Journal of Advances in Medical and Biomedical Research | ISSN:2676-6264

Protective Effects of *Cuscuta epithymum* Extract on Reproductive Health in a Pre-Pregnancy Stress Rat Models

Narjes Khavasi[1], Ehsan Saboory [2,6], Zahra Najmi [3,4], Leila Ghassemifard [1], Amir Mohammad Ghanbari [5], Saeed Sardari [1*]

1. *Department of Persian Medicine, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran*
2. *Department of Addiction Studies, School of Medicine, Zanjan University of Medical Science, Zanjan, Iran*
3. *Department of Obstetrics and Gynecology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran*
4. *Department of Clinical and Translational Science, Tufts University, Boston, MA, USA*
5. *School of Medicine, Zanjan University of Medical Science, Zanjan, Iran*
6. *Zanjan metabolic diseases research center, Health and metabolic diseases research institute, Zanjan University of medical sciences, Zanjan, Iran.*

Article Info

10.30699/jambr.34.2.98

Received: 2025/10/25;
Accepted: 2026/03/17;
Published Online: 2026/05/20

***Corresponding author:**
Saeed Sardari,
Department of Persian Medicine, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran

Email: ssardari504@zums.ac.ir

ABSTRACT

Background & Objective: Presentational stress attenuates circulating levels of sex hormones and potentially affects reproductive function. This study investigated the effects of simultaneous administration of *Cuscuta epithymum* (CE) extract on stress-related reproductive parameters in parental Wistar rats and on the serum sex hormone levels of their offspring.

Materials & Methods: In this experiment, 64 female rats and 36 male Wistar rats were divided into various categories: Control (no stress or CE extract), CE Extract (received only the extract), Stress (exposed only to stress), Stress + CE Extract (exposed to both stress and received the extract). Parental reproductive parameters were assessed across groups, including vaginal epithelial thickness and vaginal pH in female rats, sperm parameters in males, and circulating sex hormone levels in both sexes. Offspring were evaluated for sex ratio, litter size, and serum sex hormone levels in all groups.

Results: Presentational stress significantly reduced sperm parameters in adult males and decreased the thickness of the vaginal epithelium in adult females ($P< 0.01$). However, co-administration of *Cuscuta epithymum* extract in the stress-exposed groups significantly improved these parameters compared with the stress group and restored them toward control levels. Additionally, stress exposure led to a significant reduction in the number of male offspring per litter ($P < 0.01$), an effect that was significantly mitigated by CE extract administration ($P < 0.01$).

Conclusion: Concomitant administration of *Cuscuta epithymum* extract attenuated the adverse effects of stress on parental reproductive parameters, offspring sex ratio, litter size, and serum sex hormone levels. These findings suggest that CE extract may serve as a promising natural supplement for preserving reproductive function under stress before conception.

Keywords: *Cuscuta epithymum*, Reproductive health, Pre-pregnancy stress, Rat model, Herbal extract, Oxidative stress, Fertility, Persian Medicine

Copyright © 2026, This is an original open-access article distributed under the terms of the Creative Commons Attribution-noncommercial 4.0 International License which permits copy and redistribution of the material just in noncommercial usages with proper citation.

How to Cite This Article:

Khavasi N, Saboory E, Najmi Z, Ghassemifard L, Ghanbari A M, Sardari S. Protective Effects of Cuscuta epithymum Extract on Reproductive Health in a Pre-Pregnancy Stress Rat Models. J Adv Med Biomed Res 2026; 34(2):98-109.

1. Introduction

A state of stress occurs when the body experiences a negative stimulus that disrupts its natural balance (1). The likelihood of infertility increases significantly as a result of prolonged stress, which has pronounced effects on fertility (2, 3).

Stressful conditions can decrease the quantity, mobility, and progressive movement of sperm by adversely affecting the epididymis and inhibiting spermatogenesis (4, 5). Moreover, a reduction in the efficiency and quantity of Y sperm, along with a change in the acidity of the vaginal milieu, leads to a higher likelihood of delivering a female child (6), as well as causing shifts in the plasma concentration of testosterone (7) and estradiol (5, 8).

On the other hand, pre-pregnancy stress can affect the fetus and have harmful effects such as, alterations in sex hormone levels in male and female offspring. When an individual experiences stress, the hypothalamic-pituitary-adrenal (HPA) axis becomes activated. The sexual behavior of mice is impacted by immobility stress, a form of stress that combines physical and psychological factors, as it triggers the HPA axis (3, 9).

While a range of pharmaceutical and synthetic agents are routinely employed to manage stress, their use is frequently associated with adverse side effects. Consequently, there is an increasing emphasis on investigating medicinal plants and their derivatives as potentially safer and more tolerable alternatives (10).

One such plant, widely studied and utilized in Persian medicine to alleviate reproductive disorders and stress-related complications, is *Cuscuta epithymum*. Recent studies have reported significant roles of *Cuscuta epithymum* in decreasing the adverse effects of stress on the liver and spleen immune system, as well as remarkable anti-neuroinflammatory and anti-seizure effects in rats (11,12). The genus *Cuscuta epithymum* belongs to the Convolvulaceae family (Note: *Cuscutaceae* is now often merged with *Convolvulaceae*) and is distributed worldwide, comprising approximately 170 species. In Iran, 18 species of *Cuscuta epithymum* have been identified across various regions (13). *Cuscuta epithymum* is rich in phenolic compounds and flavonoids, including cuscutin, amarbelin, β-sitosterol, stigmasterol, myricetin, quercetin, cuscutamine, luteolin, and bergenin, which contribute to its antioxidant properties (14, 15). In Persian medicine, *Cuscuta epithymum* is prescribed for the treatment of excessive coldness and blood accumulation in the reproductive systems of males and females, support spermatogenesis, and for the treatment of infertility in women. *Cuscuta epithymum* is also used to prevent infertility (16, 17). Hence, taking into account the impact of stress on parental fertility and circulating sex hormone levels, along with stress effects before conception on hormonal shifts in their offspring, and considering the reported effects of *Cuscuta epithymum* species on stress and reproductive function in both sexes this study was designed to evaluate the potential protective role of *Cuscuta epithymum* extract in a rat model of stress. This study aimed to assess CE's influence on parental reproductive parameters, including vaginal epithelial thickness and vaginal pH in female rats, sperm parameters in male rats, and serum sex hormone levels in both sexes. Furthermore, offspring were assessed for sex ratio, litter size, and sex hormone levels across different groups.

2. Materials and Methods

2.1 Subjects

Sixty-four adult female and 36 male Wistar rats (180–200 g) were obtained from the Pasteur Institute, Tehran, Iran (Rats had not mated before the start of the experiment). They were maintained under standard conditions in a 12-hour light/dark cycle, 22±2 °C, relative humidity of 45–55%, pathogen-free, food and water ad libitum in the animal house at Zanjan University of Medical Sciences, Zanjan, Iran. All experiments followed the guidelines of the Medical Ethics Committee of the Iranian Ministry of Health (IR.ZUMS.REC.1399.419). The sexual cycle of female rats was equalized by being placed next to sterilized males. Subsequently, male and female subjects were divided into four groups: 1. Control (no exposure to stress or CE extract), 2. CE Extract (only received the extract), 3. Stress (only underwent stress), 4. Stress + CE Extract (exposed to both stress and the extract), as illustrated in the study diagram. Males experienced immobility stress for 2 hours each day over a period of 50 consecutive days (the duration needed for a full spermatogenesis cycle), while females were subjected to stress for 15 days (encompassing three estrous cycles). During this period, the appropriate groups were given oral doses of 100 mg/kg of CE extract. (18). The vaginal pH of female rats was also measured, and a biopsy of their vaginas was taken to assess the thickness of the vaginal epithelium. Semen samples from male rats was collected via the epididymis to assess sperm parameters. The blood of male and female rats was collected by heart puncture to investigate sex hormone levels in the serum. After the end of the stress phase, the rats entered the mating phase. The Trichus method (2 females and one male) was used for mating (all the female rats were mated on day 51 with males). The rats that were placed in a cage for mating are as follows: McFc, McFc+Ex, MsFs, MsFs+Ex (M: male, F: female, C: control, S: stress, Ex: extract). Reproductive

indices in pregnant rats after delivery (offspring sex ratio and litter size) were investigated in all groups. After the pups grew up on postnatal day (PND) 25, a male and a female pup from each mother in all groups were selected for hormonal examination.

2.2 Preparing extract

The percolation technique was employed for obtaining the extraction of the *CE* extract in this experimental study (herbarium code: 5501). The Cuscuta epithymum plant was crushed and then subjected to two standard sieves (160 and 250 micrometers) for proper sanitation. Afterwards, the 1500 g powder was transferred to the percolator, where 70% ethanol was used as the solvent.

The dark color and pungent odor indicated the purity of the *CE* extract. The solvent was separated using a Rotary Evaporator (IKA RV 05-B, Germany) at 45 to 50 °C, then subjected to vacuum drying at 45 °C (53 OT, Iran). The drying procedure persisted until a stable weight was achieved. This method was repeated three times at 24-hour intervals. After filtering the extract, concentration was performed in a water bath under vacuum conditions, followed by lyophilization (FD-5N; Eyela, Tokyo). Ultimately, the product was preserved at 4 degrees Celsius. **(19, 20)**.

2.3 Restraint stress procedure

A period of immobility stress was experienced by rats, as described by Nakhjiri et al. (21). Briefly, this process included moving the rats to the designated testing area. They were housed in a restraining device under standard room conditions. Each day, the rats were placed within the confinement chamber (a cylindrical plastic container measuring 16 cm in length and 6 cm in width) for a duration of 2 hours **(12)**.

2.4 Sperm analysis

Male rats (n = 8 in each group) were anesthetized with isoflurane on the first morning following the completion of the stress period. One epididymis was randomly selected for evaluation of sperm parameters. The caudal part of the epididymis was dissected, then floated in 5 ml of pre-heated Ham's F10 solution (Sigma, USA), then incubated up to 15 minutes at 37°C in order to allow the sperms to diffuse into the buffer.

A total of 10µl of the sperm suspension was smeared on a Neubauer chamber, finally, it was prepared to analyze sperm motility with a light microscope (Olympus BX51, Japan). After 15 minutes, 10 microscopic fields with magnifications of 400 times, counting more than 200 sperms, were observed **(5)**. Sperm motility was defined as: a) progressive (P) b) non-progressive (NP), and c) immotile (IM) when sperms had no movements. Progressive and non-progressive sperms were presented as a percentage of total sperm motility. Sperms having abnormalities in the tail, neck and head sections were considered abnormal. After diluting the suspension, one drop was placed on an erythrocytometer, and sperm count was performed with a light microscopy (Olympus, Japan) **(22)**. To assess the morphology of sperm, its suspension was smeared on a glass slide and then stained with Eosin. Finally, sperms were categorized into two major classes: a) normal and b) abnormal, sperms with dark pink are considered dead sperm as eosin penetrated into the sperm because of the breakdown of the cell membrane.

2.5 Vagina sampling

The pH of the vagina was measured using pH indicator paper **(23)**. For this purpose, the pH meter paper was inserted up to one-third of the length of the vagina using forceps, kept inside for 1 minute, and the pH change was recorded. Finally, the rats were anesthetized with isoflurane and tissue samples were prepared from the rats' vaginas. Obtained tissues fixation was performed in 10% buffered formalin, then it was sectioned and finally stained with H&E. Motic images were used to measure the thickness of vaginal *Cuscuta epithymum*. For each section, 10 different lengths were measured to obtain the average thickness **(5)**.

2.6 Mating and offspring

Twenty-four hours after the cessation of the immobilization stress protocol, each male rat was housed individually with two female rats to assess reproductive performance. After a 3-day cohabitation period, the males were removed, and the females remained in the cages until the completion of gestation. Upon delivery, offspring were evaluated for litter size, sex ratio, and serum estradiol and testosterone levels.

2.7 Blood sample collection

After the end of the stress phase for parents and at PND 25, under general anesthesia with isoflurane, blood samples were obtained at 8:00 a.m. and 11:00 a.m., through cardiac puncture.

The samples were poured into EDTA-containing tubes, and then centrifuged at 9000 rpm. The obtained plasma samples of offspring, and serum samples of adult male and female rats were placed at -80 °C. The blood samples were used for quantification of testosterone and 17-beta estradiol, using commercial ELISA kit including Zellbio GmbH (Germany, Cat. No: ZB-10259D-R9648) and Zellbio GmbH (Germany, Cat. No: ZB-10174M-R9648), respectively. The amounts were presented as pg/mL for 17-beta estradiol and ng/mL for testosterone.

2.8 Statistical analysis

The analysis was conducted using SPSS software (SPSS Inc., Chicago, IL, USA) edition 22 and presented as mean ± SEM. The Kolmogorov–

Smirnov test was utilized to check the distribution of control data. As a result, data analysis was done parametrically with the use of normal distributions and one-way analysis of variance (ANOVA) for comparisons of results for multiple groups.

When applicable, Tukey post hoc test was used. Sex ratio was analyzed using K^2 test. A $p < 0.05$ was considered significant in all instances.

3. Result

Analysis was performed on the complete data of 100 adult rats and their 131 offspring.

3.1 Sperm parameters

There was a statistically significant difference in sperm parameters including count, motility viability, and morphology between the groups (Figure 1).

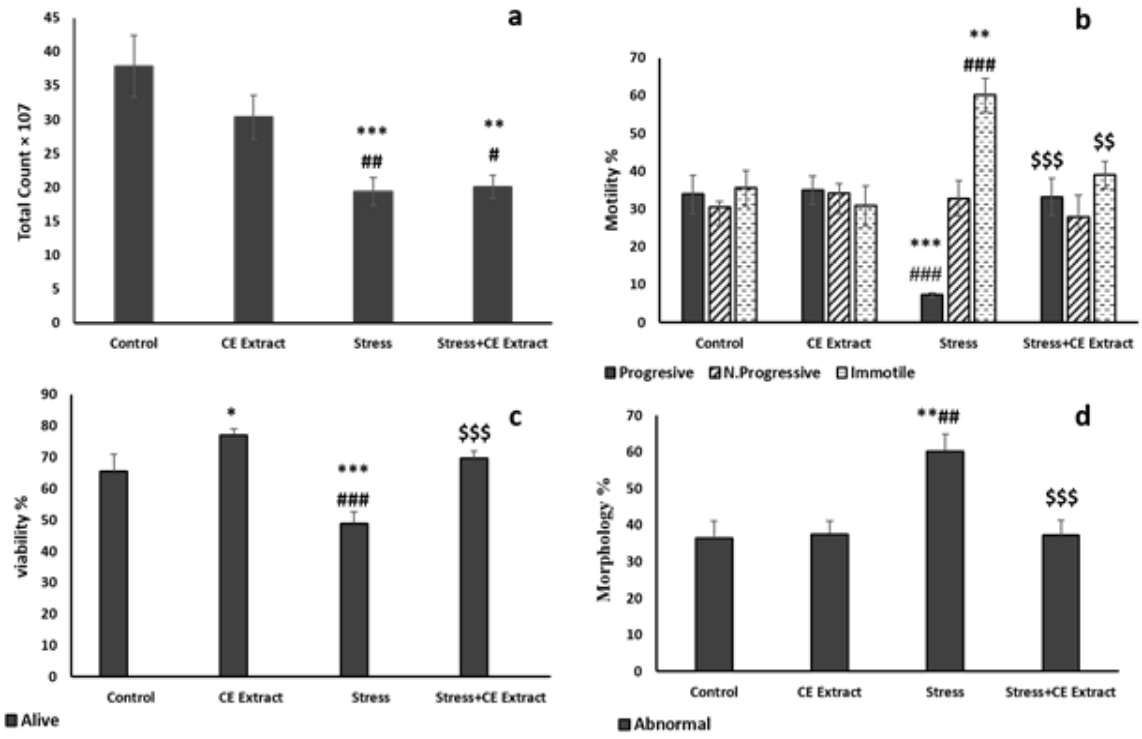

Figure 1. Sperm Parameters in Control Adult Rats, *CE* extract, stress and stress + *CE* extract groups: **Panel a;** Total count: *** p<0.001, ** p<0.01, with control group, # indicates p<0.05, ## indicates p<0.01 with CE Extract group. **Panel b;** Motility: ** indicates p<0.01, *** indicates p<0.001with control group, ### indicates p<0.001 with CE Extract group, $$ indicates p<0.01, $$$ indicates p<0.001 with Stress group. **Panel c;** Viability: * indicates p<0.05, *** indicates p<0.001with control group, ### indicates p<0.001 with CE Extract group, $$$ indicates p<0.001 with Stress group. **Panel d;** Morphology: ** indicates p<0.01 with control group, ## indicates p<0.01 with CE Extract group, $$$ indicates p<0.001 with Stress group. (**Prepared by Authors, 2026**).

3.2 Sperm count

The results demonstrated that the number of sperm in the stress group was significantly lower compared to the control, CE extract, and stress + CE extract groups (1.68±0.42, 3.78±0.9, 3.03±0.64, and 2.12±0.3; P= 0.002) (Table1). Administration of *Cuscuta epithymum* extract to the stress-exposed group resulted in an increase in sperm count from 1.68±0.42 to 2.12±0.3. However, this difference was not statistically significant (Table2).

3.3 Sperm motility

According to table 1 sperm motility revealed that the percentage of sperms with progressive movements in the stress group was significantly lower compared to the control, CE extract, and stress + CE extract groups (7.26±1.21, 33.88±11.25, 35.02±7.62, and 33.22±9.65; P= 0.001). However, no significant difference was found among the groups in the percentage of sperm with non-progressive movements. The percentage of immotile sperm in the stressed rats was significantly higher in comparison with control, CE extract, and stress + CE extract groups (60.08±8.89, 35.48±10.64, 30.85±10.54, and 39.01±6.97; P< 0.004). The percentage of progressive sperms in CE extract group significantly reached the baseline level in control group (7.26±1.21 vs. 33.22±9.65; P< 0.001); and the number of immotile sperms was significantly decreased from 60.08±8.89 to 39.01±6.97 (P= 0.008) (Table2).

3.4 Sperm viability

The percentage of sperm viability in the stress group was significantly lower compared to the control, CE extract, and stress + CE extract groups (44.75±5.78, 65.04±12.43, 77.08±3.99, and 69.6±5.49; P< 0.001)

(Table1). According to table 2, administration of CE extract to the stress group significantly increased the percentage of viable sperms from 44.75±5.78 to 69.6±5.49, P< 0.001).

3.5 Sperm morphology

The percentage of sperms with abnormal morphology in the stress group was significantly higher compared to the control, CE extract, and stress + CE extract groups (60.13±10.46, 36.37±9.34, 37.64±7.46, and 37.15±11.18; P= 0.004) (Table 1).

As it has been shown in table 2, CE extract administration to the stress group significantly decreased the percentage of sperms with abnormal morphology from 60.13±10.46 to 37.15±11.18, (P< 0.001).

Table 1. Comparison of Sperm parameters between the groups

Sperm parameters	Control	CE Extract	Stress	Stress+ CE Extract	P-value
Sperm count (million per ml)	3.78±0.9	3.03±0.64	1.68±0.42*	2.12±0.3	0.002
Sperm motility (%)					
Progressive	33.88±11.25	35.02±7.62	7.26±1.21*	33.22±9.65	0.001
Non-progressive	30.53±3.33	34.11±5.01	32.64±9.6	27.75±11.82	0.696
Immotile	35.48±10.64	30.85±10.54	60.08±8.89*	39.01±6.97	0.004
Sperm viability (%)	65.04±12.43	77.08±3.99	44.75±5.78*	69.6±5.49	0.000
Sperm Abnormal morphology (%)	36.37±9.34	37.64±7.46	60.13±10.46*	37.15±11.18	0.004

Note: Control (neither stress nor CE extract), CE Extract (just received extract), Stress (just received stress), Stress+ CE Extract (received both stress and extract). * Indicates significant difference with other groups, one-way ANOVA and Tukey post hoc test.

Table 2. Comparison of Sperm Quality between Stress and Cuscuta epithymum-treated Stressed Groups

	Stress	Stress+ CE Extract	P-value
Sperm count (million per ml)	1.68±0.42	2.12±0.3	0.333
Sperm motility (%)			
Progressive	7.26±1.21	33.22±9.65	0.001
Non-progressive	32.64±9.6	27.75±11.82	0.399
Immotile	60.08±8.89	39.01±6.97	0.008
Sperm viability (%)	44.75±5.78	69.6±5.49	0.001
Sperm Abnormal morphology (%)	60.13±10.46	37.15±11.18	0.001

3.6 Effects of pre-gestational stress and CE extract on plasma concentrations of estradiol and testosterone in parents and offspring

Significant differences in plasma testosterone and estradiol levels were observed among the experimental groups in both parents and offspring at postnatal day (PND) 26, as illustrated in (Figure 2).

3.7 Testosterone level in adult rats

In adult male rats, plasma testosterone concentration (ng/dl) was significantly lower in the stress group when compared to control, CE extract, and stress + CE extract groups (0.82±0.15, 2.12±0.48, 0.95±0.5, and 3.08±0.57; *P= 0.002*) (Table 3).

As shown in Table 4, CE extract administration to the stress group significantly increased adult male rats' serum testosterone level from 0.82±0.15 to 3.08±0.57 ng/dl, *P= 0.001*)

3.8 Testosterone level in offspring

Consistent with the findings in adult rats, male offspring in the stress group exhibited significantly lower plasma testosterone concentrations (ng/dL) compared to the control, CE extract, and stress + CE extract groups (0.24±0.09, 0.37±0.12, 1±0.24, and1.37±0.18; P= 0.031) as presented in Table3. Administration of *CE* extract to the stress-exposed group significantly increased serum testosterone levels in male offspring from 0.24±0.09 to 1.37±0.18ng/dl, P= 0.009) as shown in (Table 4).

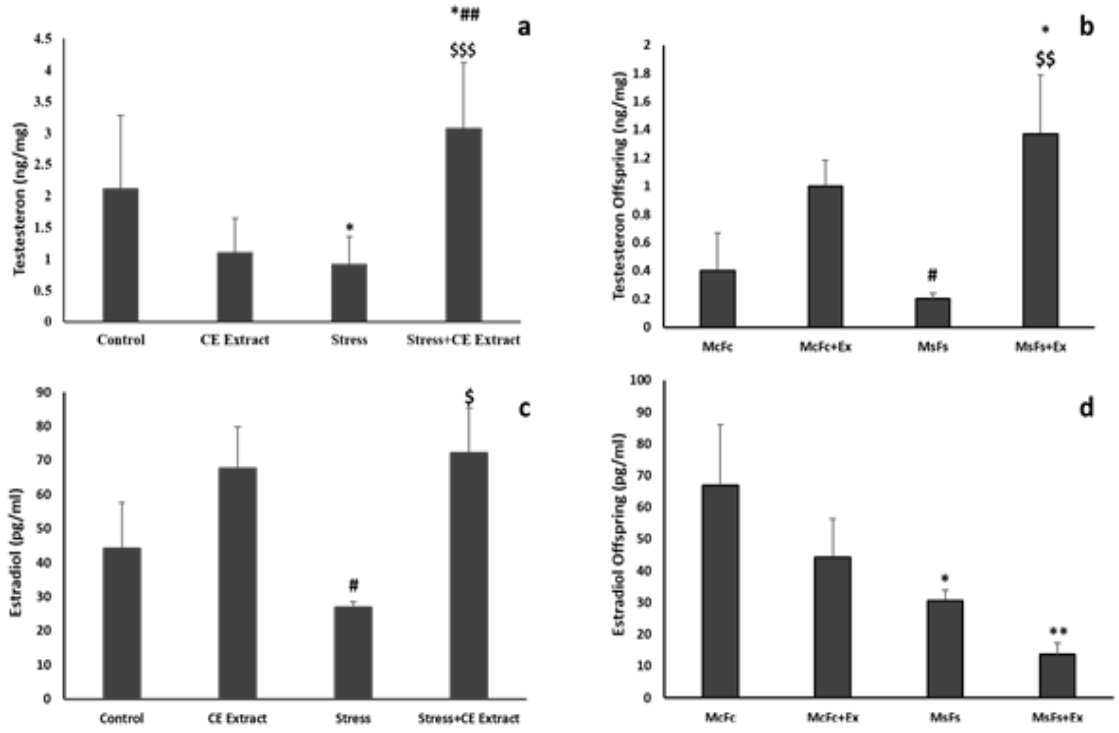

Figure 2. Serum testosterone and estradiol levels of parents (a and c) and pups (b and d) represented in the charts a, b, c, and d in the control, *CE* extract, stress and stress + CE extract groups: **Panel a;** Testosterone: * indicates p<0.05 with control group, ## indicates p<0.01 with CE Extract group, $$$ indicates p<0.001 with Stress group. **Panel b;** Testosterone Offspring: * indicates p<0.05 with control group, # indicates p<0.05 with CE Extract group, $$ indicates p<0.01 with Stress group. **Panel c;** Estradiol: # indicates p<0.05 with CE Extract group, $ indicates p<0.05 with Stress group. **Panel d;** Estradiol Offspring: * indicates p<0.05, ** indicates p<0.01 with control group. **(Prepared by Authors, 2026).**

3.9 Estradiol level in adult rats

In the adult female rats, plasma estradiol concentration (pg/ml) was significantly lower in the stress group when compared to control, CE extract, and stress + CE extract groups (26.8±3.18, 44.2±36.77, 67.62±37.31, and 72.26±13.15; P= 0.048) (Table 3). According to table 4, administration of CE extract to the stress group significantly increased adult female rats' serum estradiol level from 26.8±3.18 to 72.26±13.15 pg/mL, (P= 0.027).

3.10 Estradiol level in offspring rats

Similar to adults, in female offspring rats, plasma estradiol concentration (pg/ml) was significantly lower in the stress group when compared to control, CE extract, and stress + CE extract groups (30.56±9.72, 66.92±37.82, 44.27±23.96, and 13.62±7.42; P= 0.029) (Table3). However, administration of CE extract to the stressed group did not result in a statistically significant increase in serum estradiol levels (P = 0.28; Table 4)

Table 3. Hormone Levels in Parents and Offspring rats

Groups in Parents Offspring	Control McFc	CE Extract McFc+Ex	Stress MsFs	Stress+ CE Extract MsFs+Ex	P-value
Estradiol level Parents (pg/ml)	44.2±36.77	67.62±37.31	26.8±3.18	72.26±13.15	0.048
Estradiol Level Offspring(pg/ml)	66.92±37.82	44.27±23.96	30.56±9.72	13.62±7.42	0.029
Testosterone Level Parents (ng/dl)	2.12±0.48	0.95±0.5	0.82±0.15	3.08±0.57	0.002
Testosterone Level Offspring (ng/dl)	0.37±0.12	1±0.24	0.24±0.09	1.37±0.18	0.031

Note: Control (neither stress nor CE extract), CE Extract (just received extract), Stress (just received stress), Stress+ CE Extract (received both stress and extract).

Table 4. Comparison of serum hormone levels between stress and stress plus CE extract treated groups

Hormone level	Stress	Stress+ CE Extract	P-value
Estradiol level Parents (pg/ml)	26.8±3.18	72.26±13.15	0.027
Estradiol Level Offspring(pg/ml)	30.56±9.72	13.62±7.42	0.281
Testosterone Level Parents (ng/dl)	0.82±0.15	3.08±0.57	0.001
Testosterone Level Offspring (ng/dl)	0.24±0.09	1.37±0.18	0.009

3.11 Effects of pre-gestational stress and CE extract on vaginal thickness, vaginal pH and Offspring Number

3.11.1 Vaginal Thickness

The measurement of the vaginal epithelium thickness in mature rats revealed that the stres s group exhibited a notably reduced thickness in comparison to the control, CE extract, and stress + CE extract groups (P=0. 005; table 5). In the group subjected to stress, the application of CE extract led to a significant rise in the thickness of the vaginal epithelium in adult rats. (P<0.001; Table 6 and Figure3).

3.11.2 Vaginal PH

Vaginal pH assessment in adult female rats revealed a significant reduction in the stress group compared to the other groups ($P < 0.001$; Table 5). *CE* extract in the stress group resulted in a significant increase in vaginal pH, rising from 4.5 ± 0.109 to 5.00 ± 0.000 ($P < 0.001$; Table 6). These findings indicate that CE extract effectively counteracts the stress-induced reduction in vaginal pH.

3.11.3 Offspring Number and Sex Ratio

The total number of offspring in the control group was 29 (12 males, 17 females); in the CE extract group, 45 (26 males, 19 females); in the stress group, 20 (6 males, 14 females); and in the stress + extract group, 37 (25 males, 12 females). Although the overall number of offspring did not differ significantly among the groups (P = 0.08; Table 5), a significant difference was observed in the male-to-female ratio.

The stress group showed a markedly lower percentage of male offspring (30%) compared with the control (41.38%), CE extract (57.78%), and stress + CE extract (71%) groups (P = 0.018; Table 5). Furthermore, treatment with CE extract in the stress group significantly increased the total number of offspring (P = 0.041) and the number of male offspring (P = 0.002; Table 6).

Table 5. Comparison of vaginal epithelial thickness, vaginal pH, the number of offspring, and sex ratio between the groups

	Control	CE Extract	Stress	Stress+CE Extract	P-value
Vaginal epithelial thickness (µm)	2593.12 ± 277.88	1778.27 ± 212.8	1696.2± 182.74	2503.37 ± 205.6	0.005
pH of Vagina	5.33 ± 0.105	5.14 ± 0.922	4.5 ± 0.109	5.00 ± 0.000	0.000
Number of offspring	29	45	20	37	0.086
Sex ratio of offspring					
Female	17	19	14	12	0.235
male	12(31.48%)	26(57.78%)	6(30%)	25(71%)	0.018

Note: Control (neither stress nor CE extract), CE Extract (just received extract), Stress (just received stress), Stress+ CE Extract (received both stress and extract). Vaginal epithelial and pH of Vagina were analyzed using one way ANOVA and Tukey post hoc test; sex ratio was analyzed using K^2.

Table 6. Comparison of vaginal epithelium thickness, vaginal pH, the number of offspring, and sex ratio between stress + CE Extract groups

	Stress	Stress + CE Extract	P-value
Vaginal epithelial thickness (µm)	1696.2± 182.74	2503.37 ± 205.6	0.001
pH of Vagina	4.5 ± 0.109	5.00 ± 0.000	0.001
Number of offspring	20	37	0.041
Sex ratio of male	30%	71%	0.002
Female	14	12	0.851
male	6	25	0.002

Note: Vaginal epithelial and pH of Vagina were analyzed using one way ANOVA and Tukey post hoc test; sex ratio was analyzed using K^2 test.

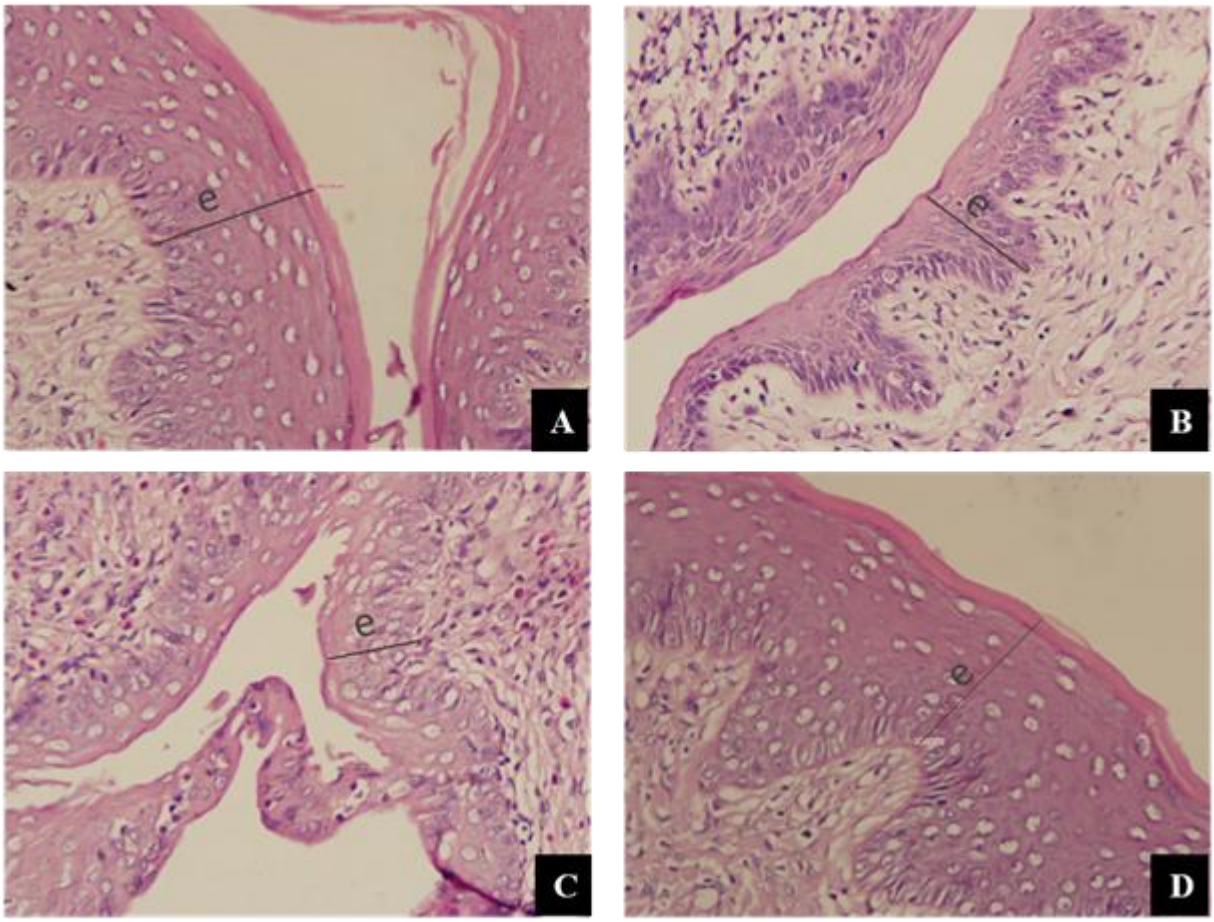

Figure 3. Histological analysis of vaginal epithelium, H&E staining with ×20 magnifications: **A.** Control group, **B.** *CE* extract group, **C.** Stress group, and **D.** Stress + *CE* extract group. The thickness of the vaginal epithelium is marked in the figure and labeled with the letter 'e' representing the epithelial layer. **(Prepared by Authors, 2026)**.

4. Discussion

Our findings indicate that immobilization stress markedly reduced sperm parameters and administration of *Cuscuta epithymum* extract resulted in an increase in sperm motility, motile sperm, progressively motile sperm, viable sperm, and sperm with normal morphology. Notably, the proportion of immotile sperm was significantly elevated in the stress group and was substantially reduced following CE extract administration. These results suggest that CE extract mitigates the deleterious effects of pre-gestational stress on sperm quality, particularly in terms of motility and viability. The findings of the present study are consistent with previous studies, which suggest that sperm quality and testicular parameters decreased remarkably after 50 days of exposure to immobility stress **(24, 25)**. The findings of Safavi et al. confirmed the adverse effects of immobility stress on epididymal tissue with increasing duration of stress exposure **(26)**. There have also been reports about the undesirable effects of stress on the quality of animal or human semen, including a decreased level in sperm volume, sperm density and motility, and normal sperm count **(27)**. Almeida et al., have shown that adult male rats, subjected to long-term immobilization, demonstrate a reduction in sperm concentration and spermatid generation and also decreased plasma testosterone content **(28)**. Kolbasi et al., (2020) showed that in the chronic stress group, sperm density and overall motility rate were not remarkably different among other experimental groups, although normal sperm morphology and progressive motility were significantly decreased **(29)**. In this study, we observed that the extract of *CE* was able to reduce the complications in sperm parameters caused by stress. Studies have also shown that reproductive organ weights, sperm count, sperm motility, the ratio of live to dead sperms and the percentage of sperms with normal morphology in the groups that received the *CE* extract increased significantly **(30, 31)**. These findings highlight the negative effects of stress on oogenesis and spermatogenesis which leads to impaired reproductive organ function in parental subjects.

Consequently, these stress-induced disruptions also affected offspring sex ratio and contributed to a reduction in the total number of offspring. This study also investigated testosterone and estradiol levels in parents and offspring. The findings demonstrated that testosterone diminished remarkably in the stress group compared to the control group. However, the group that received CE extract along with stress experienced an increase level of testosterone significantly compared to the stress group and even the control group. The level of estradiol in the stress + CE extract groups increased significantly compared to the stress group, while in offspring, estradiol dramatically decreased in the stress and stress + CE extract groups. The hypothalamic-pituitary-adrenal axis has a preventive role in the reproductive system of females. The corticotropin-releasing hormone prevents the secretion of the hypothalamic gonadotropin-releasing hormone, and causes resistance of target tissues to estradiol **(16)** and plasma estradiol contents in female rats are lowered during chronic stress **(17)**. Mahmoudkhani et al. reported findings consistent with the present study, demonstrating that serum levels of testosterone and 17β-estradiol were significantly reduced in stressed parental subjects **(32)** and Ozegbe et al. (2012) reported that the seed and stem of *C. australis*, are effective in reducing male and female reproductive disorders. *C.* australis extracts induced significant effects on the blood plasma concentrations of follicle exciting hormone(FSH), luteinizing hormone(LH), and testosterone of the adult male rats **(30)**. This stress remarkably elevated the number of female offspring in each litter **(32)**. The present findings demonstrated a significant reduction in offspring number in the stress group compared to the CE extract and stress + CE extract groups. Additionally, the stress group exhibited a higher proportion of female offspring, whereas the stress + CE extract group showed a reversal of this trend, with a superiority of male offspring. These results suggest that *Cuscuta epithymum* extract mitigates stress-induced reproductive impairments and enhances male fertility and offspring number. One of the mechanisms related to stress on fertility is that paternal exposure to environmental hazards diminishes the number of semen Y-bearing sperms or decreases their ability to get to or fertilize the oocyte **(33)**. Also, estradiol levels are lowered during chronic stressors **(34)**. Maternal predatory stress and immobility stress throughout the pre-maturation phase of oocytes reduces the developmental potential of oocytes both before and after implantation **(33, 35)**. Therefore, this may lead to a disproportionate loss of XY embryos **(33)**. Furthermore, male fetuses grow larger and therefore require a greater maternal resource and it is possible not to adapt with stressful intrauterine environment developmentally. Instead, female fetuses consume fewer resources during their development and demonstrate lower growth and needs in response to maternal stress **(36)**. These results suggest that *Cuscuta epithymum* may partially mitigate the detrimental effects of stress on reproductive outcomes. Consistent with our study, stress has also been demonstrated to impact the thickness of the vaginal epithelium across all stages of the estrous cycle **(32)**. The stress group exhibited a significant reduction in vaginal epithelial thickness compared to the control group. However, treatment with *Cuscuta epithymum* extract in the stress-exposed group resulted in a marked increase in vaginal thickness, approaching levels observed in the control group. Similarly, Kim et al. in 2016 also observed an increase in vaginal thickness with *Cuscuta epithymum* extract **(37)**. The probable mechanism of Cuscuta's action is attributed to its abundance of antioxidant compounds. The study of Ma et al. showed similar results. They reported that *Cuscuta epithymum* chinensis extracts and isolates (including flavonoid antioxidant compounds) had several impacts on male and female reproductive systems such as better fertility. The potential mechanisms of this action is the antioxidant effect, so that total flavonoids of *Cuscuta epithymum* control apoptosis and prevent abortion **(38, 39)**, since increased ROS level injures oocyte and sperm DNA directly, it contributes to lipid peroxidation **(17, 40, 41)**, inflammation and induce sperm apoptosis rapidly increased circulating glucocorticoid during stress, leads to testicular involution and subsequent remarkable decrease of testosterone release as well **(42, 43)**.

5. Conclusion

The findings may not be directly translatable to humans due to inherent interspecies biological differences, and the pathophysiology of certain diseases in animal models may not fully replicate human conditions. Therefore, additional studies involving human subjects are essential to accurately evaluate the effects of *Cuscuta epithymum* extract on pregestational stress and to assess its potential for clinical application, still, it is plausible to infer that *Cuscuta epithymum* exerts a beneficial influence on reproductive function.

6. Declarations

6.1 Acknowledgments

We appreciate Fatemeh Amiri (Persian medicine expert) for assist in animal dissection and thanks to Zanjan University of Medical Sciences for financial support.

6.2 Ethical Considerations

All experiments followed the guidelines of the Medical Ethics Committee of the Iranian Ministry of Health and Medical Education (IR.ZUMS.REC.1399.419).

6.3 Authors' Contributions

All authors contributed equally to this work.

6.4 Conflict of Interest

The authors have no financial conflicts of interest and no funding sources to disclose.

6.5 Fund or Financial Support

This work was supported by the Zanjan University of medical sciences.

6.6 Using Artificial Intelligence Tools (AI Tools)

No AI tools were used in this manuscript

References

1. Notari L. Stress in veterinary behavioural medicine. BSAVA manual of canine and feline behavioural medicine. BSAVA Libr. 2009:136-45.
https://doi.org/10.22233/9781905319879.13

2. Divyashree S, Yajurvedi HJR. Chronic stress effects and their reversibility on the Fallopian tubes and uterus in rats.Reproduct Fertill Develop. 2018;30(2):380-90.
https://doi.org/10.1071/RD17082
PMid:28743351

3. Shansky RM, Morrison JH. Stress-induced dendritic remodeling in the medial prefrontal cortex: effects of circuit, hormones and rest.Brain Res. 2009;1293:108-13.
https://doi.org/10.1016/j.brainres.2009.03.062
PMid:19361488 PMCid:PMC2748148

4. Saki G, Rahim F, Alizadeh K. Effect of forced swimming stress on count, motility and fertilization capacity of the sperm in adult rats.J Hum Reprod Sci. 2009;2(2):72.
https://doi.org/10.4103/0974-1208.57226
PMid:19881152 PMCid:PMC2800931

5. Ghassemifard L, Ramezanikhah H, Jafari P, Amiri F, Saboory E. Effects of pre-conception stress and dracocephalum moldavica extract on sperm parameters and sex hormones in parents and offspring. Birth Defects Res. 2025;117(11):e2545.
https://doi.org/10.1002/bdr2.2545
PMid:41243710

6. Barthakur IK. Choose the sex of your child: the natural way: Clarion Books associated with Hind Pocket Books; 1990.

7. Kapoor A, Matthews SG. Testosterone is involved in mediating the effects of prenatal stress in male guinea pig offspring. J Physiol. 2011;589(3):755-66.
https://doi.org/10.1113/jphysiol.2010.200543
PMid:21173081
PMCid:PMC3052442

8. Kapoor A, Matthews SG. Prenatal stress modifies behavior and hypothalamic-pituitary-adrenal function in female guinea pig offspring: effects of timing of prenatal stress and stage of reproductive cycle. Endocrinol. 2008;149(12):6406-15.
https://doi.org/10.1210/en.2008-0347
PMid:18755800

9. Hashemi P, Roshan-Milani S, Saboory E, Ebrahimi L, Soltanineghad M. Interactive effects of prenatal exposure to restraint stress and alcohol on pentylenetetrazol-induced seizure behaviors in rat offspring.Alcohol. 2016;56:51-7.
https://doi.org/10.1016/j.alcohol.2016.07.003
PMid:27542557

10. Sarris J, McIntyre E, Camfield DA. Plant-based medicines for anxiety disorders, part 2: a review of clinical studies with supporting preclinical evidence. CNS Drugs. 2013;27(4):301-19.
https://doi.org/10.1007/s40263-013-0059-9
PMid:23653088 PMCid:PMC6106973

11. Ghassemifard L, Khavasi N, Saboory E, Madani F, Sardari S. Exploring Cuscuta epithymum's effect on neuroinflammation, tyrosine kinase activity and macrophage counts in spleen and liver: Revealing their roles in stress responses. Adv Pharm Bull. 2025;15(3):657-66.
https://doi.org/10.34172/apb.025.45554
PMid:41403726 PMCid:PMC12703398

12. Khavasi N, Ghassemifard L, Ramezanikhah H, Sardari S, Saboory E. Cuscuta epithymum extract affects epileptic offspring behaviour and weight after applying preconception immobility stress on parents. Int J Dev Neurosci. 2025;85(1):e0405.https://doi.org/10.1002/jdn.10405 .
PMid:39895384

13. Jafari E, Bahmanzadegan A, Ghanbarian G, Rowshan V. Antioxidant activity and total phenolic content from aerial parts of three Cuscuta species. Analyt Chem Lett. 2015;5(6):377-84.
https://doi.org/10.1080/22297928.2016.1143394

14. Patel S, Sharma V, Chauhan NS, Dixit VK. An updated review on the parasitic herb of Cuscuta reflexa Roxb. J Chinese Integ Med. 2012;10(3):249-55.
https://doi.org/10.3736/jcim20120302
PMid:22409913

15. Tanruean K, Kaewnarin K, Suwannarach N, Lumyong S. Comparative evaluation of phytochemicals, and antidiabetic and antioxidant activities of Cuscuta reflexa grown on different hosts in northern Thailand. Nat Product Communicat. 2017;12(1):1934578X1701200114.
https://doi.org/10.1177/1934578X1701200114

16. Sun K, Zhao C, Chen X-F, Kim H-K, Choi B-R, Huang Y-R, et al. Ex vivo relaxation effect of Cuscuta chinensis extract on rabbit corpus cavernosum. Asian J Androl. 2013;15(1):134.
https://doi.org/10.1038/aja.2012.124
PMid:23147465 PMCid:PMC3739115

17. Donnapee S, Li J, Yang X, Ge A-h, Donkor PO, Gao X-m, et al. Cuscuta chinensis Lam.: A systematic review on ethnopharmacology, phytochemistry and pharmacology of an important traditional herbal medicine. J Ethnopharmacol. 2014;157:292-308.
https://doi.org/10.1016/j.jep.2014.09.032
PMid:25281912

18. Mehrabani M, Modirian E, Ebrahimabadi A, Vafazadeh J, Shahnavaz S, Heidari M. Study of the effects of hydro-methanol extracts of Lavandula vera DC. and Cuscuta epithymum Murr. on the seizure induced by pentylentetranzol in mice. J Kerman Univ Med Sci. 2007;14(1):25-32.

19. Ghassemifard L, Sardari S, Safari H, Ramezanikhah H, Khavasi N. Assessment of the antioxidant activity of the extrcat, cold press, and n-hexane oils and in vitro cytotoxic effects of Capparis spinosa L. seed on SH-SY5Y cancer cell lines. J Med Plants. 2022;21(83):87-98.
https://doi.org/10.52547/jmp.21.83.87

20. Heydari S, Hosseini SH, Kamali K, Sardari S, Parsamanesh N, Ghassemifard L, et al. Capparis spinosa L. Reduces cisplatin-induced acute liver injury in male rats: Pretreatment and single dose therapy. J Med Plant Product. 2022.

21. Nakhjiri E, Saboory E, Roshan-Milani S, Rasmi Y, Khalafkhani D. Effect of prenatal restraint stress and morphine co-administration on plasma vasopressin concentration and anxiety behaviors in adult rat offspring. Stress. 2017;20(2):205-11.
https://doi.org/10.1080/10253890.2017.1306053
PMid:28287019

22. Ramezanikhah H, Farrokhi A, Nejatbakhsh R, Shokri S, Zendedel A, Abdanipour A. Involvement of Asc and Nlrp3 inflammasomes in the testes following spinal cord injury. Endocrine Regul. 2020;54(2):96-108.
https://doi.org/10.2478/enr-2020-0012
PMid:32597153

23. Foroozanfard F, Mesdaghinia E, Tabasi Z, Sehat M, Totonian S. Prediction of preterm labor based on vaginal pH and cervical length in low risk population during the second trimester of pregnancy. Feyz J Kashan Univ Med Sci. 2014;18(3).

24. Yang J, Wang Y, Bao Y, Guo J. The total flavones from Semen cuscutae reverse the reduction of testosterone level and the expression of androgen receptor gene in kidney-yang deficient mice. J Ethnopharmacol. 2008;119(1):166-71.
https://doi.org/10.1016/j.jep.2008.06.027
PMid:18640256

25. Vallejos-Vidal E, Reyes-López F, Teles M, MacKenzie S. The response of fish to immunostimulant diets. Fish Shellfish Immunol. 2016;56:34-69.
https://doi.org/10.1016/j.fsi.2016.06.028
PMid:27389620

26. Safavi E, Khayyatnouri H. Histologic and histomorphometric study of epididymis after immobilization stress in mice. Vet Clin Pathol. 2013;7(3 (27) Autumn):193-206.

27. Almeida S, Kempinas WdG, Lamano Carvalho TL. Sexual behavior and fertility of male rats submitted to prolonged immobilization-induced stress. Brazil J Med Biol Res. 2000;33:1105-9.
https://doi.org/10.1590/S0100879X200000090009
PMid:10973146 28. Almeida S, Petenusci S, Anselmo-Franci J, Rosa-e-Silva A, Lamano-Carvalho T. Decreased spermatogenic and androgenic testicular functions in adult rats submitted to immobilization-induced stress from prepuberty. Brazil J Med Biol Res. 1998;31:1443-8.
https://doi.org/10.1590/S0100879X199800110003
PMid:9921282

29. Kolbasi B, Bulbul MV, Karabulut S, Altun CE, Cakici C, Ulfer G, et al. Chronic unpredictable stress disturbs the blood-testis barrier affecting sperm parameters in mice. Reproductive Bio Med Online. 2021;42(5):983-95.
https://doi.org/10.1016/j.rbmo.2020.12.007
PMid:33653651

30. Ozegbe P, Omirinde J. Comparative morphophysiological evaluation of the testis of adult Wistar rats fed low protein-energy diet and dosed with aqueous extracts of Cuscuta australis. Nigeria J Physiol Sci. 2012;27(2):149-55.

31. Shu J, Li L, Yu H, Zhang D. Fertility-enhancing potential of ethanol extract of Cuscuta chinensis

seeds in a rat model of unilateral cryptorchidism. Trop J Pharmaceut Res. 2021;20(5):995-1002. https://doi.org/10.4314/tjpr.v20i5.16

32. Mahmoodkhani M, Saboory E, Roshan-Milani S, Azizi N, Karimipour M, Sayyadi H, et al. Pre-pregnancy stress suppressed the reproductive systems in parents and changed sex ratio in offspring. J Apply Biomed. 2018;16(4):370-7. https://doi.org/10.1016/j.jab.2018.08.002

33. Terrell ML, Hartnett KP, Marcus MJEhtj. Can environmental or occupational hazards alter the sex ratio at birth? A systematic review. 2011;4(1):7109. https://doi.org/10.3402/ehtj.v4i0.7109 PMid:24149027 PMCid:PMC3168220

34. Shors TJ, Pickett J, Wood G, Paczynski M. Acute stress persistently enhances estrogen levels in the female rat.Stress. 1999;3(2):163-71. https://doi.org/10.3109/10253899909001120 PMid:10938577

35. Liu YX, Cheng YN, Miao YL, Wei DL, Zhao LH, Luo MJ, et al. Psychological stress on female mice diminishes the developmental potential of oocytes: a study using the predatory stress model. PLoS One. 2012;7(10):e48083. https://doi.org/10.1371/journal.pone.0048083 PMid:23118931 PMCid:PMC3485266

36. Mohagheghi SA, Poorazarang H, Elhamirad A, Dezashibi Z, Hematyar N. Extraction of phenolic compounds from potato peel (Ramus variety) with solvent and ultrasound-assisted methods and evaluation of its antioxidant activity in soybean oil.Food Sci Technol. 2010;8(1):81-91

37. Kim S, Jang BH, Chang SH, Kang H, Park KM, Ahn DK, et al. Effects of herbal mixture extracts containing angelica gigas nakai and cuscuta chinensis lam. On menopausal symptoms in ovariectomized rats. J Korean Soc Food Sci Nutr. 2016;45(8):1083-9. https://doi.org/10.3746/jkfn.2016.45.8.1083

38. Sadaghiani MM, Saboory E. Prenatal stress potentiates pilocarpine-induced epileptic behaviors in infant rats both time and sex dependently. Epil Behav. 2010;18(3):166-70. https://doi.org/10.1016/j.yebeh.2010.04.016 PMid:20483668

39. Dow C, Mancini F, Rajaobelina K, Boutron-Ruault M-C, Balkau B, Bonnet F, et al. Diet and risk of diabetic retinopathy: a systematic review. Europ J Epidemiol. 2018;33(2):141-56. https://doi.org/10.1007/s10654-017-0338-8 PMid:29204902

40. Sharma S, Hullatti K, Kumar S, Tiwari K. Comparative antioxidant activity of Cuscuta reflexa and Cassytha filiformis. J Pharm Res2012;5(1):441-3.

41. Donnapee S, Li J, Yang X, Ge AH, Donkor PO, Gao XM, et al. Cuscuta chinensis Lam.: A systematic review on ethnopharmacology, phytochemistry and pharmacology of an important traditional herbal medicine. J Ethnopharmacol. 2014;157:292-308. https://doi.org/10.1016/j.jep.2014.09.032 PMid:25281912

42. Park HJ, Koo YK, Park MJ, Hwang YK, Hwang SY, Park NC. Restoration of spermatogenesis using a new combined herbal formula of epimedium koreanum nakai and angelica gigas nakai in an luteinizing hormone-releasing hormone agonist-induced rat model of male infertility. W J Men Health. 2017;35(3):170-7. https://doi.org/10.5534/wjmh.17031 PMid:29076302 PMCid:PMC5746488

43. Adeoye O, Olawumi J, Opeyemi A, Christiania O. Review on the role of glutathione on oxidative stress and infertility. JBRA Assist Reproduct. 2018;22(1):61