

## Effects of Parsley (*Petroselinum Crispum*) Hydroalcoholic Extract on Spermatogenesis and Pituitary- Gonadal Axis in Streptozotocin-Induced Diabetic Male Rat

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### Abstract

**BACKGROUND:** Diabetes mellitus (DM), as a metabolic disease, has a high rate of mortality all over the world. In recent years, there is accumulating evidence that show this complex multifactorial disease has various effects on reproductive system.

**OBJECTIVES:** This study aimed to investigate the effect of hydroalcoholic extract of parsley (*Petroselinum crispum*) leaves on spermatogenesis and pituitary-gonadal axis in male streptozotocin (STZ)-induced diabetic rats.

**METHODS:** In the present study, 60 male rats were divided into 5 groups of 12 animals in each: control, DM control and 3 experimental groups (treated with doses 1, 2, and 4 gr/kg of hydroalcoholic extract of parsley leaves gavage for 28 days). DM was induced 48 h after intraperitoneal injection of a single dose of STZ (65 mg/kg). At the end of the treatment, rats were bled from the heart and follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone levels were measured. The testes of rats were isolated and weighed. Assessment of the sperm motility and evaluation of the seminiferous cells were performed using light microscopy.

**RESULTS:** Comparison of LH, FSH and testosterone in rats treated with doses 1 mg/kg of hydroalcoholic extract of the plant revealed a significant increase as compared to the diabetic control group ( $P \leq 0.05$ ). In addition, the results of histopathological evaluation elucidated a significant increase in the sperm cells number, and motility, testis weight, and total sperm count. It also improved condition of the seminiferous cells in the experimental group (1 mg/kg) compared to the diabetic control group ( $P \leq 0.001$ ).

**CONCLUSIONS:** It seems that parsley (*P. crispum*) hydroalcoholic extract can be effective in decreasing reproductive disorders in DM patients.

**KEYWORDS:** Diabetes mellitus, Parsley medicinal plant, Rat, Reproductive system, Sex hormone, Spermatogenesis

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## Introduction

Diabetes mellitus (DM), as a prominent public health concern, is described by hyperglycemia resulting from defects in insulin secretion, insulin action, or both of them (Ramezankhani *et al.*, 2018; Aghadavoud *et al.*, 2017; Goodarzi, *et al.*, 2016). This complex multifactorial disease has a high rate of mortality all over the world (Aqeel, 2018). Based on the prevalence of DM, it is estimated that the number of people with DM will have increased from 285 million in 2010 to 645 million by 2040 (Azarbani *et al.*, 2014).

Generally, DM is classified into three groups: type I diabetes (T1D), type II diabetes (T2D), and gestational diabetes mellitus (GDM), which among them T2D is the most prevalent form of the DM (approximately 90% of the diabetic patients) (Barter *et al.*, 2010; Vergès, 2014; Chen *et al.*, 2015). Also, T2D is started with insulin resistance. For this condition, insulin secretion from  $\beta$  cells increases, which in chronic cases reduces the mass of  $\beta$  cells. Thus, T2D is a combination of insulin resistance and insulin deficiency conditions (Cheraghi *et al.*, 2016; Elrokh *et al.*, 2010;).

In the latest research on DM, there is emerging evidence demonstrating DM could induce long-term damages, dysfunctions, and failures of various organs, including retinopathy, nephropathy, neuropathy, ulcers, amputations, genitourinary, cardiovascular complications, and sexual dysfunction (Ghasemipour *et al.*, 2007). Of these, the effect of DM on the reproductive system has received remarkable attention, as new evidence revealed a strong correlation between DM and the reproductive system (Golomb and Evans 2008).

It was reported that 27% of diabetic females and 22% of diabetic males displayed sexual dysfunction (Hillstrom *et al.*, 2003). Moreover, Rutte and colleagues found that sexual dysfunction is highly prevalent in males and females with T2D. Also, they observed that sexual dysfunction is correlated with higher age, clinical depression, and DM-related complications (Rutte *et al.*, 2014). Consequently, a critical complication of DM is the disturbance in the reproductive system.

One of the most critical metabolic pathways in spermatogenesis is glucose metabolism. Different types of DM could have harmful effects on fertility, especially on sperm quality, such as sperm motility and DNA integrity, and ingredients of the seminal plasma. Regarding the crucial role of insulin in the pathogenesis of DM, new evidence claimed that Sertoli cells of the testis that secrete insulin. Diabetic subfertility, are the result of deficiency in pancreatic insulin, testicular insulin, or both of them (Schoeller *et al.*, 2012). On the other hand, spermatogenesis as a complex sequence of events during maturation of spermatogonia into spermatozoa, implicates differential gene expression and cell-cell interplay regulated by the follicle-stimulating hormone (FSH) and luteinizing hormone (LH)-stimulated testosterone (Lee *et al.*, 2003). In this way, a study investigated the induction of oxidative damage during the early diabetic phase in testis and epididymal sperm in STZ-induced diabetic rats. It was found that oxidative stress mechanisms play a vital role in developing testicular dysfunction and degeneration under situations of experimentally induced DM in animal models (Matos *et al.*, 2005). Additionally, in T1D, the function of the Leydig cells and testosterone production was reduced due to the lack of stimulatory impact of insulin on these cells, as well as an insulin-dependent reduction in the levels of FSH and LH (Millar *et al.*, 2017).

For a long time, plants have possessed a vital role in the broad-spectrum treatment of diseases. Today, new evidence has attracted our attention to the pivotal role of medicinal plants in reducing diabetic complications and increasing the life span and quality in these patients (Montero-Bullon *et al.*, 2019; Mohsenipour and Hassanshahian, 2015; Nazni *et al.*, 2006). Parsley (*Petroselinum crispum*), as a member of the *Apiaceae* family, is a medicinal plant with various pharmacological features, including treating sexual dysfunctions, antioxidant, hepatoprotective, brain-protective, anti-diabetic, analgesic, spasmolytic, immunosuppressant, anti-platelet aggregation, gastroprotective, cytoprotective, laxative, estrogenic, diuretic, hypotensive, antibacterial and antifungal activities (Nelson, 2013). Parsley is regarded as a main source of flavonoids (apiin,

luteolin), carotenoids, ascorbic acid, tocopherol, volatile compounds (myristicin, apiole), coumarins (bergapten, imperatorin), phthalides, furanocoumarins, and sesquiter

enes (Ozsoy-sacan *et al.*, 2006).

Jalili *et al.*, reported that the application of hydroalcoholic extract of *P. crispum* can affect reproductive parameters such as weight of testis and prostate and sperm motility (Jalili *et al.*, 2015). Besides, another research has been demonstrated that parsley led to the FSH, LH, and testosterone levels elevation (Ramachandran and Wierzbicki, 2017). This study was conducted to elucidate whether the hydroalcoholic extract of parsley leaves affects spermatogenesis and pituitary-gonadal axis in male STZ-induced DM rats. For this purpose, we detected LH, FSH, and testosterone hormones and evaluated seminiferous cells in male STZ-induced DM rats treated with doses of hydroalcoholic extract of parsley.

## Materials and Methods

### Preparation of Parsley (*Petroselinum crispum*) Hydroalcoholic Extract

The *P. crispum* plant was collected from the agricultural lands of Ilam region (Iran) in the summer and recognized by the Department of Plant Medicine, School of Agriculture (Identity Number: No9070). Afterward, the plant was dried in the shade, grinded in distilled water at 25°C, and powdered. Then, the extraction process was performed as follows: The plant was added to 70% ethanol (200 mL). The obtained solution was placed in the 35°C hot water bath in darkness. The extract was then filtered, and the filtrate was evaporated using a rotary evaporator under reduced pressure to dryness. Finally, 3.42 gr of the dried extract was dissolved in normal saline and distilled water and stored at -20°C (Rouhi-Borojeni *et al.*, 2015).

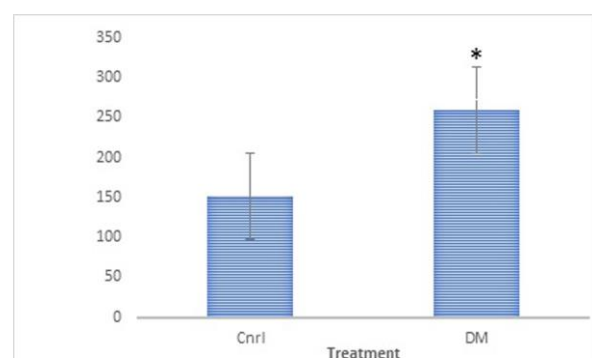
### Experimental Design

Sixty male Wistar albino rats with the weight range of 250-300 gr were purchased from Pasteur Institute of Iran. All Wistar albino rats were clinically healthy. The animals were kept in a clean cage under controlled laboratory conditions (25±2°C) and humidity (50%) with a 12/12 h light/dark cycle with free access to sufficient food and water. After 7 days

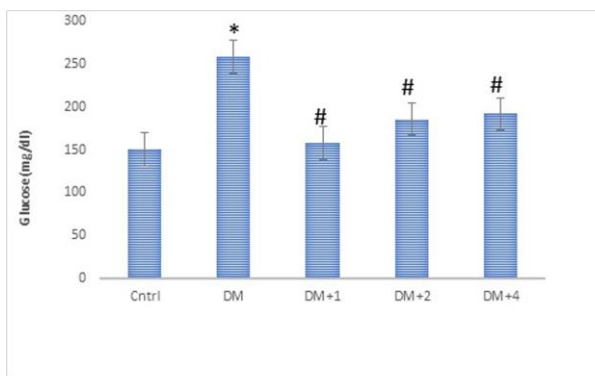
of acclimatization, animals were exposed to DM induction. STZ (Sigma Aldrich, MO, USA) was freshly prepared by dissolving in 0.1 M citrate buffer, pH 4.5. After overnight fasting, the rats in all groups except the control group were injected with a single intraperitoneal dose of STZ (65 mg/kg body weight) for the induction of diabetes. The rats were then randomly divided into 5 groups (n=12) as follows: Group I: control receiving 0.2 mL, po (orally), Group II: positive control (diabetic) administered normal saline (0.2 mL, po), and Experimental Groups (III, IV and V); diabetic groups treated with parsley extract (1, 2, and 4 gr/kg, po) by gavage, respectively.

The normal saline and plant extracts were administered orally through gavage (0.2 mL) for 28 days (at 9 a.m. every morning) in the control group and diabetic rats, respectively.

The experiments were performed in 3 steps; first, diabetic animals were separated from the healthy group (48 h after injection of STZ). Then, DM was confirmed by elevated blood glucose levels. Only rats with glucose level 220 mg/dL (Figure 1) were included in the study (Rena *et al.*, 2013). The approved diabetic rats were divided into 4 treatment groups (one diabetic control group and 3 experimental groups that were administered different concentrations of the plant extracts) (Figure 1). In the second step, blood glucose levels were compared among experimental groups (Figure 2). In the final step, the main experiment was performed, which lasted 28 days.



**Figure 1.** Comparison of the blood glucose level of diabetic rats with the healthy control group. Values are means± SEM.



**Figure 2.** Comparison of the effect of hydroalcoholic extract of parsley leaf on blood glucose in male rats in treatment groups. \* Significant level difference ( $P \leq 0.01$ ) between diabetic control group and healthy control group # Significant difference in level ( $P \leq 0.001$ ) between diabetic groups receiving the extract compared to diabetic control. Values are means  $\pm$  SEM.

A significant difference in level ( $P \leq 0.01$ ) between the diabetic group and healthy control group. Values are means  $\pm$  SEM.

Finally, the rats were euthanized using ether-soaked cotton placed in a desiccator. Then blood collection was performed quickly by cardiac puncture using needle gauge 21.

### Sample Preparation and Analysis

All the blood samples were centrifuged at 4000 g for 5 min. Then, the hormone levels (FSH, LH, and testosterone) were quantified in the serum samples by enzyme-linked immunosorbent assay (ELISA) (Diaplus, Inc., North York, Ontario, Canada) based on the manufacturer's instructions and expressed as ng/mL.

The left testes were separated and weighed, and preserved in 10% neutral buffered formalin. To prepare a solution containing epididymal sperm, the tail of the left epididymis tissue of the animals was crushed in a 5 ml normal saline. The plates were then incubated for 10 min at 37°C.

### Assessment of the Sperms' Features

The cauda epididymis was isolated and segmented in DMEM/F12 medium containing 5% FBS and incubated at 37°C and 5% CO<sub>2</sub>. The gained suspension was used for the sperm motility analysis. The spermatogonia, spermatocytes, spermatids, and

spermatozooids were counted. The epididymal sperms were counted using Neubauer's chamber and optic microscope (magnification 400X). To calculate the percentage of sperm motility, 10  $\mu$ L of the sperm-containing solution was placed on the slide, and then the motility of the sperm was counted in several levels. To evaluate the seminiferous tubules, histopathological analysis was performed on one of the testes of each rat. Clarification steps were performed by passing alcohol with ascending degrees, and xylene, respectively. The tissues were then embedded in paraffin and cut into 5  $\mu$ m microscopic sections, and processed by the conventional method of hematoxylin and eosin (H&E) staining.

### Statistical Analysis

The results were expressed as the mean  $\pm$  standard error of means (SEM). In addition, differences between 5 groups were applied by one-way analysis of variance (ANOVA) and followed by the Tukey-Kramer post-hoc test. Data were statistically analyzed by the SPSS software version 25 (SPSS Inc., Chicago, Ill., USA). P-values less than 0.05 were considered significant.

## Results

### Biochemical Parameters

The healthy male rats (n=12) were compared with male rats injected with STZ (n=48) following 48 h of STZ injection, indicating a significant increase in blood glucose levels of diabetic rats compared to the healthy rats. Diabetic rats that received doses of 1, 2, and 4 gr/kg of hydroalcoholic extract of the parsley leaves showed significant decrements in the level of glucose compared to the diabetic rats, which indicates the effect of these three different doses of parsley plant extract in lowering blood glucose (Figures 1 and 2).

The levels of LH, FSH, and testosterone were detected in DM groups treated with three doses of parsley extract. Our results revealed that, after treatment with 1 mg/kg parsley extract, the LH, FSH, and testosterone increased significantly compared to the diabetic control group ( $P \leq 0.001$ ). Moreover, in 2 mg/kg and 4 mg/kg extracts groups, a significant elevation was found in all three hormones compared to the diabetic control group ( $P \leq 0.05$ ) (Figure 3). Therefore, hydroalcoholic parsley extract in 1 mg/kg concentration was considered as the most economic

**Table 1.** Effects of different parsley extract on the weight of testes and percent of sperm motility in control and diabetic rats

	Control Mean±SD	Diabetic Mean±SD	Diabetic +1g parsley Mean±SD	Diabetic +2g parsley Mean±SD	Diabetic +4g parsley Mean±SD
<b>Weight of testis</b>	1.82±0.31 <sup>a</sup>	1.05±0.38 <sup>b</sup>	1.62±0.36 <sup>a</sup>	1.12±0.21 <sup>b</sup>	1.09±0.19 <sup>b</sup>
<b>Sperm motility</b>	22.1±1.9 <sup>a</sup>	11.4±1.8 <sup>b</sup>	21.9±4.3 <sup>a</sup>	13.5±2.84 <sup>b</sup>	12.6±2.83 <sup>b</sup>

Different letters (a and b) indicate significant differences between treatments ( $P \leq 0.001$ ).

\* Significant difference between diabetic groups receiving the extract compared to diabetic control

\*\* Significant difference between the diabetic group to control. Values are means± SEM.

and effective treatment on LH, FSH and testosterone than other concentrations (As shown in Table 1).

### The Effect of Parsley Extract on Testes Weight and Sperm Motility

The obtained data demonstrated that in the diabetic group that received parsley extract in 1 mg/kg, the weight of the left testis was significantly more compared to the diabetic control group ( $P \leq 0.001$ ). Meanwhile, after treatment with parsley extract in 2

and 4 mg/kg, the weight of the left testis showed an increase as compared to the diabetic control group ( $P \leq 0.05$ ). In sum, hydroalcoholic parsley extract in 1 mg/kg showed more positive effects on testis weight (Table 1).

Furthermore, as presented in Table 2, the mean of sperm motility increased significantly in all diabetic + parsley extract groups compared to the diabetic control group. But this increment was higher in the dose of 1 mg/kg than other doses ( $P \leq 0.001$ ).

**Table 2.** Effects of different parsley extract on spermatogonia, spermatocyte, spermatid, and spermatozooid counts in control and diabetic rats

	Control Mean±SD	Diabetic Mean±SD	Diabetic +1g parsley Mean±SD	Diabetic +2g parsley Mean±SD	Diabetic +4g parsley Mean±SD
<b>Spermatogonia</b>	39.5±5.3 <sup>a</sup>	22.6±5.08 <sup>b</sup>	38.6±1.75 <sup>a</sup>	27.4±4.14 <sup>b</sup>	<b>24.5±4.8<sup>b</sup></b>
<b>Spermatocyte</b>	50.7±2.8 <sup>a</sup>	31.1±6.01 <sup>c</sup>	41±2.28 <sup>b</sup>	37.1±6.2 <sup>c</sup>	<b>30.9±5.02<sup>c</sup></b>
<b>Spermatid</b>	40.6±5.8 <sup>a</sup>	16.2±3.5 <sup>d</sup>	39.9±1.77 <sup>a</sup>	22.3±6.1 <sup>c</sup>	<b>19±3.8<sup>d</sup></b>
<b>Spermatozoide</b>	24.9±1.9 <sup>a</sup>	14.9±2.08 <sup>b</sup>	23.4±4.21 <sup>a</sup>	16.7±3.9 <sup>b</sup>	<b>15.4±2.21<sup>b</sup></b>

Different letters (a, b, c, and d) indicate significant differences between treatments ( $P \leq 0.001$ ).

\* Significant difference between diabetic groups receiving the extract compared to diabetic control

\*\* Significant difference between the diabetic group to control

Values are means± SEM.

### Parsley Extract Effect on the Count of Spermatogonia, Spermatocytes, Spermatids, and Spermatozoides

Table 2 shows that the mean spermatogonia number increased significantly in all three groups treated with parsley extract. But this increase was more profound in 1 mg/kg dose than the other concentrations ( $P \leq 0.001$ ). In addition, the value of spermatocyte numbers was significantly increased in the diabetic

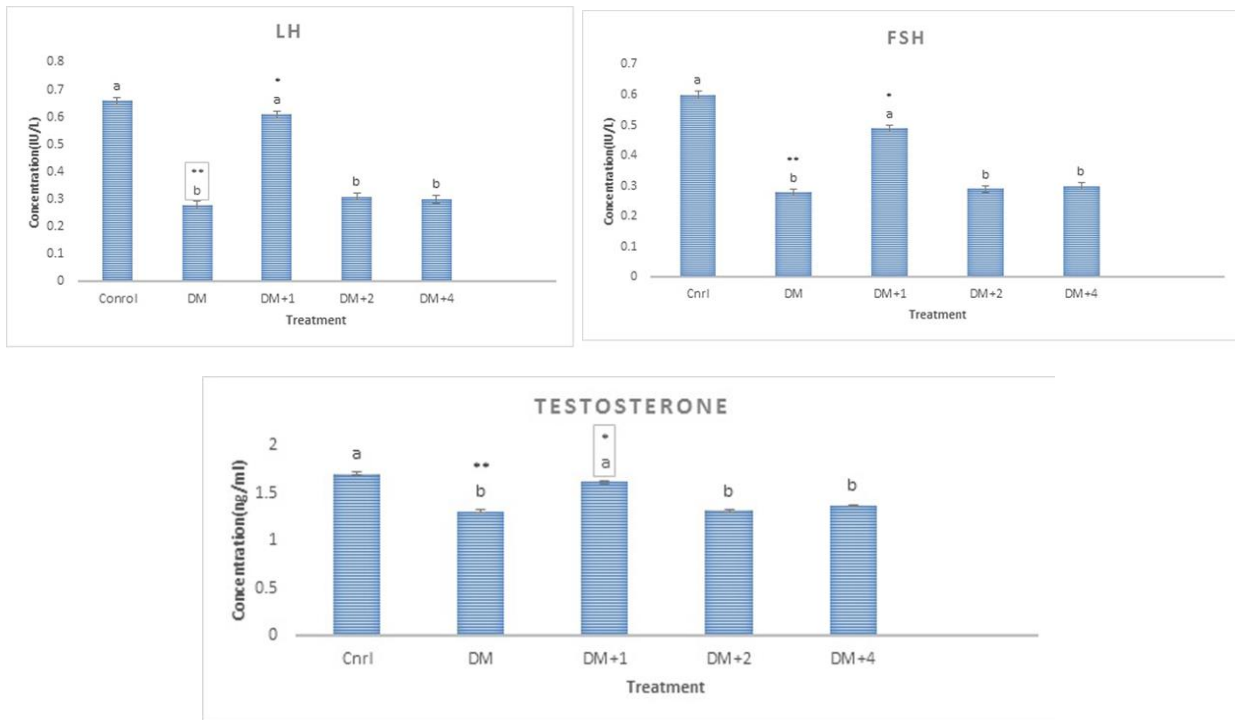
+ parsley extract group (1 mg/kg) compared to the diabetic control group ( $P \leq 0.05$ ). Furthermore, the means of spermatid and spermatozoid counts were significantly influenced by parsley extract (1 mg/kg) compared to the diabetic control group ( $P \leq 0.001$ ).

### Histopathological Findings

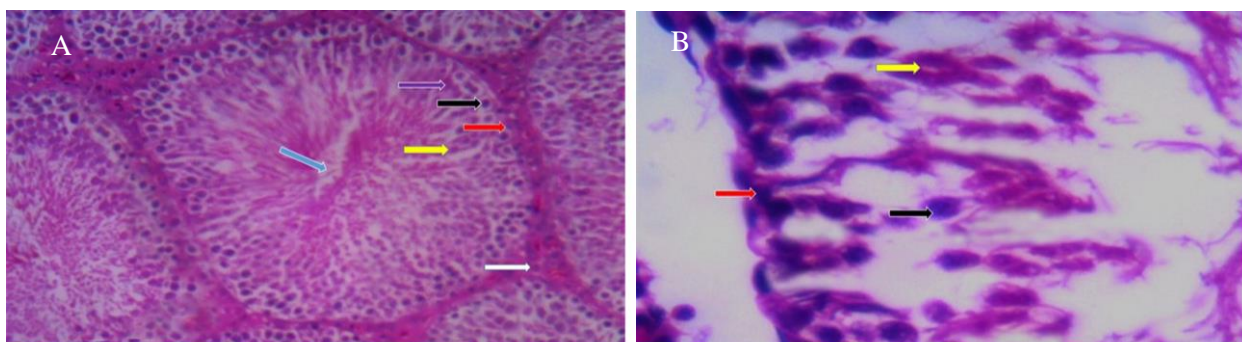
Histopathological examinations of the testicular tissue revealed that diabetic rats showed severe disruption on the order and arrangement of the cells.

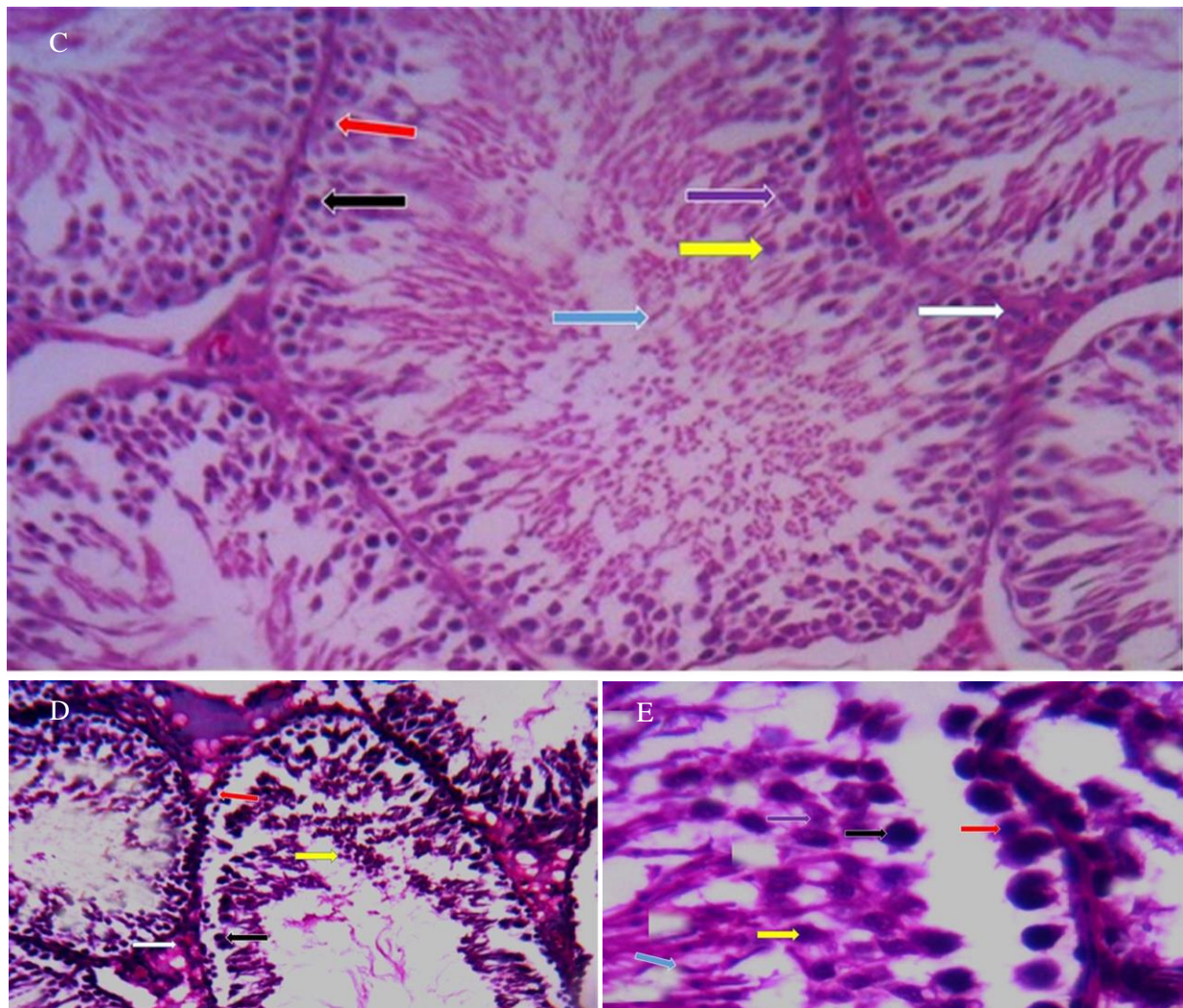
The creation of large empty spaces between different classes of cells and the process of spermatogenesis is impaired, whereas the diabetic group receiving a dose of 1 mg/kg of parsley showed fewer changes in seminiferous tubule cells compared to the diabetic rats, and the plant extract seems to have a relatively

favorable effect on these changes in diabetic rats. Thus, a partial and relative disturbance in the order and arrangement of the cells was observed compared to the healthy group. The process of spermatogenesis in the diabetic group that received extract (group 3) was almost complete (Figure 4).



**Figure 3.** Effects of different parsley extract on serum levels of LH(A), FSH(B), and Testosterone(C) hormones in control and diabetic rats. Values are means±SEM. A. LH hormone: \* Significant difference between diabetic groups receiving the extract compared to diabetic control. \*\* Significant difference between the diabetic group to control. Different letters (a and b) indicate significant differences between treatments ( $P \leq 0.001$ ). B. FSH hormone: \* Significant difference between diabetic groups receiving the extract compared to diabetic control. \*\* Significant difference between the diabetic group to control. Different letters (a and b) indicate significant differences between treatments ( $P \leq 0.001$ ). C. Testosterone hormone: \* Significant difference between diabetic groups receiving the extract compared to diabetic control. \*\* Significant difference between the diabetic group to control. Different letters (a and b) indicate significant differences between treatments ( $P \leq 0.001$ ).





**Figure 4.** Representative photograph of testicular seminiferous tubules in rats stained with H&E (×400).

A. Control group (healthy); The process of spermatogenesis is normal. (Red arrow), primary spermatocytes (black arrow), primary spermatid (yellow arrow), final spermatid (blue arrow), Sertoli cell (purple arrow), Leydig cell (white arrow). B. Diabetic group; this image shows the severe disruption of the order and arrangement of the cells. The creation of a large empty space between different classes of cells and the deformation of different cells. The process of spermatogenesis is impaired. Spermatogonia (red arrow), primary spermatocyte (black arrow), primary spermatid (yellow arrow). C. Diabetic group receiving a dose of parsley (1g/kg). A partial and relative disturbance of the order and arrangement of the cells relative to the healthy group, the creation of a large empty space between different classes of cells. The process of spermatogenesis is almost complete. Spermatogonia (red arrow), primary spermatocytes (black arrow), primary spermatids (yellow arrow), final spermatids (blue arrow), Sertoli cell (purple arrow), Leydig cell (white arrow). D. Diabetic group receiving 2 grams of parsley plant extract; In this figure, severe disruption of the order and arrangement of the cells, creating a large empty space between different classes of cells, lack of development of germ cells and the process of spermatogenesis is disrupted and incomplete. Spermatogonia cells are visible. Primary spermatocytes (black arrow), primary spermatid (yellow arrow), Leydig cell (white arrow). E. Diabetic group receiving a dose of 4 grams of parsley plant extract; Different classes of cells such as spermatogonia, primary spermatocytes, Sertoli cells, as well as primary and final spermatids have lost their natural form. As well as severe disruption of the order and arrangement of the cells, creating empty space between different classes of germ cells, impaired and defective spermatogenesis. Primary spermatocyte (black arrow), primary spermatid (yellow arrow), final spermatid (blue arrow), Sertoli cell (purple arrow).

## Discussion

Generally, there is emerging evidence showing DM can affect the function of the reproductive system. Indeed, male reproductive alterations were observed in DM subjects following usage of STZ, leading to a decrease in testosterone production. In addition, T1D and T2D could have harmful effects on sperm motility and DNA integrity (Ruel *et al.*, 2006). However, in recent years, the broad range of plant extracts have shown protective effects on different diseases, especially DM, and the attention of many researchers to these compounds is necessary to examine their critical role against diseases (Sarmarghandian *et al.*, 2016).

We noticed the evidence regarding the crucial role of medicinal plants in decreasing DM complications. Among medicinal plants, parsley (*P. crispum*) has shown therapeutic properties against sexual dysfunctions (Akrami *et al.*, 2014; Jalili *et al.*, 2015). To the best of our knowledge, this is the first study to determine the effect of parsley hydroalcoholic extract in different doses on spermatogenesis and pituitary-gonadal axis in male STZ-induced diabetic rats. Our main findings demonstrated that the levels of LH, FSH, and testosterone increased after treatment with parsley hydroalcoholic extract in 1 mg/kg dose as compared to the diabetic control group. In addition, our results showed a significant increase in the sperm cells number and motility, testis weight, and total sperm count in 1 mg/kg parsley extract group compared to the diabetic control group.

In accordance with our results, it was found that administration of hydroalcoholic extract of parsley significantly elevated the mean of sperm motility and testis weight in comparison with the control group. By contrast, no significant effect was reported for different doses of parsley extract on the sperm parameters (Tabatabaei-Malazy *et al.*, 2014). While in our experimental study, we observed that mean spermatogonia, spermatocytes, spermatids, and spermatozooids count was higher in diabetic + parsley extract groups (1 mg/kg) compared to the diabetic control group. Another study revealed that STZ-induced diabetes led to a decrease in fertility, prolificacy, and libido. Additionally, LH, FSH, and testosterone levels decreased significantly. A significant association was observed between insulin and

FSH levels. But no significant association was found between insulin/glucose and LH (Tsujimura *et al.*, 2003).

We showed that the diabetic control group had significantly lower levels of LH, FSH and testosterone than the normal group. Also, after treatment with parsley extract (1 mg/kg), the LH, FSH, and testosterone increased significantly compared to the diabetic control group.

Furthermore, it was elucidated that increasing dose of parsley in male rats taking lead acetate caused a reduction in the LH and FSH levels and an increase in the serum levels of testosterone. Meanwhile, the rats that received lead acetate with parsley extract with 200 mg/kg dose showed a significant difference in spermatogenesis. In fact, parsley with 200 mg/kg dose plays a key role in increasing the sperms numbers in the tube lumen (Khani *et al.*, 2017; Khani *et al.* 2018). In our experiment, parsley hydroalcoholic extract (1 mg/kg) affected spermatogenesis. Abdel-Wahhab *et al.*, showed that zearalenone-induced reproductive toxicity was protected by *Seudomonas crispum* oil. Also, *Seudomonas crispum* oil improved sperm motility, sperm count, and testosterone level significantly (Abdel-Wahhab *et al.*, 2006).

There is growing evidence that demonstrates free radicals cause the loss of epithelial cells, which can reduce sperm motility and sperm count (Aziz *et al.*, 2004; Kolarovic *et al.*, 2010). Moreover, there is a strong correlation between the development of DM and oxidative stress due to hyperglycemia and hyperlipidemia. On the other hand, as the parsley possess antioxidant properties; this medicinal plant can improve the sperm quality under diabetic condition by overexpression of antioxidant-related genes (Shi *et al.*, 2012, Mahmood *et al.*, 2014). In this way, it was revealed that *P. crispum* extract treatment caused to reduce the reactive oxygen species (ROS), which in turn leads to the increase in the viability of sperms (Shrilatha, 2007).

All of these available documents are compatible with our results. Therefore, this important point can be mentioned that hydroalcoholic extract of parsley with impact on the pituitary-gonadal axis may be



useful in improvement of diabetic reproductive complications. Since parsley has very low toxicity, the administration of 4 mg of its extract in male rats under treatment caused 15% deaths. However, no side effect has been reported regarding the administration of parsley extract.

## Conclusion

T2D is known as the most prevalent metabolic disease. This complex disorder is one of the main public health concerns. With respect to the effect of DM on the reproductive system, finding a way to improve the diabetic reproductive complications is of importance. Parsley (*P. crispum*) extract has been demonstrated to affect various body systems, especially the reproductive system. According to the results of the present study, the levels of LH, FSH, and testosterone increased after treatment with parsley (*P. crispum*) hydroalcoholic extract in 1 mg/kg dose as compared to the diabetic control group. Furthermore, a significant increase was found in the

sperm cells and motility, testis weight, and total sperm count in the hydroalcoholic extract of the parsley group (1 mg/kg) compared to the diabetic control group. Overall, it seems that parsley (*P. crispum*) hydroalcoholic extract can have a positive effect on diabetic reproductive complications. In sum, the results of this study highlight that the presence of natural chemical and antioxidant compounds in the hydroalcoholic extract of parsley leaves might protect animal tissues against free radical damage and improve sperm parameters and secretion of LH, FSH, and testosterone in animals.

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## Conflict of Interest

The authors declared no conflict of interests.

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## بررسی اثر عصاره هیدروالکلی گیاه جعفری بر اسپرمتوزن و محور هیپوفیز - گناد موش‌های نر دیابتی شده با استرپتوزوتوسین

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**زمینه مطالعه:** دیابت شیرین (DM)، به‌عنوان یک بیماری متابولیکی، میزان مرگ و میر بالایی در سراسر جهان دارد. در سال‌های اخیر، شواهد زیادی وجود دارد که نشان می‌دهد این بیماری چند عاملی پیچیده اثرات مختلفی روی سیستم تولید مثل دارد.

**هدف:** این مطالعه با هدف بررسی تأثیر عصاره هیدروالکلی برگ جعفری (*Petroselinum crispum*) بر اسپرمتوزن و محور هورمونی هیپوفیز - تخمدان موش صحرایی نر دیابتی شده با استرپتوزوتوسین انجام شد.

**روش کار:** در مطالعه حاضر، ۶۰ سر موش صحرایی نر به ۵ گروه ۱۲ تایی تقسیم شدند: شاهد، شاهد دیابتی و ۳ گروه آزمایشی با دریافت دوزهای ۱، ۲ و ۴ گرم به ازای کیلوگرم وزن بدن عصاره هیدروالکلی برگ جعفری به روش گاوآژ به مدت ۲۸ روز دریافت کردند. ۴۸ ساعت پس از تزریق داخل صفاقی یک دوز واحد از استرپتوزوتوسین (۶۵ میلی‌گرم در کیلوگرم) دیابت القا شد. در پایان دروه تیماری، از قلب رت‌ها خونگیری انجام شده و غلظت هورمون محرک فولیکول (FSH)، هورمون لوتئینی (LH) و تستوسترون اندازه‌گیری شد. جدا سازی و توزین بیضه‌ها و ارزیابی درصد تحرک و مطالعه سلولی لوله‌های اسپرم‌ساز با استفاده از میکروسکوپ نوری انجام شد.

**نتایج:** مقایسه میزان FSH، LH و تستوسترون در موش‌های صحرایی تحت درمان با دوز ۱ mg/kg عصاره هیدروالکلی گیاه جعفری، افزایش معنی‌داری را نسبت به گروه کنترل دیابتی نشان داد ( $P \leq 0.05$ ). علاوه بر این، نتایج بافت‌شناسی نشان داد که گروه آزمایش ۱ mg/kg افزایش قابل توجهی در شمارش اسپرم و تحرک اسپرم، وزن بیضه و همچنین وضعیت سلول‌های موجود در لوله‌های اسپرم‌ساز نسبت به کنترل دیابتی را بهبود بخشید ( $P \leq 0.01$ ).

**نتیجه‌گیری نهایی:** به نظر می‌رسد عصاره هیدروالکلی جعفری (*Petroselinum crispum*) احتمالاً در کاهش اختلالات تولید مثل در بیماران مبتلا به DM موثر باشد.

**واژه‌های کلیدی:** اسپرمتوزن، دیابت شیرین، گیاه دارویی جعفری، موش صحرایی، هورمون جنسی