorption (biguanides and thiazolines), and reducing hepatic gluconeogenesis by biguanides (16). In addition, herbal therapy is considered as an effective way for the management of diabetes (17). Some of them may contain insulin-like substances (18) or increase the number of pancreatic beta cells (β -cells) by activating their regeneration (19-22). These plants contain carotenoids, flavonoids, terpenoids, alkaloids, and glycosides and may exert anti-diabetic effects to some extent (21). *S. aromaticum*, which is usually known as clove, is originated from Asia (23) and is known

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Key Messages

- ▶ Diabetes led to kidney damage.
- ▶ Administration of clove extract protects the rat kidney against tissue damage related to diabetes.

for having antioxidant, antimicrobial, antifungal, and antiviral properties (24). Clove flower buds have 18% essential oil, 89% of which is eugenol (23). Some studies suggested the antioxidant effects of eugenol (23,25). Pinto et al showed that *S. aromaticum* has anti-oxidant and anti-fungal properties due to having eugenol (26). This study sought to evaluate the effects of hydroalcoholic extracts of cloves (*S. aromaticum*) on glucose status, lipid profile, and histopathological changes in the kidneys of diabetic rats.

Materials and Methods

Thirty-two male rats with an average weight of 200-250 g were investigated in this study. The required rates were provided by Mashhad Razi Institute and they were all kept under the standard condition (at the temperature of 25°C and 12/12 hour light/dark cycle).

Food and water were freely available for rats during the experiment. This study was run based on the instruction of Gonabad University of Medical Sciences for the maintenance and use of laboratory animals.

Animal Preparation

The rats were randomly assigned to the control and experimental groups containing 7 and 21 rats, respectively. Diabetes was induced by a single-dose injection of 50 mg/kg streptozotocin (Sigma) dissolved in 5 mmol citrate buffer (pH = 4.5) (27). Seventy-two hours after injection, a higher serum glucose level of 250 mg/dL was considered as a confirmation of DM. Diabetic rats were randomized into three balanced groups of the diabetic control group (DC) receiving normal saline as a carrier, and the remaining two diabetic groups received 4 mg/kg hydroalcoholic extract of *S. aromaticum* (DSA) and a standard drug (5 mg/kg glibenclamide, DC), respectively. For all groups, intraperitoneal treatment was performed once during 20 days. In the healthy control group, streptozotocin was substituted by an identical volume of citrate buffer. After the cessation of the treatment period on the 21st day, blood samples were taken from the anesthetized rats for measuring blood glucose, insulin, serum lipid profile, and markers of oxidant/antioxidant status and antioxidant enzymes such as malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx). Moreover, kidneys, pancreas, and liver were isolated and frozen at -70°C for subsequent assessments.

Kidney Weight

A digital scale was used for weighing the left and right kidneys of the rats.

Histopathological Studies

The samples were fixed by the formalin 10% and then dehydrated and embedded in paraffin for being cut to 5 µm by a microtome. Thereafter, hematoxylin-eosin (H&E) was used for the staining procedure. Some slides were studied for assessing the histological alteration and the others were applied for measuring the morphological parameters.

Assessment of Morphological Parameters and the Basic Membrane

The fixed samples by H&E were used for measuring the diameter and changes in the number of glomeruli and urinary spaces by ImageJ software. The thickness of the basement membrane (periodic acid-Schiff staining) was applied for measurement.

Evaluation of Biochemical Parameters

At the beginning of the study, serum glucose levels were measured using samples collected from the tails of the rats by a moveable glucometer and a commercial kit (Parsazmun, Iran), respectively. The measurement unit was milligram per deciliter. The method for measuring the lipid peroxidation or serum level of MDA used 0.20 cm³ of plasma into a microtube with 3.0 cm³ of "glacial acetic acid", to which "3.0 cm³ of 1% TBA in 2% NaOH" was added as well. The microtube containing the mixture was put into the boiling water for fifteen minutes. After cooling, the absorbance of the product was read at 532 nm, and the calibration curve was drawn by "malondialdehyde tetrabutylammonium salt" prepared from Sigma (USA). The activities of SOD and GPx in the serum were measured based on the protocols of the kits (Randox, UK) according to (28).

Assay of Serum Insulin Level

The rat insulin kit (MercoDIA) was used for measuring the blood insulin levels.

Statistical Analysis

One-way analysis of variance (ANOVA) and independent *t* test were applied for determining between-group differences based on a two-tailed analysis, and $P < 0.05$ was set for the level of significance.

Results

Kidney Weight

The DC group had a statistically higher kidney weight compared to the healthy control ($P < 0.05$) and significantly decreased in the DG and DSA groups in comparison with the DC group. There was a statistically remarkable decrease in the kidney weight in the DSA group in comparison with the healthy control group ($P < 0.05$), which is illustrated in Figure 1.

Histopathological Parameters of the Kidney

A remarkable decrease was observed in the glomerular

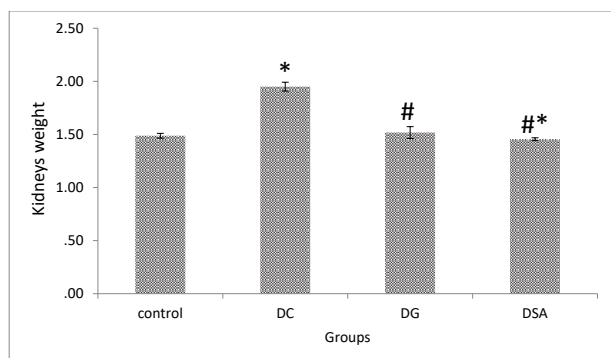


Figure 1. Comparison of the Weight of the Kidney in the Study Groups. *Note.* Normal: The control group receiving normal saline; DC: Diabetic control group receiving normal saline; DG: Treated with 5 mg/kg glibenclamide; DSA: A diabetic group receiving 4 mg/kg hydroalcoholic extracts of *Syzygium aromaticum*. The asterisk * shows a significant difference with the normal group and symbol # indicates a significant difference with the DC group ($P \leq 0.05$).

diameter in DC, DG, and DSA groups compared with healthy control ($P < 0.05$). However, it was significantly higher in DG and DSA groups compared to the DC group ($P < 0.05$). In terms of the urinary space, there was a statistically significant difference between the DC group and healthy control, as well as DG and DSA groups and DC group so that it was extremely lower in the DC group compared to healthy control while extensively higher in DG and DSA groups in proportion to the DC group ($P < 0.05$). The glomeruli number significantly decreased in DG and DSA groups compared to the healthy control although it statistically considerably increased in these two groups compared to the DC group ($P < 0.05$), the related data of which are shown in Table 1 and Figure 2.

Glomerular Basement Membrane Thickness

A statistically significant increase was observed in the thickness of the glomerular basement membrane in the DC group in comparison with the healthy control while it was remarkably lower in DG and DSA groups compared to DC control (Figure 3).

Fasting Blood Glucose and Serum Levels of Insulin

Fasting blood glucose had significantly higher levels in the DC group compared to healthy control while it was extremely lower in DG and DSA groups in comparison

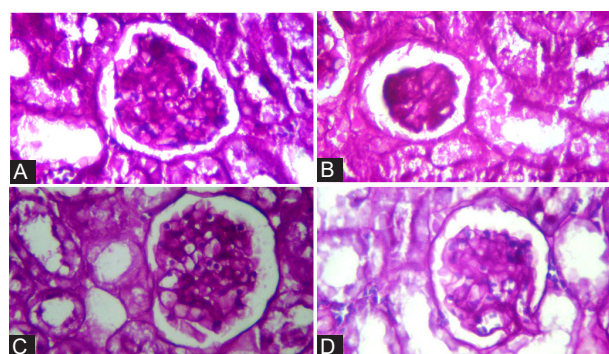


Figure 2. Histological Parameters (H&E) Staining. *Note.* H&E: Hematoxylin-Eosin; (A) Control: Control group receiving normal saline for 21 days, (B) DC: Diabetic control group receiving normal saline, (C) DG: Diabetic group treated with 5 mg/kg glibenclamide, (D) DSA: A diabetic group receiving 4 mg/kg hydroalcoholic extracts of *Syzygium aromaticum*.

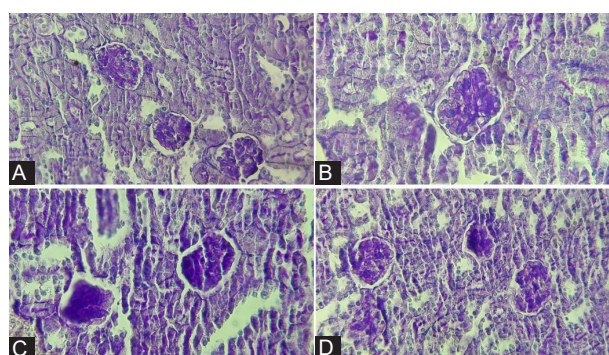


Figure 3. Histological Parameters (PAS staining). *Note.* PAS: Periodic acid-Schiff; (A) Control: Control group receiving normal saline for 21 days, (B) DC: Diabetic control group receiving normal saline, (C) DG: Diabetic group treated with 5 mg/kg glibenclamide; (D) DSA: A diabetic group that receiving 4 mg/kg hydroalcoholic extracts of *Syzygium aromaticum*.

with DC ($P < 0.05$, Figure 4). Moreover, the serum levels of insulin decreased in all three diabetic groups (DC, DG, and DSA) compared to the healthy control ($P < 0.05$), but there was a considerable difference between DC and DG and DSA groups so that the serum levels of insulin were lower in the last two groups compared to the DC group although it was significant only for the DSA group (Figure 5).

Serum Lipid Profile

The serum level of cholesterol remarkably reduced in DSA and DG groups compared to the DC group and there was a significant difference between the DC group and healthy

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

Groups	Glomerular Diameters	Urinary Space Diameters	Number of Glomeruli
Control	270.94 ± 2.85	37.4 ± 1.16	33127.5 ± 320.84
DC	325.13 ± 10.7#	20.08 ± 0.82	22531.7 ± 172.62 [#]
DSA	263.90 ± 9.68*	36.70 ± 0.65*	25297.5 ± 2350.18* [#]
DG	251.86 ± 3.88*	30.18 ± 0.44*	25469.2 ± 381.30* [#]

Note. DC: Diabetic control group receiving normal saline; DG: Treated with 5 mg/kg glibenclamide; DSA: A diabetic group receiving 4 mg/kg hydroalcoholic extracts of *Syzygium aromaticum*. The symbols * and # show significant differences with the DC and normal groups, respectively ($P \leq 0.05$).

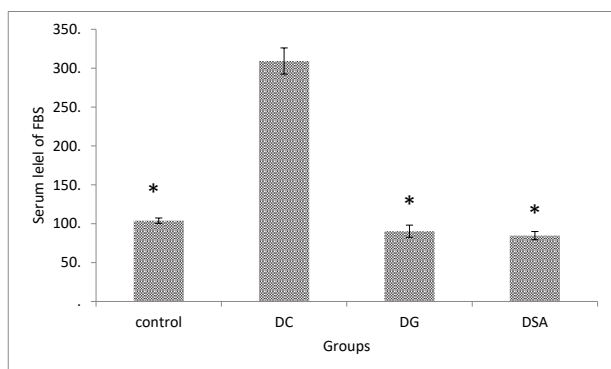


Figure 4. Comparison of the Serum Level of FBS in the Study Groups. Note. FBS: Fasting blood sugar; Normal: The control group receiving normal saline; DC: Diabetic control group receiving normal saline; DG: Treated with 5 mg/kg glibenclamide; DSA: A diabetic group receiving 4 mg/kg hydroalcoholic extracts of *Syzygium aromaticum*. The asterisk * indicates a significant difference with the normal group.

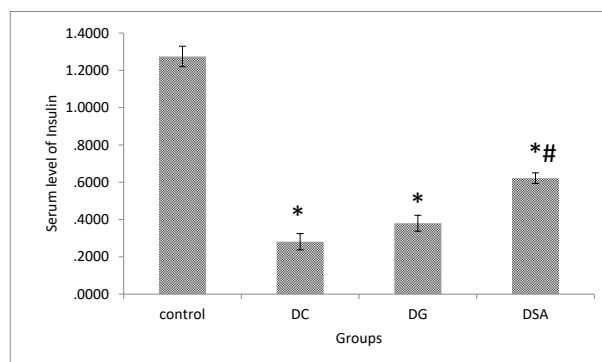


Figure 5. Comparison of the Serum Level of Insulin in the Study Groups. Note. Normal: The control group receiving normal saline; DC: Diabetic control group receiving normal saline; DG: Treated with 5 mg/kg glibenclamide; DSA: A diabetic group receiving 4 mg/kg hydroalcoholic extracts of *Syzygium aromaticum*. The asterisk * shows a significant difference with the normal group and symbol # implies a significant difference with the DC group ($P \leq 0.05$).

controls in terms of cholesterol levels ($P < 0.05$, Table 2). Regarding low-density lipoprotein (LDL) cholesterol and triglycerides (TG) levels, the highest values belonged to the DC group while the lowest LDL cholesterol and TG levels were related to DSA and DG groups, respectively (Table 2). The differences between DSA, DG, and DC groups were statistically significant in terms of TG levels ($P < 0.05$). Serum high-density lipoprotein (HDL) cholesterol was higher in DG and DSA groups compared to the DC group with a slightly higher level in the DG group compared to healthy controls with no statistically significant difference. Additionally, the DC group had an extremely lower HDL cholesterol level when compared to the healthy control ($P < 0.05$), the related data of which are presented in Table 2.

Serum Creatinine and Urea

The serum levels of creatinine and urea were significantly higher in the DC group compared to the healthy control ($P < 0.05$) and there were significantly lower levels of creatinine and urea in DG and DSA groups compared to the DC group ($P < 0.05$, Figures 6 and 7).

Serum Oxidative Stress Markers

The levels of GPx and SOD in plasma were statistically lower in the DC group in comparison with the healthy control group ($P < 0.05$). In the DSA and DG groups, the

plasma levels of GPx and SOD were significantly higher compared to the DC group ($P < 0.01$). On the other hand, the plasma level of MDA significantly raised in the DC group compared to the control group ($P < 0.01$) while the serum levels of MDA represented a significant decrease in the DSA and DG groups ($P < 0.01$, Table 3).

Discussion

Diabetes is a chronic disease with hyperglycemia and hyperlipidemia as its common complications (29). The results of this study revealed that clove has a positive effect on glycemic control by increasing the serum levels of insulin while decreasing the glucose levels, which is in line with the findings of Kuroda et al demonstrating the hypoglycemic effects of *S. aromaticum* (30). The results of another study showed that *S. aromaticum* may suppress the expression of the genes of “Phosphoenolpyruvate carboxykinase” and “Glucose 6-phosphatase”, which both have known to play an enzymatic role in gluconeogenesis. Therefore, *S. aromaticum* has insulin-like effects and decreases insulin requirements by reducing the activity of the intestinal alpha-glucosidase enzyme and thus glucose uptake (31). Adefegha et al suggested that the hypoglycemic effects of clove can be attributed to a decrease in the activity of the intestinal alpha-glucosidase that involves in intestinal glucose absorption (32). The

Table 2. Comparison of the Serum Level of the Lipid Profile in Control, DC, DG, and DSA Groups

Groups	Cholesterol \pm SD	HDL \pm SD	LDL \pm SD	TG \pm SD
Control	70.15 \pm 3.32*	45.36 \pm 1.59	20.51 \pm 1.51	34.42 \pm 5.32*
DC	84.33 \pm 4.15*	27.35 \pm 0.58	36.14 \pm 3.68	69.2 \pm 3.21
DSA	71.32 \pm 7.31*	35.96 \pm 1.61	11.48 \pm 2.99	45.75 \pm 3.6*
DG	65.19 \pm 0.10*	48.68 \pm 1.60	23.34 \pm 2.25	37.3 \pm 4.25*

Note. SD: Standard deviation; Control: The control group receiving normal saline; DC: Diabetic control group receiving normal saline; DG: Treated with 5 mg/kg glibenclamide; DSA: A diabetic group receiving 4 mg/kg hydroalcoholic extracts of *Syzygium aromaticum*. The asterisk * indicates a significant difference with the DC group and symbol # means a significant difference with the normal group ($P \leq 0.05$).

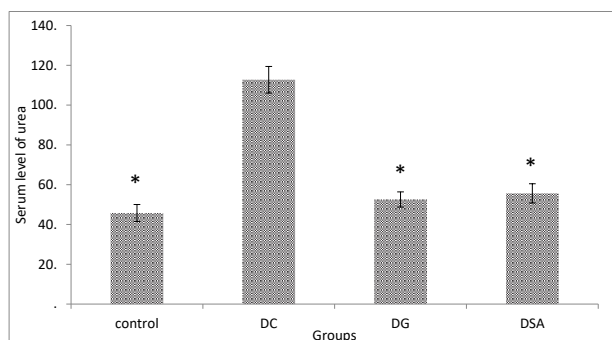


Figure 6. Comparison of the Serum Level of Urea in the Study Groups. *Note.* Normal: The control group receiving normal saline. DC: Diabetic control group receiving normal saline; DG: Treated with 5 mg/kg glibenclamide; DSA: A diabetic group receiving 4 mg/kg hydroalcoholic extracts of *Syzygium aromaticum*. The asterisk * shows a significant difference with the normal group.

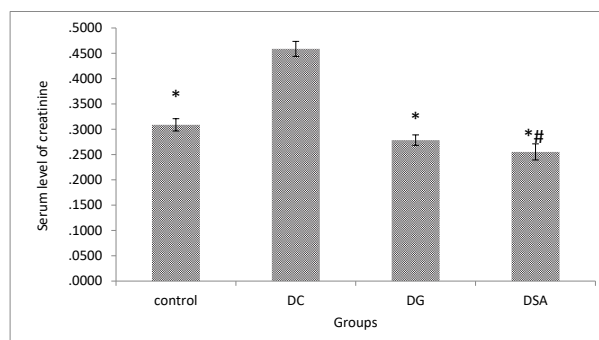


Figure 7. Comparison of the Serum Level of Creatinine in the Study Groups. *Note.* Normal: The control group receiving normal saline; DC: Diabetic control group receiving normal saline; DG: Treated with 5 mg/kg glibenclamide; DSA: A diabetic group receiving 4 mg/kg hydroalcoholic extracts of *Syzygium aromaticum*. The asterisk * represents a significant difference with the normal group and symbol # indicates a significant difference with the DC group ($P \leq 0.05$).

increased levels of this enzyme in diabetic rats can be responsible for higher blood glucose levels (33). Some phenolic compounds such as eugenol and eugenyl acetate may be responsible for this hypoglycemic effect (34). Eugenol may reduce glycogen phosphorylase activity and prevent glucose production by glucagon in the body (35). The glucose-lowering effect of *S. aromaticum* may be due to the stimulating of pancreatic beta cells for increasing insulin production (36). Lipoprotein disorders are common in patients with diabetes and they are prone to hyperlipidemia with an increase in serum cholesterol, TG, and LDL cholesterol levels while a reduction in HDL cholesterol (37). One of the reasons is insulin resistance or reduced levels of insulin (38), which results in decreasing the activity of lipoprotein lipase, which is the key enzyme in hydrolysis lipoproteins containing TG (39). Our results revealed that *S. aromaticum* decreases LDL cholesterol, total cholesterol (TC), and TG levels while increasing the serum levels of HDL. Moreover, *S. aromaticum* can prevent lipid peroxidation by lowering the levels of MDA (32,40). Therefore, due to the ability of *S. aromaticum* for restoring beta cell activities and improving the serum insulin levels, and subsequently, increasing the activity of the enzyme lipoprotein lipase, treatment with the extract of this herb may reduce the levels of TG, LDL, and TC whereas increasing the level of serum HDL in diabetes.

Renal damage and its function impairment in diabetes may be due to oxidative stress (41). In this study, the weight of the kidneys, glomerular diameter, and the thickness of the basement membrane increased while glomeruli number and the urinary space diameter reduced in the diabetic rats, showing kidney damage and lowered glomerular filtration. Sharma et al also indicated that diabetes causes an increase in the serum levels of urea and creatinine (42). Based on the results of the current study, treatment with *S. aromaticum* can protect the kidney tissue against oxidative damage, decreasing the kidney weight, the diameter of glomeruli, and the thickness of the basement membrane, and may result in decreasing the level of urea and creatinine. However, it may increase the glomeruli number and diameter of the urinary spaces in diabetic rats and may be due to the strong antioxidant properties of the plant (27). Bakour et al showed that *S. aromaticum* can reduce the damage to the kidneys and liver caused by hydrogen peroxide (43). In another study, Adam et al demonstrated that *S. aromaticum* reduced the serum levels of the urea (44). In this study, *S. aromaticum* significantly increased the serum levels of SOD, GPx, and MDA in DSA and DG groups compared to the DC control group whereas significantly decreasing the serum levels of MDA in the therapeutic groups. Similar results were reported by other studies (32,45). A decrease in the

Table 3. Serum Levels of SOD (U/mL), GPx (U/mL), and MDA (nM) in Different Groups

Groups	SOD (Mean \pm SEM)	GPx	MDA (Mean \pm SEM)
Control	2.23 \pm 0.11	220.45 \pm 5.11	1.38 \pm 0.11
DC	1.07 \pm 0.12*	95.34 \pm 7.32*	2.72 \pm 0.29*
DSA	2.09 \pm 0.17*	195.24 \pm 4.21*	1.46 \pm 0.21*
DG	1.81 \pm 0.08*	174.76 \pm 6.08*	1.65 \pm 0.14*

Note. SEM: Standard error of the mean; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; MDA: Malondialdehyde. The control group receiving normal saline. DC: Diabetic control group receiving normal saline; DG: Treated with 5 mg/kg glibenclamide. DSA: A diabetic group receiving 4 mg/kg hydroalcoholic extracts of *Syzygium aromaticum*. The asterisk * represents a significant difference with the DC group and symbol # demonstrates a significant difference with the normal group ($P \leq 0.05$).

activity of antioxidant enzymes can be due to the increase in the level of *reactive oxygen species*. Eugenol can prevent the peroxidation induced by iron through chelating it (46), and as a natural antioxidant, eugenol can have a protective effect on the active species such as (OH[•]) and (O₂[•]) (47). Antioxidant activities, radical scavenging activity, and metal chelating activity by eugenol have been reported by (46), and they may protect the kidney against oxidative damage (34).

Conclusions

Cloves (*S. aromaticum*) showed several beneficial effects for the management of diabetes-induced complications in this study and thus it can be considered as a good candidate for further research in patients with diabetes.

Authors' Contribution

MS, SHAE, MG planned and designed the experiments. HH and MS performed the experiments. MS, MH, TP, and JB analyzed the data. MS and HH wrote the manuscript. All of the authors reviewed and discussed the data.

Conflict of Interests

Authors have no conflict of interests.

Ethical Issues

This research was approved by the Animal Ethics Committee of the Gonabad University of Medical Sciences (Ethical code: IR.GMU.REC.114).

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