Herbal Medicines Journal **2019; 4(4):168-74**
DOI: 10.22087/herb%20med%20j.v4i4.719 **ISSN:** 2538-2144

Original Article

A Comparative Study of the Effects of the Hydroalcoholic Extract of Ziziphora Clinopodioides and Sesame on the Testicular Injury of Normal and Diabetic Mice

Amir Hossein Tozandeh[1], Sahar Molzemi[2*], Nahide Bolbol Haghighi[3], Farzaneh Kazemzadeh[2], Hamid Mohammad Sadeghi[4]

[1]Department of Medical Radiation Engineering, Islamic Azad University, Shahroud, Iran.
[2]Department of Experimental Sciences, Islamic Azad University, Shahroud Branch, School of Medical Sciences Shahroud, Iran
[3]Department of Midwifery, School of Nursing and Midwifery, Shahroud University of Medical Sciences, Shahroud, Iran
[4]Physiology Department, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iranic of Iran Army, Tehran, Iran

Received: 23.02.2019; Accepted: 20.04.2020

Abstract

Background and Aim: Diabetes could play a significant role in creating metabolic damages because of generating oxidative stress. It appears that the antioxidant properties of sesame and Ziziphora clinopodioides enable them to be effective in the treatment of diabetes-induced testicular injuries.

Materials and Methods: In this study, 48 male Wistar rats with the weight range of 200 to 220gr were selected and divided into the control, the diabetic and the experimental groups of 1, 2, 3 and 4 rats. Diabetes was induced by the intraperitoneal injection of 55mg/kg of sreptozotocin. The diabetic group, the first experimental group (diabetic + 100mg sesame) the second experimental group (diabetic +150 mg sesame), the third experimental group (diabetic+ 100mg Ziziphora Clinopodioides) and the fourth experimental group (diabetic + 150 mg Ziziphora Clinopodioides) whose rats became diabetic after two months were administered sesame and Ziziphora clinopodioides extract for five weeks by intraperitoneal injection. At the beginning of the fifth week, blood samples were taken from the subjects, and biochemical factors, blood hormones as well as testicle dimensions were evaluated macroscopically and histologically.

Results: The number of sertoli and spermatid cells in the diabetic group was remarkably reduced in comparison with the control group ($p<0.05$). Moreover, sesame consumption significantly increased the number of sertoli cells and spermatids compared to the other experimental groups. ($p<0.01$)**.** Testosterone and insulin also significantly decreased in the diabetic group compared to the control group ($p<0.041$)**,** and finally sesame consumption increased them compared to the other groups ($p<0.32$).

Conclusion: Sesame plays a therapeutic role in improving diabetes-induced testicular injuries due to its ability to prevent and improve oxidative stresses.

Keywords: Diabetes, Sesame, Ziziphora clinopodioides, Sertoli cells, Testicular injury

***Corresponding Author:** Sahar Molzemi, Department of Experimental Sciences, Islamic Azad University, Shahroud Branch, School of Medical Sciences Shahroud, Iran. Email: saharmolzemi@yahoo.com; Tell: (+98) 91 92739255.

Please cite this article as: Tozandeh AH, Molzemi S, Bolbol Haghighi N, Kazemzadeh F, Sadeghi HM. A Comparative Study of the Effects of the Hydroalcoholic Extract of Ziziphora Clinopodioides and Sesame on the Testicular Injury of Normal and Diabetic Mice. Herb. Med. J. 2019;4(4):168-74.

Introduction

The spermatogenic dysfunction caused by type 1 diabetes is an extensively investigated complication in both diabetic patients and animals (1-4). The International Diabetes Federation reported that diabetes entangled 451 million people throughout the world in 2017, and this figure is likely to increase to 693 million by 2045 (4) Furthermore, epidemiological surveys showed that about 50% of patients with diabetes suffer from different degrees of reproductive diseases (2) such as decreased libido and impotence, erectile dysfunction, ejaculation difficulties, and reduced sperm quality, which lead to infertility (3). In addition, decreased reproductive organ coefficient, damaged seminiferous tubules, and reduced sperm quality were observed in streptozotocin (STZ)-induced diabetic animal models (4). However, the underlying mechanisms of spermatogenesis dysfunction in patients with type-1 diabetes has remained poorly elucidated. Many plant species are being used in traditional medicine of various nations for their hypoglycemic properties to treat diabetes mellitus (3, 5).

Sesame, a species of gamopetalous dicotyledones, is typically consumed as a food. Moreover, it is used in pharmacological industry as a solvent in intramuscular injection solutions. It is also being used by various organs of the body. Sesame oil is also used as an antidote to poisons. In addition, it could be used for the treatment of burn scars on the skin. Continuous consumption of sesame could affect the memory improvement. Studies being conducted suggest that enriched antioxidants and unsaturated fat in sesame oil could assist the controlling of hypertension (6). It is the most popular oil in Asia, and is regarded as a major oil product. Due to manual harvesting restrictions, and in order to extract the oil, today modern methods are used to produce it (7).

The anti-inflammatory effect of sesame could be compared to the anti-inflammatory impact of steroid and non-steroid medicines with phenylbutazone (6). This medicine manifests its anti-inflammatory effect by inhibiting the synthesis of inflammatory prostaglandins. Flavonoid, terpenoid, saponin, alkaloid, tannin, polysaccharide and monosaccharide, including arabinose, mannose (aldohexose) and glucuronic acid derivatives have been identified (6&7). The cycle of flavonoid compounds is purified from the stem extract of this plant. Since flavonoids are able to reduce the level of free radicals in cells as antioxidant, they could be employed to reduce the destructive effects of diabetes and increase the activity of pancreatic β-cells.

Ziziphora clinopodioides is a plant, which belongs to the Lamiaceae family, ziziphora species, the aerial parts of which are used as spice. This plant grows in most areas of Iran, and encompasses 9 native subspecies in Iran. Due to having antioxidant properties, it is able to remove the destructive effects of diabetes in the body (8-13).

All parts of this plant could be used according to the teachings of traditional medicine. Many herbal species are used for the treatment of diabetes mellitus in traditional medicine of various nations for their hypoglycemic properties .In a study under the title of «The Effect of Hydroalcoholic Extract of Ziziphora Clinopodioides on the Number of Pancreatic B-Cells in streptozotocin-induced Type I Diabetic Suri Rats», its antioxidant and hypoglycemic functions have been mentioned [6]. Hence, the aim of this study was to investigate the effect of the hydroalcoholic extract of sesame on the treatment of testicular injures resulted from streptozotocin-induced diabetes in rats, and also to examine the effect of various dosages of this extract on the level of serum insulin and glucose. Moreover, we aimed to investigate the amount of testosterone hormone. The researchers expected to observe the rise of spermatogenesis and testosterone hormone as well as decrease in blood glucose due to the antioxidant property of sesame and ziziphora clinopodioides.

Materials and Methods

Sesame and ziziphora clinopodioides were harvested from Abr Village in Shahroud in 2015. They were dried in an oven or a furnace under 3^{o}C after being cleaned in the shadow. Subsequently, dried leaves were powered (about 80 grams) and placed in a Soxhlet extractor for 72 hours along with 400ml ethanol (80%). Then, the extract was filtered and dried using a rotary apparatus.

The present article is the result of the design of 1394 of Shahid Sadoughi University of Medical Sciences, Yazd. In this study 48 male Wistar rats with the weight range of 200 to 220gr were selected and divided into the control, the diabetic and the experimental groups of 1, 2, 3 and 4 rats. Diabetes was induced by the intraperitoneal injection of 55mg/kg of sreptozotocin made by Sigma Co. with the product code of S0130. The diabetic group, the first experimental group (diabetic + 100mg sesame) and the second experimental group (diabetic +150 mg sesame), the third experimental group (diabetic+ 100mg Ziziphora Clinopodioides) and the fourth experimental group (diabetic + 150mg Ziziphora Clinopodioides) in which the mice became diabetic after two months were administered sesame and ziziphora clinopodioides extract for five weeks by intraperitoneal injection. At the beginning of the fifth week, blood samples were taken from the subjects and then biochemical factors and blood hormones as well as testicle dimensions were evaluated macroscopically and histologically. The animals were kept in clean cages with the temperature of 22-24^{o}C, the light cycle of 12:12 light/dark, and the relative humidity of 40-60% in laboratory. At the beginning of the fifth week, the samples were taken directly from the heart after anesthesia (using ketamine and xylazine), and then biochemical factors and blood hormones as well as macroscopic observations (including age, diameter, length, and volume of testicles) were evaluated, and subsequently testicular sections were saved in 10% formalin to conduct histologic experiments and to be transferred to the laboratory.

Macroscopic Investigation

To investigate the testicle weight, a scale with an accuracy of 0.001g (Ahac Company) was used. The length and diameter of the testicles were measured by a caliper (Abzar Market Company) and testicular volume was measured using calibrated cylinder.

Measuring the Diameter of Seminiferous Tubules

The diameter of seminiferous tubules was measured using the Singh method. Twenty five tubules were randomly selected in each section of the testicles, and the average diameter of the tubules was calculated by measuring small and large diameters of each tubule using a calibrated micrometer connected to the eyepiece of the microscope (14).

Sertoli Cell Count

Twenty five tubules were selected in each field and each cross section of the testicles, and then sertoli cell count was conducted under the microscope. The mean of this value was calculated for each group (14).

Spermatogenesis Investigation

Following the microscopic observation of the seminiferous tubules, Table 1 was prepared in which the numbers of 2 and 1 spermatocytes, spermatids, the number of luminal sperm bundles, the thickness of basement membrane as well as the weight, length, diameter and volume of testicles, the diameter of the seminiferous tubules and the average number of sertoli cells were investigated in 25 different tubules. (14).

The Method of Testosterone Analysis

Serum testosterone is measured using ELISA Test Kits, Bovine Interferon Gamma. The basis of this kit is the RIA method with double antibody. The specified values of the sample, the marked testosterone (1251-T) and the testosterone anti-serum were added together in this kit respectively.

After incubation in normal temperature, the sample was incubated and then centrifuged to separate deposits. The number of occupied locations of the anti-serum by marked testosterone is inversely related to the concentration of the sample testosterone. Testosterone concentration was specified by counting the gamma nodes and comparing the results with standard serums.

Insulin Analysis Method

After separating the blood serum from blood contents by biochemical kits, blood insulin was investigated (14).

Blood Glucose Analysis Method

After separating the blood serum from blood contents by Pars Azmoon kits, blood glucose was investigated (14).

Statistical Analysis

Statistical calculations were conducted using SPSS 21. To compare the mean of the groups, one-way ANOVA was employed. In cases where a significant response was observed, post-hoc test was used to find the place of disputes. The significant level was defined as less than 5% ($p < 0.05$). (mean±SD).

Results and Discussion

Diabetic rats suffered from many diabetes-induced complications including bulimia, polydipsia and diarrhea.

Tissue Quantity

In the control group, testicular tissue was covered with albuginée layer, and seminiferous tubule cell collections were observed. Tissue quantity in the diabetic group was destructed, and a significant decrease was observed in cell collections. Moreover, tissue structures in the treatment group showed significant improvement by administrating 150mg/kg ziziphora clinopodioides (Table 1). The number of spermatogenic cells significantly reduced in the diabetic group in comparison with the control group, and the number of spermatogenic cells in the first and the second experimental groups significantly increased compared to the diabetic group (Figure 1).

Tissue comparison was conducted using one-way ANOVA in different groups (Table 1).

$$\text{Average Diameter} = \sqrt{L * \text{magnification}\ \ B * \text{magnification}}$$

L: Length (Large Diameter)

B: Breadth (Small Diameter)

The thickness of basement membrane increased in the diabetic group compared to the control group. Moreover, with regard to the conducted investigations, it was specified that the atrophy of seminiferous tubules in the diabetic rats was significantly increased but these injuries are reduced by administering sesame. Furthermore, a remarkable reduction was observed in the testicular weight ($p<0.02$), diameter ($p<0.023$), length ($p<0.041$) and volume ($p<0.05$) in the diabetic group compared to the control group. A significant increase was also observed in the testicular weight ($p<0.027$), diameter ($p<0.048$), length ($p<0.05$) and volume ($p<0.042$) in the first and the second experimental groups compared to the diabetic groups.

The results of immunohistochemical tests demonstrated that long-term treatment by the hydroalcoholic extract of sesame (80%) for the diabetic rats led to the improvement of diabetes-induced testicular injuries, including the macroscopic and microscopic investigations (Tables 1 and 2) compared to the diabetic rats+ziziphora clinopodioides.

Type 1 diabetes (T1D), which is also referred to as juvenile diabetes, is a type of diabetes that results in the significant reduction or cessation of insulin production by the pancreas [4]. The body needs insulin, which is a hormone that uses blood sugar (2). Prior to the treatment, this phenomenon increases the blood sugar levels in the body (1). The classic symptoms include frequent urination, increased thirst, increased hunger, and weight loss. However, this disease has secondary symptoms that might appear. These symptoms include blurred vision, fatigue, and poor wound healing (2). Symptoms typically appear after a short period of time (1).

Although the cause of type 1 diabetes has remained

Table 1: A comparison of tissue changes in the control, diabetic, the first and the second experimental groups.

PV	Control	Diabetic	1st experimental	2nd experimental	3rd experimental	4th experimental
Spermatogonium (number)	78.16±6.22	58.83±1.6*	67.34±4.24*	71.24±4.01*	65.34±2.24*	69.34±4.01*
Spermatocyte (number)	63.33±3.81	48.33±3.2*	52.83±1.9*	57.60±4*	54.83±3.9*	55.63±4*
Spermatid (number)	125.8±5.1	78.33±6.83*	96.33±2.92*	114.12±3*	100.33±3.92*	109.83±3*
Sertoli cells(number)	17.33±2.1	5.5±1.25*	11±1.87*	18±1.7*	15±1.87*	17±1.7*
Thickness of basement membrane(mm)	1.58±0.11	2.57±0.17*	1.3±0.11*	2.5±0.1*	1.3±0.11*	2±0.1*
Seminiferous tubule (diameter)(mm)	267.6±11.21	196.3±5.32*	236.1±11.64*	251.93±12.2*	236.1±11.64*	241.93±12.2*

The morphologic changes of testicle texture in various groups were investigated using one-way ANOVA (post-hoc test) which is defined in ranges $p<0.05$ (mean±SD).

Table 2: A comparison of testicular volume, length, diameter and weight between the control group and the experimental groups.

PV	Control	Diabetic	1st experimental	2nd experimental	3rd experimental	4th experimental
Testicular weight (grams)	1.52±0.048	1.18±0.048*	1.38±0.037*	2.1±0.051*	1.28±0.037*	1.4±0.051*
Testicular diameter (mm)	1.18±0.037	0.98±0.073*	1±0.057*	1.4±0.06*	1.02±0.057*	1.2±0.06*
Testicular length (mm)	2.04±0.050	1.4±0.07*	2±0.04*	2.91±0.04*	1.74±0.04*	1.89±0.04*
Testicular volume (mm^3)	2.054±0.15	1.046±0.046*	1.1±0.014*	1.5±0.015*	1.2±0.014*	1.5±0.015*

Table 3: A comparison of mean±SD of insulin and testosterone and blood glucose among the control and the experimental groups.

PV	Control	Diabetic	1st experimental	2nd experimental	3rd experimental	4th experimental
Testosterone(mg/dl)	4.1±0.509	0.34±0.05*	1.42±0.06*	2.0±0.02*	1.60±0.02*	1.70±0.02*
Insulin(mg/dl)	4.66±0.54	0.1±0.073*	2.66±0.66*	3.1±0.36*	2.7±0.36*	2.8±0.36*
Glucose(mg/dl)	85.42±5.06	300.42±9.56*	220.36±5.12*	190.74±4.98*	240.74±4.98*	230.74±9*

unknown (4), it comprises certain genetic and environmental determinants. One of the risk factors of type 1 diabetes is to have a family member suffering from this disease. The major mechanism involves an autoimmune destruction of the insulin-producing beta cells in the pancreas. The test of the level of sugar or glycated hemoglobin (HbA1C) in the blood is used to diagnose diabetes. Type 1 diabetes could be distinguished from type 2 via conducting a test to determine the presence of autoantibodies (4).

Type 1 diabetes is not a preventable disease (16). The administration of insulin is essential for survival. Insulin is injected subcutaneously but it could also be delivered by an insulin pump. A diabetic diet and exercise are considered as significant factors for controlling this disease. If left untreated, diabetes could lead to the development of several complications (17). The complications that occur relatively rapidly include diabetic ketoacidosis and nonketotic hyperosmolar coma (18). Long-term complications include heart disease, stroke, kidney failure, foot ulcers and damage to the eyes (18). Furthermore, complications might emerge due to low blood sugar caused by the excessive administration of insulin (5). In general, the results of the present research indicated that the alcoholic extract of sesame is more effective in the treatment of the destructive effects of diabetes compared to ziziphora clinopodioides. Possibly, the anti-diabetic performance of sesame is due to its activation of the peroxisome proliferator-activated receptor (PPAR)-γ. The major and effective component of this plant is gallic acid in this part of the plant (6, 7). Sperm cells of mammals contain a high amount of non-saturated fatty acids, plasmalogens and sphingomyelin, which are regarded as significant substrates in oxidation (3). Normally, there are antioxidant mechanisms in reproductive tissues that prevent oxidative injuries in mature gonadal and spermatozoa cells. Antioxidants available in sesame have removed the destructive effects of diabetes and tissue, and the macroscopic improvement of testicles is observed in the experimental groups (7).

In general, diabetes results in the oxidation of lipids, proteins and DNA by creating free radicals and oxidative stress that consequently cause extensive injuries in testicles. Diabetes mellitus brings about changes in the testicular by causing cell death, decreasing seminiferous tubules, reducing the diameter of tubules, and decreasing the collections of spermatogenic cells (19, 20). Hence, the atrophy of seminiferous tubules and the decreased number of spermatogenic cells are regarded as the morphologic signs of disorder in spermatogenesis. The aim of the present research was to reduce diabetes-induced testicular injuries by administering various doses of

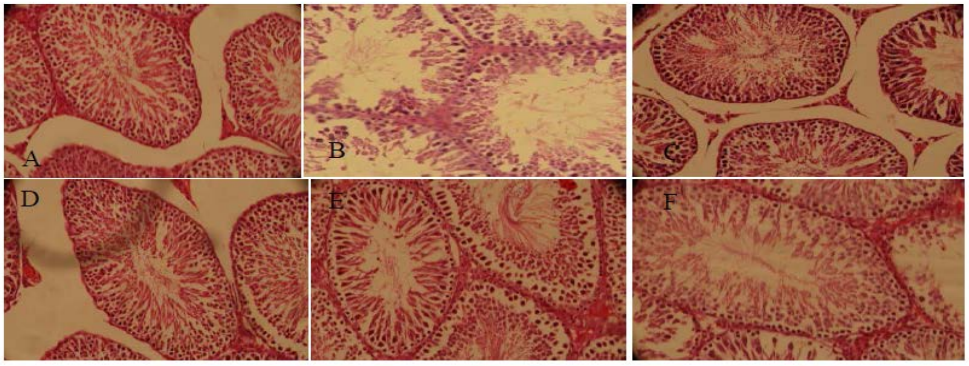

Figure 1. A, B, C, and D show seminiferous tubules (the increase in sertoli cells).

sesame.

Our results indicated a decrease in the diameter of seminiferous tubules and an increase in the diameter of basement membrane in the diabetic groups. Furthermore, it appears that in the treated diabetic groups, sesame results in increased tubular diameter, decreased basement membrane and improved spermatogenic cells (Table 3). Diabetes increases oxidative stress and creates activated oxygen that result in cell damage by peroxidation of lipids and the oxidative destruction of proteins and DNA (21, 22).

The thickness of the basement membrane of seminiferous tubules is central in spermatogenesis (2). During diabetes, the thickness of the basement membrane of seminiferous tubules increases and this increase results in decreased spermatogenesis and consequently decreased diameter of seminiferous tubules. On the other hand, there is a positive relation between the diameter of tubules and spermatogenesis activity (2).

In this study, the amount of testosterone and insulin hormones was evaluated, and a remarkable increase was observed in testosterone and insulin hormones that might be the result of the antioxidant activity of sesame (7). In line with other studies (7), the hypoglycemic effects were verified once more in the present research.

Conclusion

In general, the optical microscopic studies associated with this project indicated that in testicles of diabetic rates, the diabetes-induced increase in the thickness of the basement membrane of seminiferous tubules significantly decreases by treatment with sesame for 5 weeks. Furthermore, with regard to the results obtained from this study, the reduction of sertoli cells results in the reduction of spermatogenesis because it is responsible to feed the seminiferous cells. Sertoli cells play a vital role in spermatogenesis by providing physical and nutritional support as well as hormone signals required for successful spermatogenesis. Hence, as sertoli cells decrease, the number of generative cells significantly reduces. In this study, diabetes resulted in the reduction of sertoli cells and consequently decreased the number of generative cells. The amount of insulin hormones was evaluated, and a significant increase was observed that might be the result of the antioxidant activity of sesame. It was specified in the present research that diabetes results in the malfunction of testicles in male Wistar rats. Furthermore, sesame therapy has improved this function by protecting seminiferous tubules and spermatogenic cells. In general, all the diabetes-induced injuries in the second experimental group (diabetic+150mg/kg sesame) have been improved better compared to the first, the third, and the fourth experimental groups and therefore diabetic males with erectile dysfunction are recommended to take 100mg/kg hydroalcoholic extract of sesame despite the antioxidant properties of ziziphora clinopodioides. It is hoped that this study could be a turning point to the treatment of erectile dysfunction in diabetic men.

Acknowledgment

We would like to thank the authorities of Yazd

University of Medical Sciences and all those who contributed to the accomplishment of this project.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Maresch CC, Stute DC, Alves MG, Oliveira PF, Linn T. Diabetes-induced hyperglycemia 494 impairs male reproductive function: a systematic review, Hum. Reprod. Update. 2018;24(1):86-105.
2. Alves MG, Duarte AI, Santos MS, Moreira PI, Cavaco JE, Oliveira PF. Testosterone deficiency induced by progressive stages of diabetes mellitus impairs glucose metabolism and favors glycogenesis in mature rat Sertoli cells, International . journal. Biochem. Cell Biol. 2015;66:1-10.
3. Omolaoye TS, Skosana BT. Diabetes mellitus-induction: Effect of different streptozotocin doses on male reproductive parameters, journal Acta. Histochem. 2018;120(2):103-9.
4. Gandhi J, Dagur G, Warren K, Smith NL, Sheynkin YR, Zumbo A, et al. The Role of Diabetes Mellitus in Sexual and Reproductive Health: An Overview of Pathogenesis, Evaluation, and Management, Curr. Diabetes. Journal Rev. 2017;13(6):573-81.
5. Ghorbani A, Farahani MA, Mohammadi N. Relationship between sleepiness, physical activity,and functional outcomes in iranian patients withtype II diabetes. Jundishapur Journal Chronic Dis Care. 2014;3(1):54-60. [in Persian]
6. Eweda SM, Newairy ASA, Abdou HM, Gaber AS. Bisphenol A-induced oxidative damage in the hepatic and cardiac tissues of rats: The modulatory role of sesame lignans. journal Ther Med. 2020;19(1):33-44.
7. Tateishi N, Morita S, Yamazaki I, Okumura H, Kominami M, et al. Administration timing and duration-dependent effects of sesamin isomers on lipid metabolism in rats. Journal Chronobiol Int. 2019;13:1-17.
8. Ghafari H, Yasa N, Mohammadirad A, Dehghan G, Zamani MJ, Nikfar S, et al. Protection by Ziziphora clinopoides of acetic acid-induced toxic bowel inflammation through reduction of cellular lipid peroxidation and myeloperoxidase activity. Journal Human & Experimental Toxicology. 2006;25(6):325-32.
9. Wu Y, Wang Y, Nabi X. Protective effect of Ziziphora clinopodioides flavonoids against H2O2-induced oxidative stress in HUVEC cells .journal Biomed Pharmacother. 2019;117:109-56.
10. Sedighi S, Tehranipour M, Vaezi G, Hojati V, Hashemi-Moghaddam H. The effect of hydroalcoholic extract of Ziziphora clinopodioides L. on spatial memory and neuronal density of hippocampal CA1 region in rats with sporadic Alzheimer's disease. journal Avicenna Phytomed. 2019;9(4):362-73.
11. Allergic asthma by Ziziphora clinopodioides via upregulation of aquaporins and downregulation of IL4 and IL5. Respir. Journal Physiol Neurobiol. 2019;266:39-46.
12. Shahbazi Y. Chemical compositions, antioxidant and antimicrobial properties of Ziziphora clinopodioides Lam. essential oils collected from different parts of Iran. journal Food Sci Technol. 2017;54(11):3491-503.
13. Konyalıoglu S, Ozturk B, Meral GE. Comparison of Chemical Compositions and Antioxidant Activities of the Essential Oils of Two Ziziphora. Taxa from Anatolia. journal Pharmaceutical biology. 2006;44(2):121-6.
14. Sadeghi H, Mansourabadi A, Emami S, Nahvinejad M, Moogooei M. The effect of Ziziphora ethanolic extract on active pancreatic beta cells on streptozotocin induced diabetic mice,Iranian journal of Diabetes and Metabolism. 2015;14(6):373-8. [in Persian]
15. Mahdavi, M. Antimicrobial effects of Ziziphora clinopodioidesextracts on Salmonella typhi Vi+ in vitro, in vivo and amp; amp; cell culture. Clinical Biochemistry. 2011;44(13):326-30. [in Persian]
16. Sánchez-Gutiérrez M, Martínez-Loredo E, Madrigal-Santillán EO, Betanzos-Cabrera G, Hernández-Zavala A, Mojica-Villegas M, et al. Exposure of Fluoride with Streptozotocin-Induced Diabetes Aggravates Testicular Damage and Spermatozoa Parameters in Mice. Journal Toxicol. 2019;3;2019:5269380.
17. Liu Y, Yang Z, Kong D, Zhang Y, Yu W, Zha W. Metformin Ameliorates Testicular Damage in Male Mice with Streptozotocin-Induced Type 1 Diabetes through the PK2/PKR Pathway. Journal Oxid Med Cell Longey. 2019;28:5681701.
18. Alsemeh AE, Samak MA, El-Fatah SSA. Therapeutic prospects of hydroxytyrosol on experimentally induced diabetic testicular damage: potential interplay with AMPK expression. Journal Cell Tissue Res. 2019 Dec 14. doi: 10.1007/s00441-019-03143-2.
19. Nna VU, Bakar ABA, Ahmad A, Umar UZ, Suleiman JB, Zakaria Z, et al. Malaysian propolis and metformin mitigate subfertility in streptozotocin-induced diabetic male rats by targeting steroidogenesis, testicular lactate transport, spermatogenesis and mating behaviour. Journal Andrology. 2019 10.1111/andr.12739
20. Cheng Y, Yang Z, Shi J, Yang J, Zhao J, He Y, et al. Total flavonoids of Epimedium ameliorates testicular damage in streptozotocin-induced diabetic rats by suppressing inflammation and oxidative stress. Journal Environ Toxicol. 2020;35(2):268-76.
21. Jiang YP, Ye RJ, Yang JM, Liu N, Zhang WJ, Ma L, et al. Protective effects of Salidroside on spermatogenesis in streptozotocin induced type-1 diabetic male mice by inhibiting oxidative stress mediated blood-testis barrier damage. Journal Chem Biol Interact. 2019;2;315:108869.
22. Cho EH, Lee H, Ryu OH, Choi MG, Kim SW. Sleep Disturbances and Glucoregulation in Patients with Type 2 Diabetes. Journal Korean Med Sci. 2014;29(2):243-7.

© **Amir Hossien Tozandeh, Sahar Molzemi, Nahide Bolbol Haghighi, Farzaneh Kazemzadeh, Hamid Mohammad Sadeghi**. Originally published in the Herbal Medicines Journal (http:/www.hmj.lums.ac.ir), 30.07.2020. This article is an open access article under the terms of Creative Commons Attribution License, (https://creativecommons.org/licenses/by/4.0/), the license permits unlimited use, distribution, and reproduction in any medium, provided the original work is properly cited in the Herbal Medicines Journal. The complete bibliographic information, a link to the original publication on http://www.hmj.lums.ac.ir/, as well as this copyright and license information must be included.