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The Effect of Aerial Part of Melissa Officinalis L. Hydro-Alcoholic Extract on Pituitary- Gonadal Axis Function in Diabetic Male Mice

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ABSTRACT

Background: *Melissa officinalis* L. (lemon balm) is one of the more widely cultivated medicinal and aromatic plants that has long been used in traditional medicine to treat many disorders. The present study investigates *Melissa officinalis* L. hydro-alcoholic extract regarding pituitary-gonadal axis in diabetic mice. **Methods:** 45 NMRI mice with a mean weight of 35.6±4.5 g were divided into five groups: control group (0.2 ml of physiological serum intraperitoneally injection), diabetic mice (without treatment), and three experiment groups (diabetic groups receiving 50, 100 and 200 mg/kg of *Melissa officinalis* extract intraperitoneally injection). *Melissa officinalis* extract was injected intraperitoneally for 14 days. At the end of the experiment, blood samples were taken to determine the biochemical indicators level (glucose, LH, FSH, and testosterone), and the left testicle was weighed and examined histologically. **Results:** The results showed that the lowest amount of glucose and the highest level of LH were observed in the treatment group at a dose of 100 mg/kg of *Melissa officinalis* extract. The highest level of FSH was observed at a dose of 200 mg/kg. Histological study of the testis showed a more favorable condition in the experimental group of 200 mg/kg of lemon balm extract (P<0.05). **Conclusion:** It can be concluded that the use of *Melissa officinalis* extract is efficient in reducing glucose, improving the levels of LH, FSH, and testosterone in diabetic mice, affecting testicular weight, and improving testicular tissue indices and reproductive function of rats.

Introduction

One of the most important endocrine diseases in the world is diabetes, which affects many people annually; so it is referred to as a silent epidemic and is one of the public health problems all over the world (Standl *et al.*, 2019). This disease contributes to decreasing global birth rates (Maresch *et al.*, 2017). Research has shown that oxidative stress plays a key role in pathogenesis and complications of diabetes. Oxidative stress in diabetics leads to a wide range of diseases such as retinopathy, nephropathy, cardiovascular, sexual, and hormonal diseases (Rains and Jain, 2011).

Diabetes has different functional and structural effects on male reproductive system, such as decreased testosterone production, libido (Singh *et al.*, 2006), reduced sperm maturity, motility, and

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viability. Diabetes increases the abnormality of sperm (Mardanshahi *et al.*, 2019). It decreased sperm count, and sperm concentration (Zhong *et al.*, 2021). Diabetes also increases the detrimental effects on reproduction such as low sperm motility and DNA integrity (Diogo *et al.*, 2023)

Plants were used as traditional therapies before the discovery of insulin and anti-diabetic drugs. Traditional Persian Medicine (TPM) has a long history of the treatment of diseases by using herbal plants (Araj-Khodaei *et al.*, 2020, Naseri *et al.*, 2016). Today, various clinical and animal research has been conducted using TPM (Babaeian *et al.*, 2015).

Numerous laboratory and clinical studies have been performed on medicinal plants in the treatment of diabetes, many of which hypoglycemia have been observed among patients with diabetes (Kooti *et al.*, 2016). Nowadays, the use of medicinal plants in the treatment of diabetes is inevitable, due to their fewer side effects compared with those of chemical drugs. Prescription of herbal medicines by traditional medicine experts is common for the treatment of many diseases. Many plants are great sources of antioxidants which can reduce the negative effects of oxidants and side effects of some diseases, one of them is lemon balm (López *et al.*, 2009).

Lemon balm with the scientific name *Melissa officinalis* L. is a plant of the Lamiaceae family. It contains flavonoids, phenolic component, rosmaric acid, and gallic acid (Miraj *et al.*, 2017). Various studies on Lemon balm have revealed this plant's antiinflammatory effects, cardioprotection, antispasmodics and vasodilation (Draginic *et al.*, 2021), antiviral (Behzadi *et al.*, 2023) and antioxidant effects (Miraj *et al.*, 2017), reduction of depression (Araj-Khodaei *et al.*, 2020), anxiety and stress (Ghazizadeh *et al.*, 2021, Haybar *et al.*, 2018), and anti-diabetic effects (Naseri *et al.*, 2021). Lemon balm is sold as a substance in tea bags, food supplements, powdered herbal, and combination products on the European market (Uropean medicines agency, 2013). It is used in culinary, aromatic, and traditional medicine (Kato-Noguchi, 2003).

Lemon balm is a healthy and safe herbal medicine that improves sexual dysfunction in women (Darvish-Mofrad-Kashani *et al.*, 2018). The aqueous extract of *M. officinalis* is an effective drug in the treatment of benign heart palpitations (Alijaniha *et al.*, 2015, Naseri *et al.*, 2021). Naseri *et al*. showed the effectiveness of *M. officinalis* in improving learning and enhancing memory in diabetic rats (Naseri *et al.*, 2021). *M. officinalis* can improve cognition in older adults without hypertension (Noguchi-Shinohara *et al.*, 2023). There have also been various studies on the effects of *M. officinalis* regarding reproductive dysfunction. *M. officinalis* has a protective effect on sperm and spermatogenesis factors of rats exposed to lead (Abbasi *et al.*, 2016). Moreover, the protective effect of *M. officinalis* on malathion poisoning revealed the plant was highly efficient in the improvement of testicular tissues and normal spermatogenesis (Seif MM, 2014). Therefore, due to the antioxidant properties and effectiveness of *M. officinalis*, it is necessary to conduct comprehensive research in this regard. According to the above explanations, the present study investigates the effect of hydro-alcoholic extract of aerial parts of *M. officinalis* on the pituitary-gonadal axis, spermatogenesis, and testicular tissue in diabetic mice.

Materials and Methods

Plant material

M. officinalis was purchased from Firouzeh Botanical Garden (Tehran province, Iran). The plant was identified and authenticated by Dr. Mohammad Kamalinejad at Herbarium of the Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran (voucher number 3380).

The aerial parts of the plant were dried and macerated in hydroalcoholic solvent (40% ethanol and 60% distilled water). After extraction, the total extract was filtered, and the solvent was removed (Mariarosaria *et al.*, 2020).

Animal

In this study, 45 NMRI mice with an average weight of 36.16±4.5 g were purchased from Royan

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Research Institute (Tehran, Iran). The mice were kept for one week to adapt to storage conditions, 12 light/12 dark. Unrestricted standard water and diet were available for animals during the experimental period.

The mice were randomly divided into five groups of nine, as follows: The control group received 0.2 ml of physiological serum intraperitoneally daily. The diabetes group did not receive any treatment, and three test groups received 50, 100, and 200 mg/kg of *M. officinalis* L. extract intraperitoneally for 14 days. The care and handling of the animals were according to the NIH guidelines and ethical committee of Payame Noor University guidelines (letter No. P/7/7991 (2023/1/1) of the vice president of research and technology of the university).

The animals were included with glucose levels less than 110 mg/dl. Alloxan (Sigma, Germany) was used to make NMRI mice diabetic. 150 mg/kg of alloxan dissolved in 0.9% (w/v) saline was injected intraperitoneally and in a single dose (Verma *et al.*, 2010). Before and after the induction of diabetes, the mice were fasted overnight to measuring glucose levels. Blood was taken from the tail vein. Blood glucose levels were measured after 72 hours of alloxan injection using a digital glucometer (Emperor Prodigy Blood Glucose Monitor OK-2AJ, Taiwan), and the mice with a blood glucose level above 250 mg/dl were selected as diabetic mice and entered the experiment (Ighodaro *et al.*, 2017). The mice were excluded with blood glucose levels less than 250 mg/dl after alloxan injection.

After diabetic induction, the hydroalcoholic extract of *M. officinalis* was injected intraperitoneally for 14 days in experimental groups (Sadat *et al.*, 2019) with doses of 50, 100, and 200 mg/kg, respectively. After 14 days, the mice were anesthetized and blood was collected from the heart. Blood samples were kept at room temperature for half an hour and then the serum of blood samples was centrifuged at 3000 rpm for 20 minutes (Negahdary *et al.*, 2015); then, the blood was stored in the freezer at -20 °C until glucose, LH, FSH, and testosterone were measured.

The glucose index was measured using Bionik's kit and based on its instructions. LH, FSH, and testosterone were also measured by ELISA, the hormone diagnosis kit, and the related instructions.

After blood sampling from anesthetized animals, the right testes of mice were removed and weighed using a digital scale (A&D, Japan). The testes were washed using physiological serum (Razi serum, Iran) and then kept for 2 days at 10% formalin solution. The testicle tissues were transferred to %70 ethanol, and dehydrated by passing ascending grades of ethanol. Then, the tissues were cleared in xylene and embedded in paraffin. Five µm thickness sections were cut by microtome transferred on slide, and stained with Hematoxylin-Eosin. The slides were observed in a light microscope (Naghdi *et al.*, 2016). The maximum volume of injection was 0.2 ml in all experiments, and euthanasia of mice was done using chloroform.

Data analysis

At the end of the experiment, data were collected and statistical analysis was performed using SPSS software version 20 to ensure the normality of the data, data were first analyzed by Shapiro-Wilk test. Then, for intergroup comparison, one-way-ANOVA was used at 95% confidence level (the significance level in all tests was less than 0.05), followed by Tukey post-hoc test. Finally, the results were expressed as the mean and standard error (Means±SE).

Results

No significant difference in the weight of NMRI mice was observed in all test groups. There was a significant difference in glucose levels among the treatments, so the highest and lowest glucose levels were observed in untreated diabetic group and the control group, respectively. The highest level of LH was in the control treatment, which showed a significant difference from other treatments. The lowest levels of LH were also seen among the untreated diabetic rats. Examination of FSH in the blood serum of NMRI mice showed a significant difference among the treatments. The lowest level of FSH was observed in diabetic rats with no *M.*

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officinalis extract, and the highest level was seen in those with a dose of 200 mg/kg. Examination of testosterone changes revealed that there was no significant difference among the treatments. The control treatment had the highest level of testosterone and the lowest level in diabetic NMRI mice. Also, the highest and lowest testicular weights were experienced among the control treatment and the dose of 100 mg/kg *M. officinalis*, respectively (**Table 1**).

Table 1. Weight change and biochemical analysis in diabetic mice with different doses (50, 100, and 200 mg/kg) of *Melisa officinalis.*

Group	Initial weight (g)	Final weight (g)	Glucose (mg/dl)	LH (mu/ml)	FSH (mu/ml)	Testosterone (mu/ml)	Testis weight (g)
Control	37.22±1.33	37.22±1.32	96.00±13.81^{d}	4.27±0.25^{a}	0.57±0.04^{ab}	1.14±0.48	0.01^{a}±0.15
Diabetes	37.75±1.66	38.75±1.60	517.00±16.21^{a}	1.78±0.10^{c}	0.20±0.02^{c}	0.30±0.12	0.004^{c}±0.09
Dose 50	35.33±1.31	35.44±0.95	435.85±89.66^{c}	2.50±0.14^{bc}	0.35±0.02^{bc}	0.66±0.10	0.01^{ab}±0.14
Dose 100	33.87±1.37	34.77±1.43	279.57±67.57^{b}	3.67±0.42^{b}	0.48±0.05^{b}	0.88±0.34	0.004^{b}±0.12
Dose 200	36.66±1.14	38.77±1.41	364.57±94.98^{bc}	3.37±0.24^{b}	0.63±0.05^{a}	0.56±0.29	0.02^{ab}±0.13

Results are presented as means±SE, SE : Standard Error. Similar letters in the same column show no significant difference between results for each treatment (P>0.05).

Histological examinations

Examination of testicular tissue in untreated diabetic rats with *M. officinalis* extract showed that basal membrane of seminiferous tubules was ruptured, and in some cases, it was completely destroyed and cellular arrangement was disrupted. Moreover, in the test group receiving a dose of 50 mg/kg of M. officinalis extract, the basement membrane of seminiferous tubules ruptured in some cells, and in a small number, it was completely. In addition, in some tubes, the cellular arrangement was disrupted. Examining testicular tissue in the group with a dose of 100 mg/kg of *M. officinalis* extract revealed that in some tubes the arrangement of spermatogenesis cells was disrupted. Also, tissue analysis at the dose of 200 mg/kg group of extract revealed continuous duct-based and spermatogenesis cells in the tube.

Testicular histological studies showed that there was a significant difference among experimental treatments. The highest and lowest diameters of the urinary tract were observed in controlled treatment and diabetic rats, respectively (**Table 2**). Analyzing changes in the thickness of the epithelium of the spermatogenesis tubules showed that the lowest amount of epithelial thickness of the spermatogenesis tubules was in diabetic mice. In addition, no significant difference was observed between control treatment and the dose of 200 mg/kg of lemon balm extract. There was no significant difference in the number of spermatogonia in the control treatment and the dose of 200 mg/kg of lemon balm extract. The lowest number of primary spermatocytes was observed in diabetic mice. Furthermore, the lowest spermatid cell count was in diabetic mice. The highest number of sertoli cells was observed in the control treatment following the group with a dose of 200 mg/kg of *M. officinalis* extract. There was no significant difference between the number of leydig cells in the control treatment and the groups with 100 and 200 mg of *M. officinalis* extract, and the lowest number of leydig cells was observed in diabetic mice. The highest and lowest percentage of spermiogenesis coefficient were observed in the control treatment and diabetic rats, respectively. The lowest Johnson index was seen in diabetic mice. There was no significant difference between the control treatment and the group with a dose of 200 mg/kg of *M. officinalis* extract.

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Table 2. Histological examinations in small diabetic NMRI mice with different doses (50, 100, and 200 mg/kg) of *Melisa officinalis.*

Group	Seminiferous tubules		Spermatogonia	Primary spermatocytes	Spermatid	Sertoli cells	Leydig cells	Spermiogenesis index (SI) %	Johnson Score
	Diameter(µ)	Germinal epithelium(µ)							
Control	175.50±3.91^{a}	53.16±1.49^{a}	26.00±1.91^{a}	31.00±0.73^{a}	98.00±2.67^{a}	19.83±0.70^{a}	10.33±0.21^{a}	95.20±0.30^{a}	0.05^{a}±9.80
Diabetes	107.00±1.29^{d}	31.58±0.89^{c}	10.83±0.47^{b}	4.83±0.30^{c}	31.00±0.68^{d}	9.83±0.40^{c}	4.83±0.30^{c}	63.71±1.71^{e}	0.16^{d}±6.32
Dose 50	143.33±2.29^{b}	33.16±1.20^{c}	13.66±1.14^{b}	23.16±1.40^{b}	47.83±1.32^{c}	9.83±0.47^{c}	7.50±0.22^{b}	72.63±0.58^{d}	0.10^{c}±7.34
Dose 100	123.66±2.49^{c}	43.91±1.80^{b}	14.66±0.88^{b}	24.33±1.33^{b}	62.33±2.07^{b}	11.66±0.71^{c}	9.66±0.49^{a}	80.08±1.88^{c}	0.14^{b}±8.41
Dose 200	126.66±1.20^{c}	52.16±0.57^{a}	25.50±0.42^{a}	30.83±0.60^{a}	94.00±0.89^{a}	17.00±0.25^{b}	10.50±0.22^{a}	86.13±1.00^{b}	0.11^{a}±9.37

Results are presented as means±SE, SE = Standard Error. Similar letters in the same column show no significant difference between results for each treatment (P>0.05).

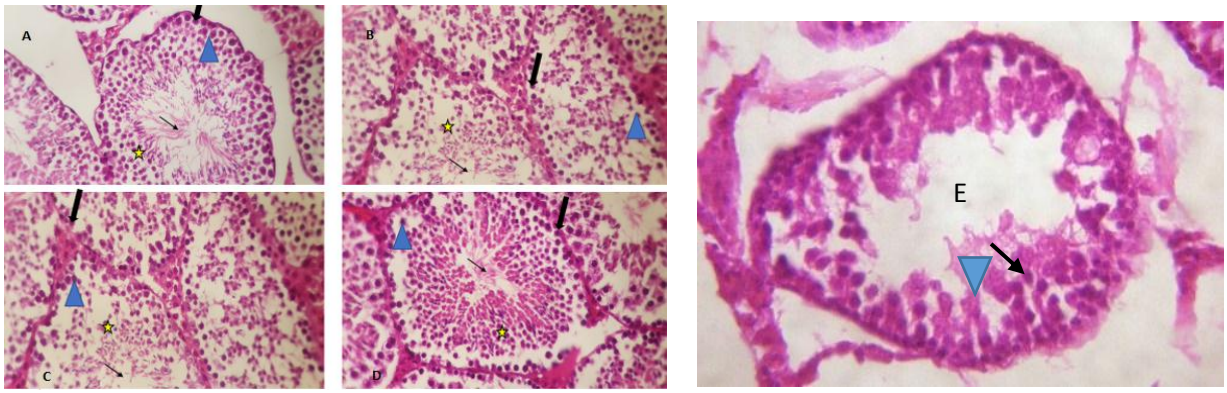

Figure 1. Comparison of testicular histology in test group samples with the magnification of 100.

Thick arrow (spermatogonia); Triangular head (primary spermatocyte); Star (spermatid); The thin arrow (sperm); Control group (A): Normal basal membrane and complete spermatogenesis were seen in the tube; The group with a dose of 50 (B): cell arrangement was disrupted and the interstitial tissue was hyalinized; The group with a dose of 100 (C): Cell disruption was observed;The group with a dose of 200 (D): Natural basal membrane and complete spermatogenesis were seen in the tube; Diabetes group (E): The lowest amount of epithelial thickness of the spermatogenesis tubules, cell disruption without sperm.

Discussion

As mentioned in the results section, no significant difference was observed in the weight of mice among all treatments. The results of the present study were similar to Ashkani-Esfahani *et al.* in that no significant difference in the weight of streptozotocin-induced diabetic rats and *M. officinalis*-treated groups were observed (Ashkani-Esfahani *et al.*, 2021).

The present study showed that hydroalcoholic extract of lemon balm could significantly reduce the serum glucose levels of alloxan-induced diabetic rats, although serum glucose levels of male diabetic rats did not return to normal levels. Various studies indicated that *M. officinalis* extract can lower blood sugar (Abolfazl *et al.*, 2016, Chung *et al.*, 2010, Weidner *et al.*, 2014). The effect of *M. officinalis* extract on beta cells and pancreas of diabetic rats showed that consumption of *M. officinalis* extract in diabetic rats can improve beta cell function by increasing cells in the pancreas, resulting in lower blood glucose (Ashkani-Esfahani *et al.*, 2021). Similar findings in the study of animal models indicated that the consumption of *M. officinalis* can prevent the destruction of pancreatic cells (Shin *et al.*, 2021). Consumption of *M. officinalis* extract increases serum insulin and reduces oxidative stress (Hsiu-Fang *et al.*, 2015).

The results of the study showed a decrease in testosterone among the group treated with *M.*

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officinalis extract compared to the control group, although no significant difference was observed among the experimental groups. *M. officinalis*, with its polyphenolic compounds, has shown high antioxidant activity due to the presence of phenolic compounds, especially rosmarinic acid, terpenoids, flavonoids, and essential oil (Petrisor *et al.*, 2022). The key role of antioxidants in the production of sex steroids was demonstrated by (Saylam and Çayan, 2020). Therefore, improvement in testosterone levels can be observed in doses containing *M. officinalis* extract. The lowest levels of FSH and LH were observed in male diabetic rats without treatment with hydroalcoholic extract of *M. officinalis*. Similar results were previously reported by Ballester (Ballester *et al.*, 2004). This research showed that serum levels of LH and FSH decreased in diabetes. Examination of FSH demonstrated that the highest amount of this hormone appears in the dose of 200 mg/kg of *M. officinalis* extract. Due to its antioxidant properties, it leads to protect the Pituitary- Gonadal axis against Malathion toxicity (Seif MM, 2014).

The lowest testicular weight was observed in diabetic rats without treatment with *M. officinalis* extract. Examination of testicular tissue in diabetic patients showed that testicular weight was reduced and led to reproductive disorders in men (Mallidis *et al.*, 2009). The weight of experimental groups containing 50 and 200 mg of *M. officinalis* extract was close to those in the control treatment, therefore, considering the antioxidant properties of *M. officinalis*, it can be said that this extract had a positive effect on male diabetic rats and may improve testicular weight over a longer period.

Diabetes causes tissue changes in the testicles, including atrophy of the spermatogenesis tubules, reducing the diameter of the tubules, low cellular density, and pathological changes (Guneli *et al.*, 2008, Korejo *et al.*, 2016). Investigation of urethra diameter among diabetic mice in this study showed that the highest sperm duct diameter was observed in the control treatment (175.50±3.91 µm) and the lowest was in diabetics' rats (107.00±1.29 µm, **Table 2**). The groups treated with *M. officinalis* extract had a better condition than diabetic mice, which may be due to the antioxidant role of *M. officinalis*. The results of the present study suggested a negative effect of diabetic mice without treatment with *M. officinalis* extract regarding the thickness of testicular spermatogenesis tubular epithelium, while the dose of 200 mg/kg of lemon balm extract was significantly different from control treatment. Similar conditions were observed in the number of spermatogonia cells, primary spermatocytes, and spermatids. Also, (Tang *et al.*, 2008) stated that the number of sex cells in diabetic patients decreased, which was similar to the findings of the present study. Hyperglycemia leads to oxidative stress and the production of free radicals, thus it caused disorders in the process of spermatogenesis and developed diabetic complications (Moudi *et al.*, 2007). Considering the presence of flavonoid and antioxidant compounds in lemon balm and that the anti-diabetic property of *M. officinalis* had been proved in various studies (Abolfazl *et al.*, 2016, Chung *et al.*, 2010, Weidner *et al.*, 2014), it was concluded that *M. officinalis* extract could reduce the negative effect of diabetes in mice. One of the complications of diabetes is an increase in free radicals in the body (Moussa, 2008), so with an increase in free radicals, leydig cells will not be able to activate androgenesis (Khaneshi *et al.*, 2013). As mentioned earlier, some laboratory studies demonstrated that insulin deficiency in diabetic rats reduces the secretion of LH and FSH (Schoeller *et al.*, 2012). In addition, insulin is necessary to maintain LH receptors in leydig cells and regulating division and metabolism in leydig cells (Noori Roshnavand *et al.*, 2019). Thus, a decrease in insulin in male diabetic rats can impair the leydig cells activity and reduce testicular steroid hormones (Schoeller *et al.*, 2012). Diabetes through oxidative stress (production of free radicals), cell damage by lipid peroxidation, and oxidation of proteins, disrupts the process of spermatogenesis. Oxidative stress was induced by reactive oxygen species (ROS) and antioxidant therapy could be a central approach in the treatment of male infertility (Sadeghi *et al.*, 2023). Also, Antioxidants provide protected from the disruptions caused by diabetes (Sheikhpour, 2013). Antioxidant therapy can

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increase sperm concentration (Yamasaki *et al.*, 2022), and oxidative stress is the main cause of male fertility. Therefore, the production of oxidative compounds should be limited (Hussain *et al.*, 2023). According to the results of the spermiogenesis coefficient and antioxidant properties of lemon balm extract (López *et al.*, 2009), this extract has improved the process of spermatogenesis in diabetic rats.

The limitation of the current study was taking care of diabetic mice and the death of a limited number of them due to diabetes. According to this investigation, the constituents of the *Melissa officinalis* extract as a natural antioxidant might be an alternative to some antidiabetic drugs; however, clinical trials are recommended to evaluate its beneficial effects. For further research, the authors suggest a survey of the impact of *Melissa officinalis* extract on embryos and female mice during pregnancy.

Conclusion

Hydroalcoholic extract of Lemon balm (*Mellisa officinalis*) as a natural antioxidant is effective in lowering glucose, improving the levels of LH, FSH, and testosterone in male diabetic mice, influencing testicular weight, and improving testicular tissue indices and reproduction system function of the mice.

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Contributions

Nasri S designed research; Arab M, Nasri S, Naseri M, and Shahi Sadr Abadi F conducted research; Naseri M and Shahi Sadr Abadi F analyzed data; Nasri S wrote the paper; Nasri S had primary responsibility for final content. All authors read and approved the final manuscript.

Conflicts of interest

The authors declared no conflict of interests.

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References

Abbasi MM, Dadkhah N, Shahnazi M & Parvin N 2016. Protective Effects of Melissa officinalis (Lemon Balm) on Sperm Parameters and Spermatogenesis Quality in Rats Exposed to Lead. *Iranian Red Crescent Medical Journal.* **18 (12)**: 20-.

Abolfazl S, Amirhossein S & Behjat J 2016. Melissa officinalis L. – A review of its traditional uses, phytochemistry and pharmacology. *Journal of ethnopharmacology.* **188**: 204-228.

Alijaniha F, et al. 2015. Heart palpitation relief with Melissa officinalis leaf extract: double blind, randomized, placebo controlled trial of efficacy and safety. *Journal of ethnopharmacol.* **164**: 378-384.

Araj-Khodaei M, et al. 2020. A double-blind, randomized pilot study for comparison of Melissa officinalis L. and Lavandula angustifolia Mill. with Fluoxetine for the treatment of depression. *BMC complementary medicine and therapies.* **20 (1)**: 207.

Ashkani-Esfahani S, et al. 2021. The Effect of Melissa officinalis Extract on Streptozotocin-Induced Diabetes in Rats: A Stereological Study on Pancreatic Islets and Beta-cells. *Journal of advances in medical and biomedical research.* **29 (132)**: 34-40.

Babaeian M, et al. 2015. Herbal Remedies for Functional Dyspepsia and Traditional Iranian Medicine Perspective. *Iran Red Crescent Medical Journal.* **17 (11)**: e20741.

Ballester J, et al. 2004. Insulin-dependent diabetes affects testicular function by FSH-and LH-linked mechanisms. *Journal of andrology.* **25 (5)**: 706-719.

Behzadi A, et al. 2023. Antiviral Potential of Melissa officinalis L.: A Literature Review. *Nutrition and metabolic insights.* **16**: 11786388221146683.

Chung MJ, Cho SY, Bhuiyan MJ, Kim KH &

[DOI: 10.18502/jnfs.v9i4.16888]

Lee SJ 2010. Anti-diabetic effects of lemon balm (Melissa officinalis) essential oil on glucose- and lipid-regulating enzymes in type 2 diabetic mice. *British journal of nutrition.* **104 (2)**: 180-188.

Darvish-Mofrad-Kashani Z, et al. 2018. Effect of Melissa officinalis (Lemon balm) on Sexual Dysfunction in Women: A Double- blind, Randomized, Placebo-controlled Study. *Iranian journal of pharmaceutical research.* **17 (Suppl)**: 89-100.

Diogo P, et al. 2023. Type I Diabetes in Zebrafish Reduces Sperm Quality and Increases Insulin and Glucose Transporter Transcripts. *International journal of molecular sciences.* **24 (8)**: 7035.

Draginic N, et al. 2021. Melissa officinalis L. as a Nutritional Strategy for Cardioprotection. *Frontiers in physiology.* **12**.

Ghazizadeh J, et al. 2021. The effects of lemon balm (Melissa officinalis L.) on depression and anxiety in clinical trials: A systematic review and meta-analysis. *Phytotherapy research.* **35 (12)**: 6690-6705.

Guneli E, et al. 2008. Effect of melatonin on testicular damage in streptozotocin-induced diabetes rats. *European surgical research.* **40 (4)**: 354-360.

Haybar H, et al. 2018. The effects of Melissa officinalis supplementation on depression, anxiety, stress, and sleep disorder in patients with chronic stable angina. *Clinical nutrition ESPEN.* **26**: 47-52.

Hsiu-Fang Y, Chi-Ting H, Tusty-Jiuan H, Fang-Rong C & Chin-Kun W 2015. In vitro anti-diabetic effect and chemical component analysis of 29 essential oils products. *Journal of food and drug analysis.* **23 (1)**: 124-129.

Hussain T, et al. 2023. Unraveling the harmful effect of oxidative stress on male fertility: A mechanistic insight. *Frontiers in endocrinology.* **14**: 1070692.

Ighodaro OM, Adeosun AM & Akinloye OA 2017. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. *Medicina* **53 (6)**: 365-374.

Kato-Noguchi H 2003. Assessment of allelopathic potential of shoot powder of lemon balm. *Scientia horticulturae.* **97**: 419-423.

Khaneshi F, Nasrolahi O, Azizi S & Nejati V 2013. Sesame effects on testicular damage in streptozotocin-induced diabetes rats. *Avicenna journal of phytomedicine.* **3 (4)**: 347-355.

Kooti W, Farokhipour M, Asadzadeh Z, Ashtary-Larky D & Asadi-Samani M 2016. The role of medicinal plants in the treatment of diabetes: a systematic review. *Electron physician.* **8 (1)**: 1832-1842.

Korejo NA, Wei QW, Shah AH & Shi FX 2016. Effects of concomitant diabetes mellitus and hyperthyroidism on testicular and epididymal histoarchitecture and steroidogenesis in male animals. *Journal of Zhejiang University SCIENCE B.* **17 (11)**: 850-863.

López V, et al. 2009. Neuroprotective and neurological properties of Melissa officinalis. *Neurochemical research.* **34 (11)**: 1955-1961.

Mallidis C, Agbaje I, O'Neill J & McClure N 2009. The influence of type 1 diabetes mellitus on spermatogenic gene expression. *Fertility and Sterility.* **92 (6)**: 2085-2087.

Mardanshahi T, Rezaei N, Zare Z, Malekzadeh Shafaroudi M & Mohammadi H 2019. Effects of L-Carnitine on the sperm parameters disorders, apoptosis of spermatogenic cells and testis histopathology in diabetic Rats. *International journal of reproductive bioMedicine.* **17 (5)**: 325-336.

Maresch CC, et al. 2017. Diabetes-induced hyperglycemia impairs male reproductive function: a systematic review. *Human reproduction update.* **24 (1)**: 86-105.

Mariarosaria L, et al. 2020. Impact of extraction processes on phytochemicals content and biological activity of Citrus × clementina Hort. Ex Tan. leaves: New opportunity for under-utilized food by-products. *Food research international.* **127**: 108742.

Miraj S, Rafieian K & Kiani S 2017. Melissa officinalis L: A Review Study With an Antioxidant Prospective. *Evidence-based complementary and alternative medicine.* **22 (3)**: 385-394.

[DOI: 10.18502/jnfs.v9i4.16888]

Moudi B, Heydari Z, Mahmoudzadeh Sagheb HR & Harati M 2007. Biochemical and Histological Study of Protective Effect of Sodium Tungstate on Oxidative Stress Induced by Streptozotocin in Pancreas of Diabetic Rats. *Anatomical sciences journal*. **5 (21)**: 0-0.

Moussa S 2008. Oxidative stress in diabetes Mellitus. *Romanian journal of biophysics.* **18**: 225-236.

Naghdi M, et al. 2016. Effects of Common Fig (Ficus carica) Leaf Extracts on Sperm Parameters and Testis of Mice Intoxicated with Formaldehyde. *Evidence-based complementary and alternative medicine.* **2016**: 2539127.

Naseri M, et al. 2021. The effect of Melissa officinalis L. extract on learning and memory: Involvement of hippocampal expression of nitric oxide synthase and brain-derived neurotrophic factor in diabetic rats. *Journal of ethnopharmacology*. **276**: 114210.

Naseri M, et al. 2016. Bloating: Avicenna's Perspective and Modern Medicine. *Evidence-based complementary and alternative medicine.* **21 (2)**: 154-159.

Negahdary M, Arefian Z, Dastjerdi HA & Ajdary M 2015. Toxic effects of Mn2O3 nanoparticles on rat testis and sex hormone. *Journal of natural science, biology and medicine.* **6 (2)**: 335-339.

Noguchi-Shinohara M, et al. 2023. Effects of Melissa officinalis Extract Containing Rosmarinic Acid on Cognition in Older Adults Without Dementia: A Randomized Controlled Trial. *Journal of Alzheimer's disease*. **91 (2)**: 805-814.

Noori Roshnavand F, Hojati V, Vaezi G & Rahbarian R 2019. The Comparison of Green Tea Aqueous Extract and Catechin Effect on Pituitary-Gonadal Axis in Rat Models of Type 1 Diabetes. *Journal of advances in medical and biomedical research.* **27 (125)**: 9-15.

Petrisor G, et al. 2022. Melissa officinalis: Composition, Pharmacological Effects and Derived Release Systems-A Review. *International journal of molecular sciences.* **23 (7)**.

Rains JL & Jain SK 2011. Oxidative stress, insulin signaling, and diabetes. *Free radical biology and medicine*. **50 (5)**: 567-575.

Sadat SS, et al. 2019. Effects of Carob Fruit Extract on Spermatogenesis, Antioxidant Status, and Apoptosis in Adult Male Mice. *Pharmaceutical sciences.* **25 (3)**: 184-189.

Sadeghi N, Boissonneault G, Tavalaee M & Nasr-Esfahani MH 2023. Oxidative versus reductive stress: a delicate balance for sperm integrity. *Systems biology in reproductive medicine*. **69 (1)**: 20-31.

Saylam B & Çayan S 2020. Do antioxidants improve serum sex hormones and total motile sperm count in idiopathic infertile men? *Turkish journal of urology*. **46 (6)**: 442-448.

Schoeller EL, Schon S & Moley KH 2012. The effects of type 1 diabetes on the hypothalamic, pituitary and testes axis. *Cell and tissue research* **349 (3)**: 839-847.

Seif MM KF, Abou Arab AAK, Abdel-Aziz AS, Abou Donia MA. and Mohamed Sh. R 2014. Protective effect of Melissa officinalis L. against malathion toxicity and reproductive in male rats. *International scholarly and scientific research & innovation* **8**:931-936.

Sheikhpour R 2013. Diabetes and Oxidative Stress: The Mechanism and Action. *Iranian journal of diabetes and obesity*. **5 (1)**: 40-45.

Shin Y, et al. 2021. The herbal extract ALS-L1023 from Melissa officinalis reduces weight gain, elevated glucose levels and β-cell loss in Otsuka Long-Evans Tokushima fatty rats. *Journal of ethnopharmacol*. **264**: 113360.

Singh S, et al. 2006. Effects of streptozotocin-diabetes and insulin replacement on androgen and estrogen receptor concentrations in the epididymis of Wistar rats.

Standl E, Khunti K, Hansen TB & Schnell O 2019. The global epidemics of diabetes in the 21st century: Current situation and perspectives. *European journal of preventive cardiology*. **26 (2_suppl)**: 7-14.

Tang XY, et al. 2008. Effects of strontium fructose 1,6-diphosphate on expression of apoptosis-related genes and oxidative stress in testes of diabetic rats. *International journal of urology*. **15 (3)**: 251-256.

Uropean medicines agency 2013. Assessment

[DOI: 10.18502/jnfs.v9i4.16888]

report on Melissa officinalis L. folium.

Verma L, et al. 2010. Effect of ethanolic extract of Cassia occidentalis Linn. for the management of alloxan-induced diabetic rats. *Pharmacognosy research.* **2 (3)**: 132-137.

Weidner C, et al. 2014. Lemon balm extract causes potent antihyperglycemic and antihyperlipidemic effects in insulin-resistant obese mice. *Molecular nutrition & food research.* **58 (4)**: 903-907.

Yamasaki K, et al. 2022. Effects of antioxidant co-supplementation therapy on spermatogenesis dysfunction in relation to the basal oxidation-reduction potential levels in spermatozoa: A pilot study. *Reproductive medicine and biology.* **21 (1)**: e12450.

Zhong O, Ji L, Wang J, Lei X & Huang H 2021. Association of diabetes and obesity with sperm parameters and testosterone levels: a meta-analysis. *Diabetology & metabolic syndrome.* **13 (1)**: 109.

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