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Effect of glabridin on sperm traits, testicular oxidative status, and *in vitro* fertilization in diabetic mature mice: a controlled experimental study

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Abstract

Diabetes is a metabolic disorder characterized by hyperglycemia due to the defects in insulin secretion and function, or both. Glabridin (GBD) is one of the natural anti-oxidants used for infertility treatment. This study was planned to evaluate the effects of GBD on testicular oxidative status, sperm characteristics, and early embryo development in diabetic mature mice. Forty mature male mice were allotted to five equal groups, including control group received no treatment, diabetic group received intraperitoneal streptozotocin (50.00 mg kg^{-1}), and three experimental groups receiving 12.50, 50.00, and 200 mg kg^{-1} GBD by gavage daily for 30 days, respectively. Serum levels of testosterone, sperm parameters, and testicular malondialdehyde, total anti-oxidant capacity, and catalase levels, as well as pre-implantation embryo development were determined. The diabetic group exhibited significantly reduced sperm motility, viability, and count, testosterone level, and testicular total anti-oxidant capacity and catalase levels, and increased testicular malondialdehyde level, and DNA-damaged and immature sperms along with poor *in vitro* fertilization outcomes compared to the control group. In contrast, the GBD administration, particularly at the highest dose, caused a pronounced improvement in the above-noted parameters. These findings suggest that GBD may play a role in impeding diabetes-induced male reproductive complications through oxidative stress repression, and sperms and early embryos protection.

Introduction

Diabetes mellitus (DM), types 1 and 2, has been associated with various male and female reproductive complications. The DM can affect fertility through triggering hormonal imbalances and oxidative stress, or inducing reproductive disorders, such as polycystic ovary syndrome. It can disturb hormonal regulation, affecting ovulation and menstrual cycles, leading to fertility challenges. Accordingly, it has been reported that women with DM are at a higher risk of pregnancy complications, such as miscarriage, preeclampsia, and congenital anomalies.[1] Diabetes is also linked to the reduced sperm quality, being related to the oxidative damage and metabolic disruption. Correspondingly, it has been shown that diabetic men often have lower testosterone levels, negatively affecting reproductive health.[2]

Oxidative stress, being characterized by excessive reactive oxygen species production and impaired anti-oxidant defense mechanisms, plays a key role in the pathophysiology of DM.[3] Additionally, empirical evidence has indicated that oxidative stress-evoked cyto-destruction leads to sperm functional impairments, capacitation failure, and eventually unsuccessful fertilization.[4]

Glabridin (4-[(3R)-8,8-dimethyl-3,4-dihydro-2H-pyrano [2,3-f]chromen-3-yl]benzene-1,3-diol, $C_{20}H_{20}O_4$; GBD), a prenylated isoflavan being extracted from *Glycyrrhiza glabra* L., is known for its various pharmacological properties, including anti-oxidant, anti-inflammatory, anti-tumor, anti-microbial, osteo-protective, cardio-protective, hepato-protective, neuro-protective, anti-obesity, and anti-diabetic activities.[5] Earlier reports have stated that potent anti-oxidative effects of GBD are mainly involved in its

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salutary role in protection against DM-elicited organopathies.[6,7] Further, it was found previously that *G. glabra* root extract can abate methotrexate-induced male reproductive toxicity in mice, owing its anti-oxidant, anti-inflammatory, and anti-apoptotic activities.[8] Likewise, *G. glabra* extract has shown to improve spermatogenesis in high-fat diet-induced obese mice.[9]

On this account, this study was aimed to provide mechanistic insights into the possible repro-protective effects of GBD against DM-related male reproductive dysfunction in mature mice.

Materials and Methods

Animals. Forty mature male mice (age: 8 weeks and weight: 23.00 ± 2.00 g) were provided from the Animal Resource Center, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. The mice were placed in a well-ventilated environment with monitored light (12-hr light/dark cycle), temperature (24.00 ± 2.00 ˚C), and humidity (50.00 ± 60.00%). They had free access to the rodent laboratory chow and tap water. The 2-week acclimatization period was also considered. All animal care and experimental procedures were conducted following guidelines set by Animal Research Ethics Board, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. Efforts were made to minimize animal suffering and reduce the number of animals used (Ethical Code: IR-UU-AEC-3/95).

Experimental design. Animals were randomly divided into five equal groups, including control group including healthy animals received no treatment, diabetic group received intraperitoneal (IP) streptozotocin (50.00 mg kg^{-1}; Sigma, St. Louis, USA), DM + $GBD_{12.50}$ group received daily oral administrations of GBD (Sigma) at a dosage of 12.50 mg kg^{-1}, DM + GBD_{50} group received daily oral administrations of GBD at a dosage of 50.00 mg kg^{-1}, and DM + GBD_{200} group received daily oral administrations of GBD at a dosage of 200 mg kg^{-1}. The experimental period was 30 days.

Sampling. All animals were sacrificed using IP injection of 10.00 mg kg^{-1} xylazine (Alfasan, Woerden, Netherlands) and 150 mg kg^{-1} ketamine (Alfasan).[10] Approximately 2.00 mL blood was collected directly from the left ventricle using a sterile syringe and needle, transferred to the serum separator tubes, allowed to clot and then, centrifuged to separate serum for future analyses.

Sperm count. Epididymal sperms were harvested through slicing the caudal part of the epididymis into the small pieces and incubated in 1.00 mL of human tubal fluid (HTF; Sigma) supplemented with 4.00 mg mL^{-1} bovine serum albumin (Sigma). The incubation was executed for 30 min at 37.00 ˚C in an atmosphere of 5.00% CO_2, allowing the sperms to swim out of the epididymal tubules. Sperm concentration was quantified using a hemocytometer (Brand™, Berlin, Germany), and expressed as 10^6 *per* mL.[11]

Sperm viability. To examine sperm viability, 10.00 µL of Eosin-Nigrosin staining solution was mixed with an equal volume of the sperm suspension. After a 2-min incubation at the room temperature, slides were evaluated under a light microscope (CHT; Olympus, Tokyo, Japan) at 400× magnification, and the percentage of live sperms was recorded.[10]

Sperm motility. A drop of sperm suspension was put on a glass slide, covered with a coverslip, the sperms showing rapid progressive forward, slow progressive forward, and circumferential movements in 10 microscopic fields of view were counted using a light microscope (Olympus) at 400× magnification, and the percentage of motile sperms was reported.[11]

Sperm DNA damage. Acridine Orange staining was used to determine sperm DNA denaturation. A drop of sperm suspension was placed on a glass slide and allowed to be air-dried. Then, the smears were fixed in methanol-acetic acid (1 : 3 v/v; Sigma) for two hr, stained with 3.00 mL of 19.00% Acridine Orange solution in phosphate citrate (Sigma) for 5 min, and rinsed with deionized water. Using a fluorescence microscope (GS7, Nikon, Tokyo, Japan), the percentage of sperms with single-stranded DNA appearing orange-red was calculated.[12]

Sperm chromatin quality. A drop of sperm suspension was put on glass slides and allowed to be air-dried. The smears were then fixed in 3.00% glutaraldehyde (Sigma) in phosphate-buffered saline, stained with 5.00% aqueous Aniline Blue mixed with 4.00% acetic acid (pH: 3.50) for 5 min, and the percentage of immature sperms having blue-stained heads was enumerated.[13]

Testicular malondialdehyde (MDA) level. Testicular tissue samples were minced and homogenized under ice-cold conditions. After that, 300 µL of 10.00% trichloroacetic acid (Sigma) was added to 150 µL of the homogenized sample and centrifuged at 1,000 rpm for 10 min at 4.00 ˚C. The supernatant was then transferred to a test tube with 300 µL of 67.00% thiobarbituric acid (Sigma) and incubated at 100 ˚C for 25 min. After 5 min of cooling, a pink color appeared due to the MDA- thiobarbituric acid reaction. Absorbance was recorded using a spectrophotometer (Novaspec II; Biochrom Ltd., Cambridge, UK) at the wavelength of 535 nm.[14]

Testicular catalase (CAT) activity analysis. Catalase activity was measured based on its ability to decompose hydrogen peroxide (Sigma) in the homogenized testicular tissue, following Aebi's method. Briefly, the testicular tissue was homogenized in phosphate-buffered saline and centrifuged at 3,400 rpm for 15 min. Then, 1.00 mL of hydrogen peroxide was added to 2.00 mL of the supernatant, and the absorbance was recorded at 240 nm using the Novaspec II spectrophotometer.[15]

Testicular total antioxidant capacity (TAC) measurement. Testicular total anti-oxidant capacity was measured according to the Katalinic *et al*. method based on ferric reduction anti-oxidant power assay, and the values were reported as μmol g^{-1} tissue.[16]

Testosterone level determination. The serum levels of testosterone hormone were determined *via* rat/mouse enzyme-linked immuno-sorbent assay kit (Cosmo Bio Co., Tokyo, Japan) based on the manufacturer's protocols and expressed as ng mL^{-1}.

Oocyte pick-up. Each female mouse received an IP injection of pregnant mare's serum gonadotropin (10.00 IU; Folligon, Boxmeer, Netherlands) 48 hr prior to an IP administration of human chorionic gonadotropin (10.00 IU; Folligon). The animals were euthanized 14 hr after human chorionic gonadotropin injection, and the ampullary portions of the oviducts were excised and transferred to a Petri dish containing 1.00 mL HTF being supplemented with 4.00 mg mL^{-1} bovine serum albumin. Using a stereo microscope (TL2; Olympus), the ovulated oocytes were meticulously dissected and placed into fertilization droplets under mineral oil in the HTF + bovine serum albumin medium.[11]

***In vitro* fertilization (IVF).** The capacitated sperms (1.00×10^6 *per* 1.00 mL HTF) were added to the medium. Fertilization rate was recorded 6 hr after sperms addition through male and female pronuclei observation. After that, granulosa cells were denuded and rinsed, and zygotes were transferred to the fresh pre-equilibrated medium for an additional 5 days of culture. Two-cell embryos and blastocysts formations were reported 24 hr and 5 days after fertilization, respectively.[14]

Statistical analyses. The variables were analyzed by one-way analysis of variance followed by Tukey multiple range *post hoc* analyses using SPSS Software (version 22.0; IBM Corp., Armonk, USA). The data were expressed as the mean ± standard deviation, and the *p*-value of less than 0.05 was considered statistically significant.

Results

Spermatological parameters. A significant ($p < 0.05$) reduction was observed in sperm count following DM induction compared to the control group. In the DM + GBD groups, an increase in the sperm count was observed; however, significant ($p < 0.05$) differences were only noted in the DM + GBD_{50} and DM + GBD_{200} groups compared to the diabetic group (Table 1). The percentage of motile sperms in the diabetic group was significantly ($p < 0.05$) lower than the control group. In the DM + GBD groups, an elevation in the sperm motility was observed; but, significant ($p < 0.05$) differences were only noted in the DM + GBD_{50} and DM + GBD_{200} groups compared to the diabetic group (Table 1). In the diabetic group, a significant ($p < 0.05$) decrease in the percentage of viable sperms was observed compared to the control group. While, in the DM + GBD groups, sperm viability showed a marked ($p < 0.05$) improvement compared to the diabetic group. No significant differences were observed among the DM + GBD groups ($p > 0.05$; Fig. 1A and Table 1).

Diabetic mice showed a significant ($p < 0.05$) increase in the immature sperm's percentage compared to the control group. In the DM + GBD groups, reductions in this parameter were recorded; although, significant ($p < 0.05$) differences were only noted in the DM + GBD_{50} and DM + GBD_{200} groups compared to the diabetic group (Fig. 1B and Table 1). It was found that DM led to significant ($p < 0.05$) DNA damage increase in sperms compared to the control group. In spite of decrease in the percentage of DNA-damaged sperms in the DM + GBD groups, the significant ($p < 0.05$) reduction compared to the diabetic group was only observed in the DM + GBD_{200} group (Fig. 1C and Table 1).

Biochemical parameters. Diabetes caused a significant ($p < 0.05$) increase in the testicular MDA level compared to the control group. The testicular MDA levels in the DM + GBD groups were lower than the diabetic group; however, this reduction was statistically significant ($p < 0.05$) only in the DM + GBD_{200} group (Table 2). Testicular TAC level was significantly ($p < 0.05$) decreased after DM induction in comparison with the control group. While, the DM + GBD_{200} group showed pronounced increase in testicular TAC level compared to the diabetic group ($p < 0.05$; Table 2). There was a significant decrease ($p < 0.05$) in the testicular CAT level in the diabetic group compared to the control group. In the DM + GBD groups, increases in the level of testicular CAT were seen; however, significant ($p < 0.05$) differences were only recorded in the DM + GBD_{50} and DM + GBD_{200} groups compared to the diabetic group (Table 2).

Table 1. Spermatological parameters in different experimental groups.

Groups	Sperm count ($\times 10^6$ *per* mL^{-1})	Sperm motility (%)	Sperm viability (%)	DNA-damaged sperms (%)	Immature sperms (%)
Control	53.16 ± 3.07[a]	81.11 ± 7.57[a]	83.05 ± 7.32[a]	8.48 ± 1.75[a]	7.33 ± 0.39[a]
DM	28.11 ± 3.69[b]	52.63 ± 5.97[b]	53.44 ± 5.17[b]	25.96 ±2.43[b]	21.47 ± 0.97[b]
DM + $GBD_{12.50}$	31.90 ± 4.55[b]	55.85 ± 6.07[b]	57.53 ± 9.82[c]	22.11 ± 0.95[b]	19.33 ± 1.08[b]
DM + GBD_{50}	45.64 ± 3.623[c]	63.82 ± 3.53[c]	68.26 ± 5.59[c]	19.22 ± 0.82[b]	10.16 ± 0.85[c]
DM + GBD_{200}	47.46 ± 4.05[c]	64.94 ± 8.61[c]	74.70 ± 4.96[c]	9.78 ± 0.77[c]	6.53 ± 0.84[c]

DM: Diabetes mellitus; GBD: Glabridin.

[abc] Values with different superscripts within one column differ significantly at $p < 0.05$.

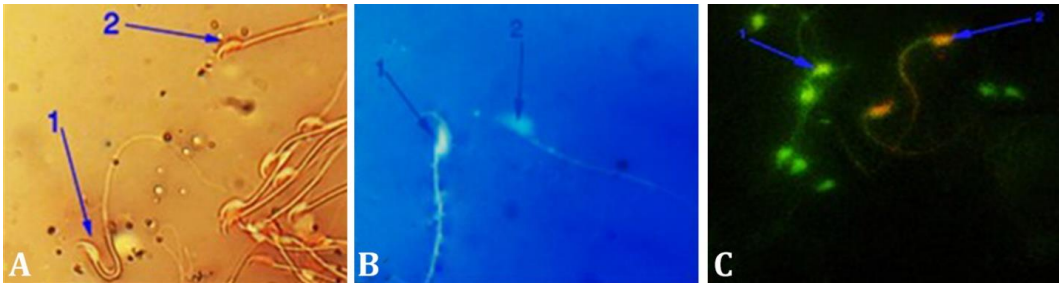

Fig. 1. Sperm viability, chromatin quality, and DNA damage analyses. **A)** Eosin-Nigrosin staining: Live sperm (1), with uncolored head and dead sperm (2), with pink-red head can be seen (400×); **B)** Aniline Blue staining: Sperm head with mature nuclear chromatin (1), appears light blue and sperm head having immature nuclear chromatin (2), is dark blue (1,000×); **C)** Acridine Orange staining: Sperm with normal DNA integrity (1), appears green and reddish-orange stained sperm (2), has damaged DNA (400×).

In the diabetic group, testosterone levels decreased significantly ($p < 0.05$) compared to the control group. In the DM + GBD groups, marked ($p < 0.05$) increases in serum levels of testosterone were observed compared to the diabetic group. There were no significant differences in testosterone levels among the DM + GBD groups ($p > 0.05$; Table 2).

Embryological parameters. The fertilization rate in diabetic group showed a significant decrease ($p < 0.05$) compared to the control group. In the DM + GBD groups, improvement in the IVF rate was observed; however, this increase was only significant ($p < 0.05$) in the DM + GBD_{200} group compared to the diabetic group (Table 3). The percentages of two-cell, blastocyst-stage, and hatched embryos were reduced significantly ($p < 0.05$) in the diabetic group compared to the control group. In the DM + GBD groups, higher percentages of two-cell, blastocyst-stage, and hatched embryos than diabetic group were found; but, DM + GBD_{50} and DM + GBD_{200} groups exhibited significant ($p < 0.05$) improvements (Fig. 2 and Table 3).

Discussion

Diabetes as a chronic disease, causing serious health problems through glucose metabolism disruption, has several negative effects on different tissues. It has been highlighted that men are more affected by the DM complications than women, particularly in terms of

Table 2. Biochemical findings in different experimental groups.

Groups	Catalase (µmol g^{-1} tissue)	Malondialdehyde (µmol g^{-1} tissue)	TAC (µmol g^{-1} tissue)	Testosterone (ng mL^{-1})
Control	37.34 ± 2.95[a]	1.28 ± 0.39[a]	3.38 ± 1.19[a]	1.14 ± 0.12[a]
DM	8.24 ± 1.84[b]	2.63 ± 1.07[b]	1.78 ± 0.71[b]	0.65 ± 0.37[b]
DM + $GBD_{12.50}$	13.61 ± 1.90[b]	2.35 ± 0.86[b]	1.99 ± 0.47[b]	0.91 ± 0.10[c]
DM + GBD_{50}	31.53 ± 2.91[c]	2.21 ± 0.58[b]	2.09 ± 0.84[b]	0.98 ± 0.17[c]
DM + GBD_{200}	33.51 ± 2.66[c]	1.39 ± 0.83[c]	3.07 ± 0.94[c]	1.02 ± 0.19[c]

DM: Diabetes mellitus; GBD: Glabridin; TAC: Total anti-oxidant capacity.
[abc] Values with different superscripts within each column differ significantly at $p < 0.05$.

Table 3. Embryological parameters in different experimental groups.

Groups	Fertilization rate (%)	Two-cell embryos (%)	Blastocysts (%)	Hatched embryos (%)
Control	88.35 ± 4.27[a]	81.22 ± 8.15[a]	64.52 ± 4.47[a]	56.99 ± 5.17[a]
DM	53.19 ± 5.06[b]	43.49 ± 4.07[b]	36.29 ± 4.06[b]	25.74 ± 3.02[b]
DM + $GBD_{12.50}$	57.38 ± 5.59[b]	48.10 ± 6.72[b]	41.28 ± 5.61[b]	29.88 ± 5.65[b]
DM + GBD_{50}	62.31 ± 7.92[b]	61.64 ± 5.08[c]	55.17 ± 3.29[c]	48.31 ± 4.60[c]
DM + GBD_{200}	73.94 ± 3.81[c]	69.48 ± 7.91[c]	57.94 ± 5.33[c]	49.13 ± 6.62[c]

DM: Diabetes mellitus; GBD: Glabridin.
[abc] Values with different superscripts within each column differ significantly at $p < 0.05$.

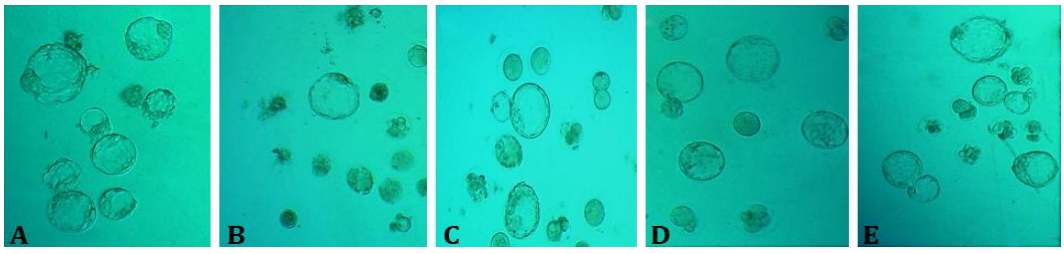

Fig. 2. Embryos in different growth stages in the day five post-fertilization (200×). **A)** Control; **B)** Diabetic group; **C)** Diabetes + low dose (12.50 mg kg^{-1}) of glabridin; **D)** Diabetes + medium dose (50.00 mg kg^{-1}) of glabridin; **E)** Diabetes + high dose (200 mg kg^{-1}) of glabridin.

reproductive system issues.[17] A significant body of evidence has indicated that DM is associated with reductions in testosterone and gonadotropins levels, leading to spermatogenic disorders and unfavorable sperm quality and quantity.[2,18] It is well-grounded that DM leads to sperm concentration reduction, sperm deformity increase, and improper sperm kinematic parameters.[19] It has been well-established that oxidative stress has a prominent position in the pathophysiology of DM complications. Diabetes-induced hyperglycemia can result in excessive reactive oxygen species generation, leading to cyto-damages and inflammatory responses.[3] Furthermore, an overwhelming body of scientific evidence shows that inflammation and oxidative stress are interconnected in the male infertility pathogenesis.[20]

In the present study, sperm count, motility, and viability decreased in the diabetic group, while the percentages of immature and DNA-damaged sperms increased. Also, the IVF rate, as well as pre-implantation embryonic development reduced following experimental DM induction. These findings are in accordance with the earlier published reports.[18-21]

Recently, anti-oxidants have gained great interest in the DM complications treatment due to their ability to curb oxidative stress and prevent over-generated reactive oxygen species-educed organ dysfunction.[22] Moreover, it becomes increasingly clear that anti-oxidants can help boost fertility.[23]

Being in conformity with former reports regarding anti-oxidative potential of GBD,[5-7] it was found in this study that GBD administration in diabetic mice reduced MDA level and increased the CAT activity along with TAC level in the testicular tissue, and improved testosterone level, sperm characteristics, IVF success rate, and *in vitro* early embryonic development. In agreement with the findings of the current study, recent evidence indicated that GBD alleviated ultra-violet B-induced skin damage in mice through pro-inflammatory cytokines levels attenuation and anti-oxidant defense system reinforcement.[24] Correspondingly, it was also found that *G. glabra* extract could improve ovarian morphology and oocyte maturation in a mouse model of polycystic ovary syndrome.[25] Accordingly, it has been revealed that GBD retains anti-inflammatory abilities to down-regulate inducible nitric oxide synthase expression under high-glucose stress, introducing it as a promising anti-inflammatory agent against diabetes-related vascular dysfunction.[26]

To wrap up, these findings postulate that GBD may play a beneficial role in impeding DM-induced male reproductive complications through oxidative stress repression, and repro-protection. Nonetheless, further research is imperative to delve into the precise underlying mechanisms, laying the groundwork for the development of GBD-based therapeutic strategies to promote fertility status in diabetic patients.

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Conflicts of interest

There are no financial or non-financial competing interests to declare.

References

1. Qin X, Du J, He R, et al. Adverse effects of type 2 diabetes mellitus on ovarian reserve and pregnancy outcomes during the assisted reproductive technology process. Front Endocrinol (Lausanne) 2023; 14: 1274327. doi: 10.3389/fendo.2023.1274327.
2. Lotti F, Maggi M. Effects of diabetes mellitus on sperm quality and fertility outcomes: clinical evidence. Andrology 2023; 11(2): 399-416.
3. Bhatti JS, Sehrawat A, Mishra J, et al. Oxidative stress in the pathophysiology of type 2 diabetes and related complications: current therapeutics strategies and future perspectives. Free Radic Biol Med 2022; 184: 114-134.
4. Hussain T, Kandeel M, Metwally E, et al. Unraveling the harmful effect of oxidative stress on male fertility: a mechanistic insight. Front Endocrinol (Lausanne) 2023;14: 1070692. doi: 10.3389/fendo.2023.1070692.
5. Zhang J, Wu X, Zhong B, et al. Review on the diverse biological effects of glabridin. Drug Des Devel Ther 2023; 17: 15-37.
6. Wu F, Jin Z, Jin J. Hypoglycemic effects of glabridin, a polyphenolic flavonoid from licorice, in an animal model of diabetes mellitus. Mol Med Rep 2013; 7(4): 1278-1282.
7. Tan H, Chen J, Li Y, et al. Glabridin, a bioactive component of licorice, ameliorates diabetic nephropathy by regulating ferroptosis and the VEGF/Akt/ERK pathways. Mol Med 2022; 28(1): 58. doi: 10.1186/s10020-022-00481-w.
8. Aldhahrani A, Soliman MM, Althobait F, et al. The modulatory impacts of *Glycyrrhiza glabra* extract against methotrexate-induced testicular dysfunction and oxidative stress. Toxicol Res (Camb) 2021; 10(4): 677-686.
9. Nguyen TH, Ly HHV, Nguyen TM, et al. The effects of *Glycyrrhiza glabra* extract in high-fat diet-induced obese male mice: a controlled experimental study. Obes Med 2023; 44: 100525. doi: 10.1016/j.obmed.2023.100525.
10. Sadeghirad M, Soleimanzadeh A, Shalizar-Jalali A, et al. Synergistic protective effects of 3,4-dihydroxyphenylglycol and hydroxytyrosol in male rats against

induced heat stress-induced reproduction damage. Food Chem Toxicol 2024; 190: 114818. doi: 10.1016/j.fct.2024.114818.
11. Babaei M, Najafi G, Shalizar Jalali A, et al. Effects of unilateral iatrogenic vas deferens trauma on fertility: an experimental *in vitro* fertilization mice model study. Bull Emerg Trauma 2015; 3(4): 122-127.
12. Armand Z, Najafi G, Farokhi F, et al. Attenuation of cyclosporine-induced sperm impairment and embryotoxicity by *Crataegus monogyna* fruit aqueous extract. Cell J 2013; 15(3): 198-205.
13. Mahdivand N, Najafi G, Nejati V, et al. Royal jelly protects male rats from heat stress-induced reproductive failure. Andrologia 2019;51(3):e13213. doi: 10.1111/and.13213.
14. Aaly-Gharibeh Z, Hosseinchi M, Shalizar-Jalali A. Effect of nanocurcumin on fertility in murine model of polycystic ovary syndrome. Vet Res Forum 2024; 15(2): 113-117.
15. Aebi H. Catalase *in vitro*. Methods Enzymol 1984; 105: 121-126.
16. Katalinic V, Modun D, Music I, et al. Gender differences in antioxidant capacity of rat tissues determined by 2, 2'-azinobis (3-ethylbenzothiazoline 6-sulfonate; ABTS) and ferric reducing antioxidant power (FRAP) assays. Comp Biochem Physiol C Toxicol Pharmacol 2005; 140(1): 47-52.
17. Nellaiappan K, Preeti K, Khatri DK, et al. Diabetic complications: an update on pathobiology and therapeutic strategies. Curr Diabetes Rev 2022; 18(1): e030821192146. doi: 10.2174/1573399817666210309104203.
18. Huang R, Chen J, Guo B, et al. Diabetes-induced male infertility: potential mechanisms and treatment options. Mol Med 2024; 30(1): 11. doi: 10.1186/s10020-023-00771-x.
19. Ali BR, Alameri AN, Al-Rumaidh S, et al. Correlation between reproductive hormones levels and semen quality in patients with diabetes. J Med Life 2022; 15(12): 1507-1510.
20. Dutta S, Sengupta P, Slama P, et al. Oxidative stress, testicular inflammatory pathways, and male reproduction. Int J Mol Sci 2021; 22(18): 10043. doi: 10.3390/ijms221810043.
21. Zhong O, Ji L, Wang J, et al. Association of diabetes and obesity with sperm parameters and testosterone levels: a meta-analysis. Diabetol Metab Syndr 2021; 13(1): 109. doi: 10.1186/s13098-021-00728-2.
22. Fatima MT, Bhat AA, Nisar S, et al. The role of dietary antioxidants in type 2 diabetes and neurodegenerative disorders: an assessment of the benefit profile. Heliyon 2022; 9(1): e12698. doi: 10.1016/j.heliyon.2022.e12698.
23. Smits RM, Mackenzie-Proctor R, Fleischer K, et al. Antioxidants in fertility: impact on male and female reproductive outcomes. Fertil Steril 2018; 110(4): 578-580.
24. Peng G, Li Y, Zeng Y, et al. Effect of glabridin combined with bakuchiol on UVB-induced skin damage and its underlying mechanism: an experimental study. J Cosmet Dermatol 2024; 23(6): 2256-2269.
25. Shamsi M, Nejati V, Najafi G, et al. Protective effects of licorice extract on ovarian morphology, oocyte maturation, and embryo development in PCOS-induced mice: an experimental study. Int J Reprod BioMed 2020; 18(10): 865-876.
26. Yehuda I, Madar Z, Leikin-Frenkel A, et al. Glabridin, an isoflavan from licorice root, downregulates iNOS expression and activity under high-glucose stress and inflammation. Mol Nutr Food Res 2015; 59(6): 1041-1052.