

Assessment the Effect of Aqueous Extract of *Peganum harmala* Seeds on Fertility of Male Mice

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Abstract

Peganum harmala is one of the medicinal plants, it is a widely used in a traditional medicine to treat several diseases, so the aim of this study was to evaluate of effect of aqueous extract of *Peganum harmala* seeds on fertility in male mice. Sixteen male mice were used in this study at age of 6 – 10 weeks, they were divided into two groups of 8 mice each, the control group was given distilled water by mouth, while another group was received aqueous extract of *Peganum harmala* seeds at a dose of (300 mg/kg) for three weeks. The results showed a significant decline in body and epididymis weight in the treated group comparing to the control group. There was no any effect of aqueous extract of *Peganum harmala* on sperm parameters and testicular tissue. Also no significant change was observed in percent of deformed embryos and dead embryos of females impregnated by males treated with the aqueous extract when compare to control.

Key words: *Peganum harmala*, Male mice, Testis, Fertility.

Introduction:

Peganum harmala (*P. harmala*) is known as a medicinal plant, it is widely used in traditional medicine which belongs to the family Zygophyllaceae (Aslam *et al.*, 2014). Also it is widely spread in Central Asia but nowadays it grows in Australia, North Africa and South western America (Bahman *et al.*, 2012), and can grow in sandy soils, semi-arid lands and mountainous areas (Mahmoudian *et al.*, 2002). It is known as Harmal and Espand in Iran, Harmal in North Africa and the middle east, Maxican rue or African rue in USA (Mahmoudian *et al.*, 2002). This plant contains several effective alkaloids compounds especially in the seeds and roots that include beta-carbolines such as harmaline, harmine, harmalol and harman, in addition to quinazoline derivatives like vassicine and vasicinone (Moloudizargari *et al.*, 2013).

The seeds of *P. harmala* are useful for epilepsy, loss of memory, chronic headache, kidney stone, jaundice, colic and sciatica (Niroumand *et al.*, 2015), also to treat urinary tracts, strengthen the hair and relieve joint pain (Lamchouri *et al.*, 2002). Aqueous of the seeds has anticholinergic, antihistaminic and antiadrenergic effects (Agel and Hadidi, 1991). Several studies revealed that the alkaloids of *P. harmala* have antiparasidal (Astulla *et al.*, 2008), antibacterial (Nenaah, 2010) and antileishmanial

effects (Rahimi-Moghaddan, 2011), they also have antitumor activities where inhibit DNA topoisomerase and interfere with DNA synthesis (Li *et al.*, 2007).

P. harmala and its alkaloids possess a wide range effects on the nervous system including antidepressant, analgesia (Monsef *et al.*, 2004), hallucination, excitation (Nasehi *et al.*, 2010) and strongly inhibit of monoamine oxidase (MAO) (Massaro, 2002). There are little studies were conducted about effect of *P. harmala* seeds on fertility in male mice, so this research aims to evaluate impact of aqueous extract of *P. harmala* seeds on sperm parameters, testicular tissue also on the embryos.

Material and Methods:

2.1 plant material:

Peganum harmal specimens were collected from the western mountain of Libya, the seeds were separated, grinded and stored in a dark glass bottle until the time of use.

2.2 Experimental animals and treatment:

Sixteen male mice weighing (25 -30 g), were reared in the animal house of Zoology department, Faculty of Science, Tripoli University. Mice were housed in plastic cages in an air-conditioned room (temperature approximately 25 c°) under a normal photoperiod of 12 h dark / 12 h light and they were given the food and drinking water. The animals were divided into two groups of 8 mice each, group I: control mice were received distilled water orally, whereas the group II was given the aqueous extract of *Peganum harmala* seeds with a dose of (300 mg/kg) for three weeks, during the course of the treated period, animals were observed a day for any abnormal behavior. The animals were weighed daily during the dosing period to adjust the administered dose according to body weight changes. At the end of the treatment, the mice were weighed prior to sacrifice, then killed, some sex organs (vas deferens, testis and epididymis) were dissected and were used for evaluation sperm parameters as well as histological examination.

2.3 Sperm function evaluation:

Sperm were collected by squeezing vas deferens in 37 c° normal saline (0.9 NaCl). Sperm count and motility were assessed by using Neubauer hemocytometer. The examination of sperm morphology was carried out by putting a drop of sperm suspension on a slide, after that air-dried and stained with 1 % eosin, then washed and air-dried again, the smears on the slides were observed under a light microscope.

2.4 Histological analysis:

The testes were dissected and fixed in 10 % formalin, after that processed in paraffin, the sections were cut (at 7 µm thickness), deparaffinized, hydrate, stained with eosin and hematoxylin and observed using a light microscope.

2.5 Test of effect of aqueous extract of *Peganum harmala* seeds on fertility in male mice:

The females were mated with treated males (3:1), the mating was confirmed by the presence of vaginal plug. Once the vaginal plug was observed, that day was considered as day zero of gestation (GD₀), the pregnant mice were killed on 18 day of gestation, the embryos were removed and weighed, the number of implantation sites, deformed embryos, viable and dead embryos were recorded.

2.6 Statistical analysis:

This analysis was performed using SPSS (version 20), one-way ANOVA was used to determine the significant difference between the control and treated group, (P < 0.05) was considered statistically significant.

Results and discussion:

3.1. Effect of aqueous extract of *P. harmala* seeds on body and some sex organs weight in male mice:

The results in Table (1) show a significant decrease of body weight ($P < 0.05$) in treated group comparing to control, also the average weight of epididymis revealed a significant decrease ($P < 0.05$) in treated mice compare to control. No significant changes were observed in testes weight ($P > 0.05$) between the control and treated group. These results are similar to previous study reported that the extract of harmala caused decline in body and some sex organs weight (EL-Dwairi and Banihani, 2007). The reduction in body weight may be attributed to anorexia, while the decrease in epididymis weight may be due to the low synthesis of testosterone (El-Dwairi and Banihani, 2007; Yousefvand *et al.*, 2017).

Table 1. Effect of aqueous extract of *P. harmala* seeds on body and some sex organs weight in male mice.

parameters groups	Body weight (g)	Epididymis (g)	Testes (g)
control	29.56±1.14	0.103±0.01	0.25±0.04
Group treated with (300mg/kg)	28.27±0.26 *	0.087±0.01*	0.22±0.01

* ($P < 0.05$) significantly different from control group. Value expressed as mean ± sd.

3.2. Effect of aqueous extract of *P. harmala* seeds on sperm parameters in male mice:

The statistical data in Table (2) show non-significant decline ($P > 0.05$) in sperm count and the percent of motile sperm. Non-significant increase in the percent of abnormal sperm where was (35 %) in the treated mice and 33 % in the control. The microscopic examination of sperm shapes showed some abnormalities were as following, sperm with irregular tail, sperm with bent neck, sperm with coiled tail and sperm with tailless head compare to normal morphology of sperm figure (1). The results of this study do not agree with a previous study demonstrated reduction in sperm count and motility in rats treated with *Peganum harmala* seeds extract for 60 days (EL-Dwairi and Banihani, 2007), also with another study showed that evaporation of *Peganum harmala* seeds for 7, 14, and 21 days caused increase in sperm count in chlorpromazine treated rats (AL-Mushhadani *et al.*, 2014). This difference may be attributed to the extraction method or the time period of the treatment.

Table 2. Effect of aqueous extract of *P. harmala* seeds on sperm parameters in male mice.

parameters groups	Motility %	Sperm count × 10 ⁶ / ml	Abnormal sperm %
The Control	79.33 ± 0.14	27.25 ± 16.05	0.33 ± 0/05
The Group treated with (300mg/kg)	78.77 ± 0.12	24.92 ± 15.31	0.35 ± 0.06

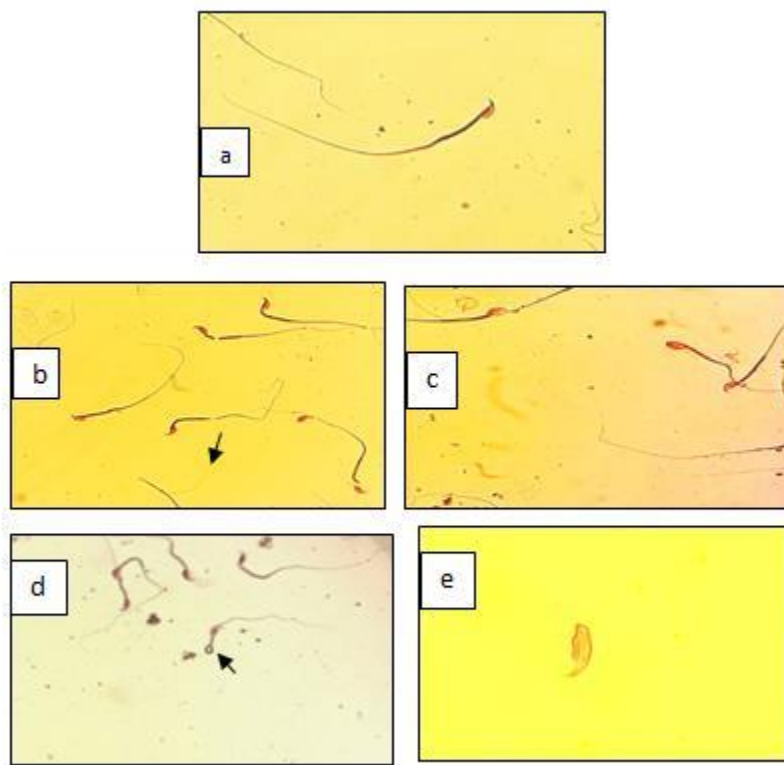


Figure 1. Photomicrograph of sperm morphology in treated group.

a) normal sperm, b) irregular tail c) sperm with bent neck
d) coiled tail, e) tailless head [Eosin - 40 ×]

3.3. Effect of aqueous extract of *P. harmala* seeds on the embryos of females impregnated by treated males.

The results table (3) revealed that male mice that were given orally aqueous extract of harmala seeds for 3 weeks did not cause influence on the number of females impregnated by these males, its fertility rate was 100 %. No significant difference ($P > 0.05$) in the percent of deformed embryos and dead embryos between the control and treated group. These results are not in agreement with a previous study indicated that harmala seeds extract caused reduction in the number of impregnated females by treated males and also decrease in the number of viable embryos and increase in the number of dead embryos (EL-Dwairi and Banihani, 2007). This difference may be attributed to the extraction method or the time period of treatment.

Table 3. Effect of aqueous extract of *P. harmala* seeds on the embryos of females impregnated by treated males.

Parameters groups	No. of males	No. of females	No. of implantation	Deformed embryos %	Dead embryos %
The Control	2	6	9.17 ± 0.98	0.033 ± 0.05	0.02 ± 0.05
The Group treated with (300mg/kg)	2	6	9.17 ± 2.04	0.18 ± 0.16	0.04 ± 0.07

3.4. Effect of aqueous extract of *P. harmala* seeds on testicular tissue in male mice.

In terms of control mice, the testes revealed normal cellular arrangement of seminiferous tubules, the seminiferous tubules separated from each other by interstitial space containing Leydig cells and blood capillaries. The germinal epithelium of seminiferous tubules including (spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids). The lumens contain spermatozoa figure (2 a). In mice treated with aqueous extract of *Peganum harmala* seeds demonstrated normal structure similar to the control group figure (2 b). These results are not in agreement with a previous study reported that *P. harmala* caused histopathological changes in testes of male rats (EL-Dwairi and Banihani, 2007). This difference may be due to the extraction method or the time period of treatment.

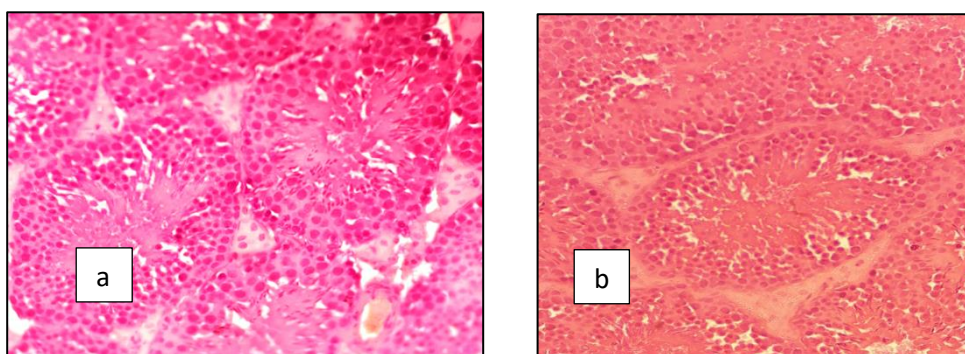


Figure 2. Photomicrograph of testicular tissue.

(a) the control mice.

(b) the mice treated with extract of *P. harmala* seeds. [H and E- 40 ×]

Conclusion:

The current study showed that administration of aqueous extract of *Peganum harmala* seeds for male mice at a dose of (300 mg/kg) caused a significant decrease in body and epididymis weight. There were no any detrimental effects on sperm parameters, testicular tissue and the embryos. This study represents just a preliminary study about the effects of aqueous extract of *Peganum harmala* seeds on sperm parameters and fertility. Further studies should be conducted to confirm that the safe dose on fertility in male mice was (300 mg/kg).

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تقييم تأثير المستخلص المائي لبذور نبات الحرمل *Peganum harmala* في الخصوبة في ذكور الفئران

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الملخص

يعتبر نبات الحرمل من النباتات الطبية المستعملة بشكل واسع في الطب الشعبي لعلاج العديد من الأمراض، لذا كان الهدف من الدراسة تقييم تأثير المستخلص المائي لبذور الحرمل على الخصوبة في ذكور الفئران. استخدم في هذه الدراسة 16 فأراً، تراوحت أعمارهم ما بين 6-10 أسابيع، قسمت الفئران عشوائياً إلى مجموعتين، احتوت كل مجموعة على 8 فئران، أعطيت المجموعة الأولى (الشاهد) الماء المقطر عن طريق الفم، بينما أعطيت المجموعة الثانية مستخلص بذور الحرمل بجرعة (300 مغ/كغ من وزن الجسم) ولمدة 3 أسابيع. أظهرت النتائج انخفاض في وزن الجسم والبربخ في المجموعة المعاملة مقارنة مع الشاهد، ولم يظهر أي تأثير للمستخلص في صفات النطف، والتركيب النسيجي للخصية وكذلك على أجنة الإناث المخصبة بواسطة الذكور المعاملة.

الكلمات المفتاحية: نبات الحرمل (*Peganum harmala*)، ذكور الفئران، الخصي، الخصوبة.