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Original article

Assessment of Sperm Function and Embryo Morphology Following Exposure to *Peganum Harmala* Seeds Decoction in Mice

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Abstract
Harmala *(Peganum harmala* L.) is one medicinal plant used in traditional medicine for the treatment of a lot of diseases in many countries, including Libya. This study aimed to determine the effect of harmala plant seeds decoction on testosterone levels, sperm quality, and the embryos' morphology. In this study, fifteen male mice were used at the age of 6 -10 weeks and divided into three groups (each group contained five mice). Group I (control) was given distilled water orally, while groups II and III received harmala plant seeds decoction at doses of 300 and 400 mg/kg, respectively, for three weeks. The results showed a significant decline in the mean body weight in the treated groups compared with the control, and also a significant increase in the percentage of abnormal sperm was observed in the group treated with a dose of 400 mg/kg. There was no effect of harmala seeds decoction on embryo morphology, the mean weight of testes, testosterone hormone, sperm counts, and its motility.
Keywords. Male Mice, Harmala Seeds, Testosterone Hormone, Sperm Quality, Embryos.

Introduction

Harmala *(Peganum harmala* L.*)* is one of the medicinal plants used in traditional medicine for treating numerous diseases. This plant belongs to the Family: Zygophyllaceae [1], and spreads in North Africa, the Middle East, Central Asia, and South Europe [2], as it grows in semi-arid, steppe areas and sandy soils [3]. Many studies indicated that the toxicological effects of harmala plant are attributed to the presence of bioactive compounds like saponins [4], polyphenols [5], and alkaloids, which are composed of beta-carbolines and quinazoline derivatives such as vasicine and vasicinone [6]. The beta-carbolines have inhibitory effects on monoamine oxidase type A and serotonin [7], while quinazoline alkaloids have abortive activity because of their stimulatory effects on the uterine tissues, and this effect is mediated by prostaglandins [8]. The seeds of the harmala plant possess abortive, anti-spasmodic, anti-rheumatism, anticancer, and anti-asthmatic properties [9], as well as they are useful for treating chronic headache, loss of memory, epilepsy, kidney stones, and colonic diseases [10].
Several studies indicated that harmala plant extracts have anti-leishmania [11], anti-inflammatory [12], anti-fertility [13], anti-bacteria [14], anti-depression [15], anti-diabetic [16], antitumor [17-19], antioxidants [5,20], anti-helminthic [21], and anti-HSV properties [3]. Another study revealed that the alkaloids of harmala plant inhibit DNA topoisomerase and interfere with DNA synthesis [22].
Shirani-Borougeni *et al* [23] found that administration of harmala as capsules is useful in reducing urinary tract symptoms in patients with prostate enlargement. Other research studies demonstrated that harmala seed extracts caused histological disturbances in the hepatic tissue of treated animals [24,25]. Consequents of a previous study revealed that administration of aqueous extract of harmala plant to rats six days a week for 3 months led to an increase in transaminase, as well as to histological changes were observed in central nervous system (cortex of cerebrum and cerebellum) at the dose 2 g/kg [17]. There are few research studies were carried out about the effect of harmala plant on reproductive function in male mice, therefore, this study aims to assess the impact of harmala seeds decoction on sperm function, testosterone hormone and the embryo morphology.

Methods

Experimental animals

This study was conducted on 15 adult male mice; their ages ranged between 6-10 weeks, and their weight ranged between 25-30g. Animals were maintained in a plastic cage under standard conditions and given food and drinking water continuously. Mice were reared in the animal house, Department of Zoology, Faculty of Science, University of Tripoli, Libya.

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Preparing harmala seeds
Harmala (*Peganum harmala* L.) plant samples were collected from the Western Mountain in Libya, the seeds were separated, grinded, and then maintained in a dark bottle until the time of use. Boiled harmala seeds were prepared by boiling a certain amount of seeds powder with a limited amount of distilled water for 5 min according to the required dose. The mixture was cooled, filtered. Then, the filter was used for different treatments.

Experimental design
Mice were divided into 3 groups; each group is composed of 5 males. The first group served as a control and received distilled water orally, while the second and third groups were given harmala seeds decoction with doses of 300 and 400 mg/kg, respectively, for three weeks.

Sample collection and preparation of sperm suspension
At the end of the treatment, animals were weighed, sacrificed, and dissected. The testes were removed, weighed, and kept in 10% formalin. The vas deferens was cut from each mouse and scraped with forceps in a petri dish containing 0.9 % saline solution. Sperm suspension was placed in a 37 C° incubator for 10 min. After that, the suspension was mixed well, and motile and non-motile sperm were counted under a light microscope at 400x magnification.

Sperm morphology assessment
Two smears were prepared from the suspension of each mouse, dried and stained with 1% eosin for 10 min, and the percentage of abnormal sperm was recorded.

Testosterone measurement
The blood samples were collected from facial vein of all mice in EDTA tubes, and then, centrifuged at 3000 rpm for 15 min. Testosterone concentration in serum was measured using an ELISA hormone test kit.

Embryo morphology evaluation
In the second week of the treatment, non-treated females were placed with treated males (2:1) for mating, and left together for one week. Pregnant females were dissected on the 18th day of gestation and the embryos were extracted and examined.

Statistical analysis
The data obtained from this study were analyzed by one-way analysis of variance (ANOVA) followed by Duncan' test for multiple comparisons. Differences were considered statistically significant at $P < 0.05$.

Ethical approval
All ethical procedures about dealing with animals were conducted according to the rules of the ethics committee of Tripoli University (Ref. No 20-2024).

Results

Body and testis weights in male mice exposed to harmala seeds decoction.
The results of this study revealed a significant decline ($P < 0.05$) in the body weight of treated mice with doses of 300 and 400 mg/kg of harmala seeds decoction when compared with mice from the control group Table 1. There was no significant difference ($P > 0.05$) in testis weight of treated animals compared to the control ones Table 1.

Table 1. Body and testis weight in male mice treated with harmala seeds decoction.

Groups	Body weight (g)	Testis weight (g)
The control	29.87 ± 0.93	0.26 ± 0.05
300 mg/kg treated mice	28.27 ± 0.68 *	0.22 ± 0.01
400 mg/kg treated mice	28.32 ± 0.55 *	0.21 ± 0.03

* *Significantly different from control at (p<0.05). Data are presented as mean ± SD*

Sperm quality in male mice exposed to harmala seeds decoction.
The results of statistical analysis Table 2, indicate a non-significant reduction ($P > 0.05$) in the percentage of sperm motility and sperm counts between the control and treated groups. The data on sperm abnormality show that the treated group with 400 mg/kg has the highest average abnormality compared to the control and the 300 mg/kg treated group Table 2. The sperm morphology test of treated mice revealed different abnormalities, such as coiled tail, crooked tail, amorphous head, and hairpin tail, compared to normal sperm Figure 1.

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Table 2. Sperm quality in male mice treated with harmala seeds decoction.

Groups	*Sperm motility %*	*Sperm count × 10^6 ml*	*Abnormal sperm%*
The control	77.6 ± 0.15	31.5 ± 13.7	0.33 ± 0.05
300 mg/kg treated mice	76.0 ± 0.13	21.0 ± 11.5	0.34 ± 0.06
400 mg/kg treated mice	76.8 ± 0.09	20.2 ± 2.68	0.74 ± 0.05 *

** Significantly different from control at (p<0.05). Data are presented as mean ± SD*

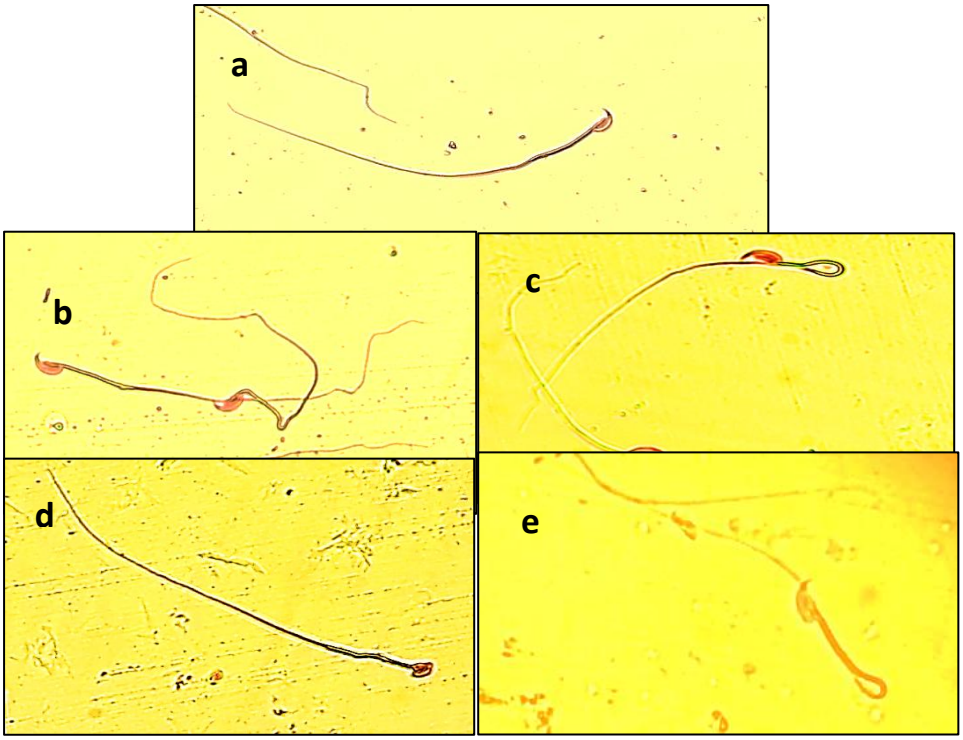

Figure 1. Different shapes of sperm morphology. (a): Normal sperm; (b): Crooked tail;(C): coiled tail; (d): Amorphous head; (e): hairpin tail. (Eosin - 40x).

Testosterone level in mice exposed to harmala seeds decoction.

The recorded findings in Table 3 demonstrated that oral administration of harmala seeds decoction at doses of 300 and 400 mg/kg to male mice for 3 weeks did not cause any influence on testosterone hormone (P > *0.05).*

Table 3. Testosterone level in mice treated with harmala seeds decoction.

Hormone	**G1 (control)**	**300 mg/kg treated mice**	**400 mg/kg treated mice**
Testosterone (ng/ml)	3.25 ± 0.04	3.24 ± 0.03	3.20 ± 0.03

Values expressed as mean ± SD.

Effect of harmala seeds decoction on the embryo morphology.

The visual examination of embryos from untreated females impregnated by treated males with harmala seeds decoction showed that they were normal, free from any deformation, and also did not differ from those from the control group.

Discussion

The results of this study revealed a significant decrease in the body weight of treated mice with doses of 300 and 400 mg/kg of harmala seeds decoction when compared with mice from the control group. This result was in line with the results of previous studies found that the harmala plant caused a decrease in the body weight of treated rats [26; 27]. Previous research also demonstrated a significant drop in the body weight of treated rats with a methanolic extract of the harmala plant at the dose of 400 mg/ kg [28]. The reduction in body weight may be attributed to the dose or the extraction method, or the period of exposure. No significant change was observed in the testis weight of treated animals compared to the control. This result is not by previous studies [13,27]. This difference among various studies may be a result of the treatment period or the method of extraction, or the dose.

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Our results also showed a non-significant decrease in sperm motility and count in treated groups compared to the control group. The boiled harmala plant seeds had a significant effect on the percentage of abnormal sperm in the treated group with 400 mg/kg. Different results have been reported in other studies: El-Dwairi and Banihani [27] found that aqueous extract of the harmala plant resulted in a marked reduction in sperm count and motility in rats treated orally for 60 days. A study demonstrated that evaporation of chlorpromazine treated rats with harmala seeds for 7, 14, and 21 days led to a significant increase in sperm count [29]. Derbak *et al* [30] stated that treatment of male mice with alkaloid extract of harmala seeds did not produce any significant difference in sperm motility. This contradiction between the studies might be attributed to the doses or the method of extraction or the part of the plant, or the period of treatment. The recorded abnormalities in this study may have resulted from sperm DNA damage that gives rise to malformations in the sperm head or dysfunction in microtubules that leads to distortions in the sperm tail [31,32].

The recorded findings in this study demonstrated that oral administration of harmala seeds decoction at doses of 300 and 400 mg/kg to male mice did not produce any impact on the testosterone hormone. This outcome is not consistence with studies were carried out by Al-Mushhadani *et al* [29] and Derbak *et al* [30], whose findings indicated that harmala plant led to a significant elevation in the level of testosterone hormone. On the contrary, a Previous study illustrated that an aqueous extract of harmala seeds gave rise to a marked drop in the level of this hormone in male mice treated for 60 days. The result of our current study also differs from previous research was conducted by Benbott *et al* [13], who stated that injection of male mice intraperitoneally with alkaloid extract of harmala seeds led to a reduction in the level of testosterone hormone. The differences observed among various studies could probably be due to the exposure method or the doses or the extraction method, or the treatment period.

The visual examination of embryos from untreated females impregnated by treated males with harmala seeds decoction showed that they are normal and similar to those from the control group. a previous research study reported that no significant change was observed in the percentage of deformed embryos of females impregnated by males treated with the aqueous extract [19]. Different results were documented by Sasi *et al* [33], who recorded deformity embryos from treated females with different doses of aqueous extract of harmala seeds. The difference among the studies may be attributed to animal gender or the treatment period, or the doses.

Conclusion

The findings of the current study showed that administering harmala seeds decoction at doses of 300 and 400 mg/kg to male mice resulted in a significant decrease in the body weight of treated mice and also a significant increase in the percentage of abnormal sperm in mice treated with 400 mg/kg. There was no effect of harmala seeds decoction on the embryos, testosterone hormone, sperm count, and its motility.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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