



Protective Effect of Hydroalcoholic Extracts of *Galega officinalis* and *Cornus mas* on Spermatogenesis and Oxidative Stress Associated With Diabetes in the Testes of Adult Rats: An Experimental Study

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Abstract

Objectives: In this research, we evaluated the protective effect of hydroalcoholic extracts of *Galega officinalis* and *Cornus mas* on spermatogenesis and oxidative stress associated with diabetes in the testes of adult rats.

Materials and Methods: In this experimental study, a total of 64 adult male Wistar rats were divided into eight equal groups (n=8 in each) as follows: (1) control group, (2) diabetic control group, (3) diabetic group receiving *Galega* extract daily at a dose of 50 mg/kg, (4) healthy group receiving *Galega* extract daily at a dose of 50 mg/kg for 14 days, (5) diabetic group receiving *Cornus mas* extract daily at a dose of 100 mg/kg, (6) healthy group receiving *Cornus mas* extract daily, (7) diabetic group receiving *Cornus mas* and *Galega* daily, and (8) healthy group receiving *Galega* and *Cornus mas* extract daily. Diabetes was induced by single intraperitoneal injection of streptozotocin (50 mg/kg). At the end of treatment period, all animals were anesthetized and blood samples were taken to measure the serum levels of insulin, glucose, and oxidative stress markers. Finally, the testicles and epididymis were removed and sperm parameters were assessed.

Results: *Galega* and *Cornus mas* extract significantly reduced the oxidative stress, sperm parameters, glucose, and insulin plasma levels ($P=0.001$). Furthermore, the malondialdehyde (MDA) level was increased in diabetic rats. The activity of superoxide dismutase (SOD) and catalase (CAT) enzymes decreased in testicular tissue ($P=0.001$). Administering *Galega* and *Cornus mas* extract significantly improved these parameters ($P<0.05$).

Conclusions: Our results confirmed the antioxidant effect of administering *Galega* extract and *Cornus mas* extract on improving the sperm parameters and testicular oxidative damage caused by diabetes.

Keywords: Oxidative stress, Diabetes, *Galega officinalis* extract, *Cornus mas* extract, Testis, Sperm parameters

Introduction

Diabetes is one of the most common metabolic disorders associated with increased serum levels of glucose. Diabetes is ensued either from insulin generation defects (insulin-dependent diabetes) or due to hormone resistance in peripheral tissues (non-insulin-dependent diabetes) with a decrease in the secretion of hormone from pancreatic islets β -cells. Sexual dysfunction is one of the significant complications of diabetes in men, which includes the declined weight of testis, low sperm quality, low level of testosterone in plasma, enhanced abnormal sperm, and infertility (1,2).

In diabetic patients, the high blood glucose causes an enhanced advanced glycation end-products (AGEs), changes in the protein kinase C activity, a disturbance in the prostanoids balance, and enhanced manufacturing of mitochondrial superoxide, leading to enhanced oxidative stress due to excess free radicals. Several studies have shown that amplifying the antioxidant system can reduce the complications of diabetes (3-5).

Although several chemical medicines have been applied

to treat diabetes, recently some herbal components have been used as low-risk remedies for diabetes. *Galega officinalis*, often called goastro, is a member of the Fabaceae family, growing in southwest Asia and southern Europe. It is one of the favorite plants of Europeans and Americans for cultivation due to its therapeutic and traditional uses. *G. officinalis* is a plant about one to two meters long. The stems are approximately 22 cm long and about 20-50 white to purple flowers, each one cm long at the end of the stems. Hydroalcoholic extract of *G. officinalis* contains flavonoids, tannins, saponins, glycosides, resins, and steroids. While these compounds (alkaloids, flavonoids, and phenols) have been shown to have anti-diabetic properties in research, the presence of tannins may also act as an anti-diabetic agent in the *G. officinalis* plant (6,7).

The Cornelian cherry medicinal plant, with the scientific name *Cornus mas*, belongs to the Cornaceae family. Blueberries are used in traditional medicine to treat diarrhea, inflammation of the intestines, fever, malaria, kidney stones, and kidney and bladder infections (8). Bioflavonoids, vitamins such as vitamin C and



Key Messages

- ▶ Diabetes led to damage in the testis tissue of rats.
- ▶ Galega and *Cornus mas* extract protect the testis tissue against diabetes-related damage.
- ▶ Galega and *Cornus mas* extract improve the sperm quality in diabetic rats.

ursolic acid (9) have antioxidant compounds, including anthocyanins and flavonoids. Therefore, the antibacterial, antimicrobial, antiallergic, antihistamine, anti-diabetic, and anti-inflammatory properties of *C. mas* extract might affect atherosclerosis.

Considering the antioxidant and anti-inflammatory effects of *G. officinalis* and *C. mas* extract, this study aimed to investigate the protective effect of hydroalcoholic extracts of *G. officinalis* and *C. mas* on spermatogenesis and oxidative stress associated with diabetes in the testes of adult rats.

Materials and Methods

Animals and Experimental Design

In this experimental study, a total of 64 adult (8-week-old) male Wistar rats weighing 200–250 g were used. All animals were kept under the same environmental and nutritional situation and accommodated in standard cages. The rats were accommodated in a room with a normal temperature of $23 \pm 2^\circ\text{C}$ and 40-50% humidity on a 12-hour photo cycle with free access to water and food. The samples were randomly divided into eight equal groups (n=8 in each) as follows: Group 1: Healthy control group (Control or G1); Group 2: Diabetic control group (G2); Group 3: Diabetic group treated with *G. officinalis* extract (50 mg/kg) daily (13) for 2 weeks (G3); Group 4: Healthy control group treated with *G. officinalis* extract (50 mg/kg) daily for 2 weeks (G4); Group 5: Diabetic group treated with *C. mas* extract (100 mg/kg) for 2 weeks; Group 6: Healthy group treated with *C. mas* extract 100 mg/kg; Group 7: Diabetic group treated with *G. officinalis* extract (50 mg/kg) and *C. mas* extract (100 mg/kg); and Group 8: Healthy group treated with *G. officinalis* extract (50 mg/kg) and *C. mas* extract (100 mg/kg).

Based on a previous study, diabetes was induced in rats of experimental groups by an intraperitoneal single injection of 50 mg/kg streptozotocin solution (STZ, Sigma-Aldrich, Germany) in 0.01 M citrate buffer (pH = 4.5). After 72 hours of STZ injection, the blood glucose levels were checked by drawing some blood from the vein of the rats' tail. The rats were considered as diabetic if the blood glucose level was higher than 250 mg/dL (10).

Histopathological Examination of the Testes

To estimate the histopathological changes in seminiferous tubules, after stabilization, the tissue was dehydrated and purified and then embedded in paraffin. We prepared testicular tissue slides and assessed histopathological

damage in each slide. Then, the number of germ cells was counted at $\times 400$ magnification (11).

Biochemical Assays

To assess the changes in the plasma insulin and glucose levels, blood samples were centrifuged immediately after sampling and stored at -80°C until analysis. The glucose concentration was measured using the Iran Pars Azmoon kit (Tehran, Iran,). Plasma insulin levels were measured by the enzyme-linked immunosorbent assay (ELISA) method using the commercial insulin kit for rat (Merckodia, USA)

Measurement of Lipid Peroxidation

The lipid peroxidation level was demonstrated by the malondialdehyde (MDA) value in the testis tissue. To this goal, firstly, 375 mg of thiobarbituric acid (TBA) was solved in 2 mL of hydrochloric acid (HCL) to prepare a TBA-trichloroacetic acid (TCA)-HCL solution. This solution was added to 100 mL of 15% TCA. To finalize the sediment dissolution, we utilized water bath at 50°C . Then, to achieve a 10% homogenized mixture, a slice of testis tissue was weighed and immediately homogenized with a potassium chloride 5.1% solution. Next, 1 cc of homogenized tissue mixture was added to 2 mL of TBA-TCA-HCL solution and heated with boiling water for 45 minutes (pink-orange solution). Then, the solution was centrifuged at 1000 rpm for 10 minutes after cooling. A spectrophotometer was used to read the absorption at 535 nm (12).

Determination of Superoxide Dismutase Activity

The concentration of superoxide dismutase (SOD) in the testis tissue was assessed using an ELISA reader (Antus) according to the manufacturer's protocols (Ransod, UK).

Catalase Activity Assays

The catalase (CAT) activity was determined by evaluating the reduction in absorbance of a reaction mixture, including 30 mM H₂O₂ sodium phosphate buffer (pH=7) and prerequisite volume homogenized tissue at 240 nm. The specific activity was calculated and expressed as units/mg of total protein.

Evaluation of Sperm Parameters

The epididymis of all rats was removed from both testes, then washed and placed in 5 mL HAM's F10 medium and pieced. After that, we put it in the incubator CO₂ 37°C for 30 minutes and also 100 μL removed from the solution, then added into 900 μL from HAM's F10. Next, this procedure was frequented for new solutions. After mixing carefully, one drop of the solution was placed into Neubauer's chamber, and the sperms count was conducted based on the standard protocol. At the end, the total count of sperms was calculated by the correction factor $\times 10^6$ m. Firstly, the smears of the sperms were provided, and the slides were fixed with 96% alcohol and dried under

exposure to air. This enabled to access the morphometry of sperms. Then, all the slides were stained with hematoxylin and eosin (H & E) method. For this purpose, we counted 100 sperms from each sample in each slide. Finally, the number of normal and abnormal sperms was determined and expressed as percentage (13,14).

Statistical Analysis

All the statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS) software (version 19), and the results were expressed as mean \pm SD. One-way analysis of variance (ANOVA) followed by Tukey's range test were applied to analyze the data. P values ≤ 0.05 were considered as statistically significant (15).

Results

Serum Glucose Levels

There was a significant increase in serum glucose levels during the two weeks of the study in the diabetic group compared to the control group ($P < 0.001$). Additionally, we observed a significant decrease in serum glucose levels in the third and sixth weeks in the diabetic group under treatment with *G. officinalis* extract (50 mg/kg) and *C. mas* extract (100 mg/kg) compared to diabetic control group (Table 1).

Serum Insulin Levels

According to the serum insulin levels, inducing diabetes significantly decreased the serum insulin levels compared

to the control group ($P < 0.001$). However, treating the diabetic rats with *G. officinalis* extract (50 mg/kg) and *C. mas* extract (100 mg/kg) improved the decrease of serum insulin level compared to the diabetic group ($P < 0.05$) (Table 1).

Histopathological Examination

According to the results of germ cell counting, the number of germ cells significantly decreased in the diabetic group compared to the control group ($P < 0.05$). On the other hand, in the diabetic group receiving *Galega officinalis* extract (50 mg/kg) and *Cornus mas* extract (100 mg/kg), the number of germ cells significantly increased ($P < 0.05$). The number of germ cells in treated groups was significantly higher than diabetic control group ($P < 0.05$). Histopathological examination showed that the seminiferous tubule in the diabetic group was irregular with few diameters and thickness. Also, we observed hemorrhage in the seminiferous tubule. This damage improved in the group treated with *Galega officinalis* extract (50 mg/kg) and *Cornus mas* extract (100 mg/kg) (Figure 1, Table 2).

The Oxidative Stress Levels in the Testis Tissue

There was a significant increase in the MDA levels in the testes of diabetic rats compared to the control group ($P = 0.001$). The diabetic rats treated with *G. officinalis* extract (50 mg/kg) and *C. mas* extract (100 mg/kg) remarkably declined the MDA level, which increased the MDA level in testicular tissue caused by diabetes

Table 1. Serum Level of Glucose and Insulin in the Study Groups

Groups	Glucose (mg/dL)	Insulin (μ g/L)
Control	82 \pm 6.5	1.63 \pm 0.21
Diabetic	325 \pm 7.35 ^a	0.32 \pm 0.11 ^a
Diabetic + <i>Galega officinalis</i> extract	177.2 \pm 5.7 ^{ab}	0.65 \pm 0.18 ^{ab}
Diabetic + <i>Cornus mas</i> extract	185.5 \pm 5.25 ^a	0.57 \pm 0.24 ^{ab}
Diabetic + <i>Galega</i> + <i>Cornus mas</i> extract	195 \pm 2.25 ^a	0.67 \pm 0.14 ^{ab}
<i>Galega officinalis</i> extract	70.5 \pm 6.5 ^b	1.64 \pm 0.21 ^b
CME	92 \pm 2.2 ^b	1.60 \pm 0.15 ^b
<i>Galega officinalis</i> + <i>Cornus mas</i> extract	80.8 \pm 3.5 ^b	1.65 \pm 0.25 ^b

Data are expressed as mean \pm SD.

^a Compared to the control group; ^b Compared to the diabetic group.

Table 2. The Count of Germ Cells in the Study Groups

Groups	Round Spermatid	Primary Spermatocytes	Spermatogonia Cells	Sertoli Cells
Control	230.11 \pm 3.85	125.41 \pm 4.25	41 \pm 1.05	30.14 \pm 2.17
Diabetic	89.15 \pm 5.07	54.12 \pm 2.60	18.7 \pm 2.35	17.12 \pm 1.65
Diabetic + <i>G. officinalis</i> extract	165.65 \pm 2.45 ^{ab}	98.41 \pm 3.17 ^{ab}	24.4 \pm 2.0325	22.12 \pm 2.35
Diabetic + <i>Cornus mas</i> extract	158.34 \pm 1.90 ^{ab}	88.54 \pm 4.36 ^{ab}	24.54 \pm 1.42 ^a	21.41 \pm 1.15
Diabetic + <i>G. officinalis</i> + <i>C. mas</i> extract	180.55 \pm 4.35 ^b	95.71 \pm 3.45 ^{ab}	25.39 \pm 1.0926	22 \pm 161.07
<i>Galega officinalis</i>	227.55 \pm 6.35 ^b	108.71 \pm 5.45 ^b	37.41 \pm 1.05	29.06 \pm 1.17
<i>Cornus mas</i> extract	219.45 \pm 5.15 ^b	114.71 \pm 7.45 ^b	42.25 \pm 2.35	31.56 \pm 1.7
<i>Galega</i> + <i>Cornus mas</i> extract	215.33 \pm 3.25 ^b	110.55 \pm 4.15 ^b	43.54 \pm 2.03	32.44 \pm 1.2

Data are presented as mean \pm SD.

Letter *a* shows a significant difference between the control group and diabetic group, and Letter *b* shows a significant difference between the diabetic and diabetic treatment groups ($P < 0.05$).

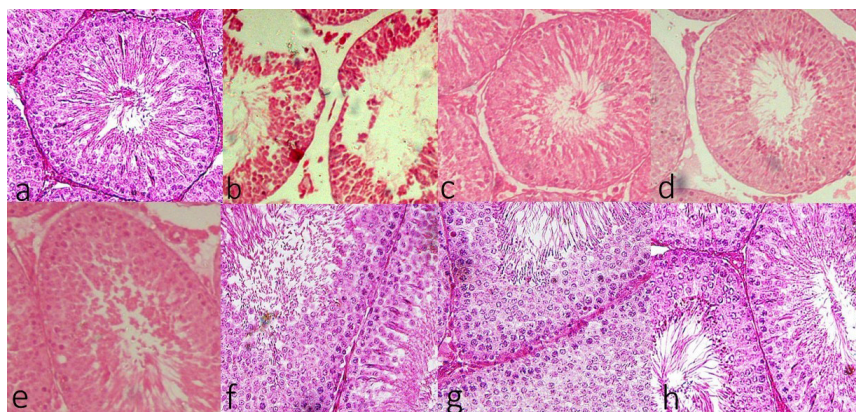


Figure 1. Histological Examination of Study Groups. (a) Control group, (b) Diabetic group, (c) Diabetic group treated with *Galega officinalis* extract, (d) Diabetic group treated with *Cornus mas* extract, (e) Diabetic group treated with *Galega officinalis* extract + *Cornus mas* extract, (f) Healthy group treated with *Galega officinalis* extract, (g) Healthy group treated with *Cornus mas* extract, and (h) Healthy group treated with *Cornus mas* extract + *Galega officinalis* extract.

($P=0.001$). These findings indicated that diabetes significantly decreased the CAT enzyme activity compared to the control group ($P=0.001$). Treating diabetic rats with *Galega officinalis* extract (50 mg/kg) and *C. mas* extract (100 mg/kg) led to significant differences compared to the untreated diabetic group ($P=0.001$). Also, the activity of SOD significantly decreased in the diabetic rats compared to control group ($P=0.001$). In addition, the comparison between the treatment and the diabetic groups indicated a significant increase in the activity of SOD enzyme ($P=0.001$) (Table 3).

The Sperms Count, Morphology, and Motility

The concentration of sperms significantly decreased in the diabetic group compared to the control group ($P=0.001$). In both treatment groups, the number of sperms significantly increased compared to the diabetic group ($P=0.001$). Diabetes led to an increase in the number of abnormal sperms compared to the control group ($P=0.001$). Also, treatment with *G. officinalis* extract (50 mg/kg) and *C. mas* extract (100 mg/kg) significantly decreased the number of abnormal sperms compared to the diabetic group ($P=0.001$). On the other hand, the motility of sperms significantly decreased in the diabetic group compared to the control group ($P=0.001$).

Treatment of diabetic rats with *G. officinalis* extract (50 mg/kg) and *C. mas* extract (100 mg/kg) significantly improved the sperm motility in both treatment groups (Table 4).

Discussion

The present study examined the meliorative effect of *Galega* extract (50 mg/kg) and *C. mas* extract (100 mg/kg) against injuries in the male reproductive system induced by diabetes. Diabetes produces testicular dysfunctions in the male reproductive organ. According to our results, *G. officinalis* extract (50 mg/kg) and *C. mas* extract (100 mg/kg) improved these functional deficiencies due to their antioxidant and antidiabetic properties. We also witnessed that the *G. officinalis* extract (50 mg/kg) and *C. mas* extract (100 mg/kg) decreased the blood glucose levels and the level of oxidative stress markers in the testes of diabetic rats. The hypoglycemic effect of *G. officinalis* extract (50 mg/kg) and *C. mas* extract (100 mg/kg) is related to increasing the sensitivity of tissues to insulin (16,17).

In diabetic patients, in addition to an enhanced amount of blood glucose, the balance between generation and resolution of free radicals is also suspended. As a result, free radicals increment and cause oxidative stress (5,6).

Table 3. The Concentration of MDA, CAT, and SOD in the Rats' Testis Tissue in Four Groups

Groups	MDA	CAT	SOD
Control	0.62 ± 0.011	5.85 ± 0.057	1.72 ± 0.021
Diabetic	1.85 ± 0.035 ^a	2.88 ± 0.048 ^a	0.68 ± 0.011 ^a
Diabetic + <i>Galega officinalis</i>	0.95 ± 0.02 ^{ab}	4.22 ± 0.072 ^b	1.27 ± 0.018 ^{ab}
Diabetic + <i>Cornus mas</i> extract	1.00 ± 0.025 ^a	3.95 ± 0.10 ^b	1.16 ± 0.024 ^{ab}
Diabetic + <i>Galega officinalis</i> + <i>Cornus mas</i> extract	0.92 ± 0.011 ^a	4.32 ± 0.084 ^b	1.32 ± 0.014 ^{ab}
<i>Galega officinalis</i>	0.605 ± 0.01 ^b	5.97 ± 0.037 ^b	1.68 ± 0.021 ^b
<i>Cornus mas</i> extract	0.652 ± 0.02 ^b	5.50 ± 0.077 ^b	1.60 ± 0.015 ^b
<i>Galega officinalis</i> + <i>Cornus mas</i> extract	0.601 ± 0.05 ^b	6.05 ± 0.059 ^b	1.75 ± 0.025 ^b

Data are presented as mean ± SE.

^a in comparison with control group ($P=0.001$)

^b in comparison with diabetic group ($P=0.001$).

Table 4. The Comparison of Sperms Parameters after the Treatment Period

Groups	Sperm Parameters		
	Sperms Count $\times 10^6$	Morphology	Sperm Motility
Control	45.60 \pm 3.48	75.4 \pm 1.07	63.6 \pm 1.13
Diabetic	12.5 \pm 2.59 ^a	26.4 \pm 0.50 ^b	12.25 \pm 1.03 ^b
Diabetic + <i>Galega officinalis</i>	22.45 \pm 2.98 ^a	50.12 \pm 1.17 ^a	38.45 \pm 0.84 ^a
Diabetic + <i>Cornus mas</i> extract	20.85 \pm 3.18 ^{a,b}	49.62 \pm 0.59 ^{a,b}	35.5 \pm 0.75 ^{a,b}
Diabetic + <i>Galega officinalis</i> + <i>Cornus mas</i> extract	24.6 \pm 3.48 ^{a,b}	44.4 \pm 1.07 ^{a,b}	33.6 \pm 1.13 ^{a,b}
<i>Galega officinalis</i>	44.5 \pm 9.16 ^b	70.87 \pm 0.51 ^b	60.75 \pm 1.20 ^{a,b}
<i>Cornus mas</i> extract	42.5 \pm 6.07 ^b	65.87 \pm 0.45 ^b	64.75 \pm 1.06 ^{a,b}
<i>Galega officinalis</i> + <i>Cornus mas</i> extract	46.32 \pm 4.25	72.25 \pm 2.11	65.24 \pm 2.11

Data are presented as mean \pm SE.

Letter a shows a significant difference between the control group and diabetic group, and Letter b shows a significant difference between the diabetic and diabetic treatment groups ($P < 0.05$).

Oxidative stress results in cell injury via mechanisms such as lipid peroxidation, and DNA and oxidative protein damage (18-23). The results of this research showed that diabetes remarkably incremented the MDA levels (as lipid peroxidation marker) in the testicles of diabetic rats, which is in line with the findings of several previous studies (5,18). Hence, the level of MDA increase in the testes of diabetic rats indicates the increase of lipid peroxidation. In this research, treating diabetic rats with *G. officinalis* extract and *C. mas* extract significantly decreased the MDA concentration in the testis tissue. Several studies reported that flavonoids in *G. officinalis* extract and *C. mas* extract scavenge the free radicals generated during lipid peroxidation (19,20). Thus, decline in testis MDA concentration in the groups treated with *G. officinalis* extract and *C. mas* extract may be related to the antioxidant effects of these two plants.

In our research, the activity of SOD was extremely decreased in diabetic rats, which is similar to a previous study (6). SOD is one of the most important enzymes of the antioxidant system, while its main action is the catalysis of superoxide anion radicals to H₂O₂. Through this procedure, the toxicity of superoxide decays and no free radicals from superoxide are produced (21). Again, in our research, the activity of SOD significantly increased in the testes of diabetic rats receiving *G. officinalis* extract and *C. mas* extract compared to the diabetic group.

CAT, as an antioxidant enzyme, is another enzyme with detoxification effects against free radicals (22). In our research, the activity of CAT enzyme in diabetic rats decreased more significantly compared to the control group. Also, in the groups treated with *G. officinalis* extract and *Cornus mas* extract, the activity of CAT enzyme increased more significantly compared to the diabetic group. A decline in the activity of CAT in this study can be resulted from the increment in H₂O₂ generation because of autoxidation of glucose and non-enzymatic protein glycation that cause the production of oxygen-free radicals (3). It is known that antioxidant therapy can increase the activity of CAT, as confirmed in our study.

The present study findings indicated that diabetes reduced the sperms parameters (count, motility, and

morphology), and treatment with *G. officinalis* extract and *C. mas* extract enhanced the sperms count and ameliorated sperm motility and morphology in diabetic rats. This may be related to the antioxidant effects of *G. officinalis* extract and *C. mas* extract on activating antioxidant enzymes, that can counteract free radicals. Consistent with our findings, a previous study indicated that medical plants with flavonoids components could improve sperms quality and testosterone levels (5,18,23).

The possible mechanisms complicated in the recovery of oxidative stress markers in diabetic rats testis by *G. officinalis* extract and *C. mas* extract can be expressed as follows: *G. officinalis* extract and *C. mas* extract have antioxidant compounds, which reduce the levels of blood glucose and enhance insulin secretion; they can also activate the antioxidant enzyme (19,20,24,25).

Conclusion

The findings of present study revealed that diabetes can negatively affect testes and sperms quality through oxidative stress. *G. officinalis* extract and *C. mas* extract have potent effects on antioxidant system activation in reducing the oxidative stress induced by diabetes. However, further studies are needed to confirm these results.

Authors' Contribution

Conceptualization: ES, IP
 Methodology: ES, IP, HG, SH
 Validation: ES, IP, HG, SH
 Formal Analysis: ES
 Investigation: ES, IP
 Resources: ES, IP, HG, SH
 Data Curation: ES, IP, HG, SH
 Writing—Original Draft Preparation: ES
 Writing—Review and Editing: ES, IP
 Visualization: ES, IP, HG, SH
 Supervision: IP
 Project Administration: ES
 Funding Acquisition: ES, IP, HG, SH

Conflict of Interest

The authors declare they have no conflict of interest.

Ethical issues

All the experimental processes of this research were approved by the

Ethical Committee of Islamic Azad University, Tehran, Iran (Code:).

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