Histo-functional effects of *Peganum harmala* on male rat's spermatogenesis and fertility

Qasim A. EL-Dwairi & Saleh M. Banihani

Department of Anatomy, Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan

Correspondence to: Assistant Prof. Qasim A. EL-Dwairi, MBBS, PhD. (McGILL)
Department of Anatomy, Faculty of Medicine,
Jordan University of Science & Technology
P.O. Box 3030, Irbid, Jordan
PHONE: +962 02-7201000
FAX: +962 02-7205010
EMAIL: qadwairi @yahoo.com

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Abstract

BACKGROUND: The *Peganum harmala* is currently used by the Jordanian populations systemically for its antispasmodic, diuretic, sedative and analgesic effects and externally for its antirheumatic effect.
OBJECTIVE: To study effects of *Peganum harmala* on the reproductive system and fertility using adult male albino rats.
MATERIAL & METHOD: A total of 20 rats were involved in this study and were divided into two groups. Group (A) a vehicle-treated control and group (B) a treated group with aqueous extract of *Peganum harmala* at a dose of 300 mg/kg body weight for 60 days.
RESULTS: This dose induces a significant decrease in the weight of reproductive organs ($p<0.01$) when compared to controls. The sperm motility and density in cauda epidydimides and testicular ducts were significantly decreased ($p<0.01$). Furthermore treatments have remarkably altered the histoarchitecture of testes. Spermatogenesis was inhibited at both primary and secondary spermatocyte stages. Epididymides showed reduced number of spermatozoa. Lumen of vas deferentia were devoid of sperms. The secretary activities of seminal vesicle and ventricular prostate were also reduced.
Spermatocytes parameters were altered which included a significant decrease count ($p<0.001$). In addition counts of developing spermatocytes in treated rats showed a decrease in number of spermatocytes and spermatids ($p<0.001$) when compared to controls. Serum hormonal assay indicated a decrease in Testosterone and Follicular Stimulating Hormone (FSH) levels in treated rats. A decreased in number female rats impregnated by males receiving treatment was observed and demonstrated by a decrease in the implantation sites and viable fetuses number ($p<0.01$).
CONCLUSION: The aqueous extracts of *Peganum harmala* might have adverse effects on the processes of spermatogenesis due to direct or indirect effects on somnferous tubules and or the pituitary testicular axis.

ORIGINAL ARTICLE

INTRODUCTION

Medicinal plants are considered as one of the main sources for developing new drugs with potential therapeutic effects. Thus, study of traditional plant species that been used as pain killers should still be seen as a logical search strategy, in research for new analgesic drugs [1–4]. The *Peganum harmala* L. (Syrian rue) is a wild-growing flowering plant belonging to the *Zygophylaceae* family and is found abundantly in Middle East and North Africa [5]. From ancient times, it has been claimed to be an important medicinal plant. Its seeds are known to possess hypothermic and hallucinogenic properties [6,7]. It has been used traditionally as an emmenagogue and an abortifacient agent in the Middle East and North Africa [8]. There are several reports in the literature indicating a great variety of pharmacological activities for *Peganum harmala* L. such as anti-bacterial, antifungal and MAO-inhibition [9]. It has also been known to interact with α_2-Adrenoceptor subtypes [10] and have hallucination potency and to be effective in the treatment of dermatosis [11], hypothermia [12] and cancer [13].

Fertility regulation with plants or plant preparations have been reported in the ancient literature of herbal medicine in India. A number of plant species have been tested for fertility regulation beginning about 50 years ago and were subsequently fortified by national and international agencies [14–15]. The role of plant products in the induction of male and female infertility in experimental animals has drawn the attention of researchers over the turn of the century [16–18]

In the light of this facts this work was conducted to monitor the effects of *Peganum harmala* on the reproductive system and fertility in adult male rats.

MATERIAL AND METHOD

Animals and treatment

Adult male and female albino rats of Sprague Dawley strain, weighing about 300 gm. were raised in the Animal House Unit in Jordan University of Science and Technology under controlled temperature of 21±1 °C and 12 hours light:12 and hours darkness schedule (lights on 06.00 AM–18.00 PM hr). Food and Water were avialable *ad labitum*.

Plant material

Samples of *Peganum harmala* plant were collected from Mafraq area, between September to December, of 2006. The plant were identified in our laboratory, then the plant was dried and grinded with a grinder into powder preparation for extraction.

The *Peganum harmala* powder was extracted by water-ethanol mixture (70|30 v|v) for 6 hours and this step was repeated three times. The filtrate was pooled and concentrated under vacuum (not exceeding 50 °C), and dissolved in freshly prepared normal saline to a final concentration of 300 mg/kg for further use.

The extract administered orally to rats using animal feeding intubations needles (Popper and Sons, New York) in concentration of 300 mg/kg.

Experimental Design

Male rats were divided into following groups:

Group 1 – Intact (Control): The rats of this group received vehicle (Normal Saline) for 60 days.

Group 2 – Intact + Aqueous extracts *Peganum harmala* [300 mg/kg body weight of extract for a period that covers one spermatogenic cycle of 60 days.

After 24 hours of the last dose, the animals were weighed and autopsied under light ether anaesthesia. The blood was collected through cardiac puncture using a dry and clean syringe, for serum was separated and used for hormonal analysis. Simaltenously, specimens were collected fro different reproductive organs as testicles, epididymis and prostate for histological studies.

Fertility Test

Fertility was estimated in adult male rats treated with Aqueous extracts of *Peganum harmala* and in the control males counterparts. Each male was placed in an individual cage with two virgin untreated females of the same strain for 10 days during which two estrous cycles had elapsed (19). One week after the removal of the exposed males, pregnant females rats were killed by cervical dislocation under light ether anesthesia and the number of implantation sites, number of viable fetuses and the number of resorption sites were recorded.

Body and Organ Weights

The initial and final body weights of the animals were recorded. The reproductive tract was taken out trimmed free of fat and each organ was weighed separately on electronic balance. The reproductive organ taken into account in this study include testicles, epididymides, ventral prostrate, seminal vesicle and vas deferens. These organs were weighed and kept in 90% formaldehyde for further analysis.

Sperm Motility and Count

To determine the sperm count and motility, a 100 mg of cauda epididymides was minced in 2 ml of physiological saline and one drop of the evenly mixed sample was applied to a Neubauer's counting chamber under cover slip. Quantitative motility expressed as percentage was determined by counting both motile and immotile spermatozoa per unit area. Cauda epididymal and testicular sperm counts were made by routine procedures and expressed as million/ml of suspension (20).

Histological analysis

The Bouin's fixed reproductive organs (testes, epididymides, seminal vesicle, ventral prostate, and vas deferens) along were cut into small pieces and processed for histological slides. After dehydration using different

concentration of alcohol, specimens were embedded in paraffin blocks and sectioned at 5 µm, placed on a clean histological glass slide and stained using routine Haematoxyline and Eosin stains. The structures of these organs were studuied under light microscopy.

Histometry

With the help of Camera Lucida, one hundred of circular appearing somniferous tubules were traced at 80× magnification and the diameter of each tubule was measured separately. The measurement was expressed in terms of mean of all the traced tubules. Similarly, Leydig cell and their nuclei were traced at 800× magnification. In addition, epithelial cells height of cauda epididymides, caput epididymides and seminal vesicle were also traced at 360× and recorded.

Testicular Cell Population Counting

Spermatogenic elements namely spermatogonia, spermatocytes and spermatids were counted in 5 µm thick cross sections of 10 somniferous tubules obtained from 10 animals of each group. All raw counts were transformed to 'true' counts by an adaptation of Abercrombie formula [21] from germ cell diameter measurement. Interstitial cell types (such as fibroblast, immature and mature Leydig cells and degenerating cells) were estimated, applying a differential count over 200 cells population and statistically verified by the binomial distribution [22].

Hormonal Assays

Blood Plasma FSH and Testosterone concentrations was measured by Radioimmunoassay using two commercial kits (Cis BIO International Gif sur Yvette, France).

Statistical Calculation

All the values of body/organ weights and histometry were expressed in terms of mean value ± S.D. The different treatment groups were compared with control group using Chi-Square Test and Student's "t" Test [25].

RESULTS

Histological Observations

TESTES

Intact control: (Figure 1A)

The testes at control animals present a normal picture with all successive stages of spermatogenesis, i.e. spermatogonia, primary and secondary spermatocytes, spermatid and spermatozoa. In the centre the tubule, bunches of spermatozoa can be seen adhered to Sertoli cells. In the intertubular spaces healthy Leydig cells, connective tissues and blood vessels are present. Control testes showed active spermatogenesis.

P. harmala Treated Group (Figure 1B)

After 60 days of treatment of *Peganum harmala* in albino rats the relative amount of interestitial tissue was decreased. Spermatocytes were completely absent. Functional Leydig cells in the interstitial tissue could be seen. The seminiferous tubule diameter was decreased when compared with control.

Cauda Epididumes

Intact control: (Figure 1C)

Cauda epididymides show enlarged tubules. The structure is lined with pseudostratified epithelium with low columnar cell. The lumen was filled with a large number of mature spermatozoa.

P. harmala Treated Group (Figure 1D)

In *Peganum harmala*-treated rats the tubular diameter was reduced. Epithelial cell heights were low in comparison with the control group. Stereocillia were less in number and the sperms were greatly reduced in number. Intertubular stroma was increased.

Effects of P. harmala on Body and Organ Weight

Table 1 shows that intragastric administration of *P. harmala* caused a decrease in body weight, when initial and final body weight were compared in experimental group vis the control group. The weight of the testes, epididymides, seminal vesicle, ventral prostate and vas deferens were significantly decreased ($p<0.01$) in treated male rats compared to control group.

Effects of P. harmala on Sperm Dynamics and Histometrical Parameters

Table 2 shows that the motility of sperm in cauda epididymis was significantly ($p<0.001$) decreased in treated animals that *P. harmala* in comparison with control. Sperm density in treated animals, the seminiferous tubule diameter and leydig cell nuclear diameter of treated male was also decreased significantly ($p<0.01$). Epithelial cell heights in epididymides (cauda and caput) and seminal vesicle were significantly decreased ($p<0.01$). The levels of plasma FSH and testosterone a significant decrease in the treatment group as compared to the control group.

Effects of P. harmala on Testicular Cell Population Dynamics

Table 3 shows that the administration of *P. harmala* extract caused a significant decreased in the germinal cell population: spermatocytes (primary and secondary) and spetmatids were decreased to significant level ($p<0.001$). Similarly the immature and mature Leydig cells number were also decreased significantly.However the degenerating cells number was significantly increased ($p<0.001$). Decreased fibroblast and spermatogonia numbers were not altered significantly.

Effect of P. harmala on Male Rat Fertility

The results presented in Table 4 shows that intragastric administration of (*P. harmala)* diet at dose (300 mg/kg body weight) for 60 days to male rats had significantly

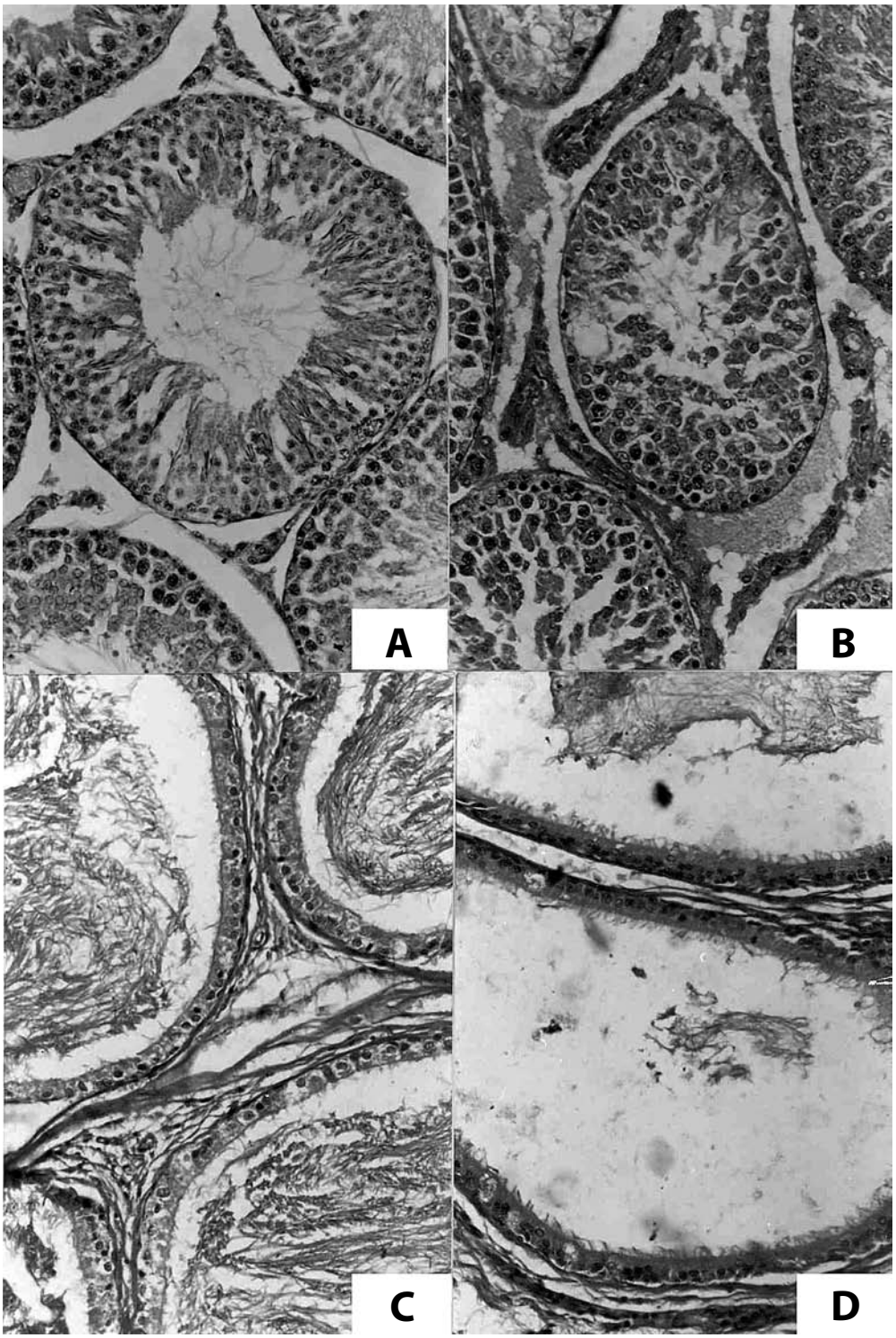

Figure 1. Histological observations.

decrease ($p<0.01$) the number of impregnated females. The number of implantations and number of viable fetuses were significantly ($p<0.01$) decreased in female rats impregnated by treated males. On other hand, the number of resorption sites was significantly ($p<0.05$) increased in females impregnated by treated-males.

DISCUSSION

The *P. harmala* is currently used by Jordanian population as a phrodisiac and fertility promoting agent. The animal model used in this work has been used previously by several workers to assess the adverse effects of extract

Table 1. Body and organ weights of *Peganum harmala* fed male rats.

Condition	Body weight [g]		Testes	Epididymides	Seminal vesicle	Ventral Prostate	Vas deferens
	Initial	Final	(mg/100 g body weight)				
Control group	304±2.80	319±2.65	895±25.21	367±21.61	376±14.38	226±4.1	113±4.36
Peganum harmala	343±4.55	382±4.55	705±14.66	326±16.33	284±17.36	206±4.55	58.75±3.55

Results are expressed as mean ± S.D.; Ten rats were included per group.
*p<0.05, **p<0.01, ***p<0.001 - significantly different from control group (Student's "t" test).

Table 2. Histometerical parameters and sperm dynamics of of *Peganum harmala* fed male rats and Hormonal Assays.

Condition	Sperm density million/ml		Sperm motility %	Seminiferous tubule	Leydig cell nuclear	Epithelial cell height			Testesteron	FSH
	Testes	Cauda		diameter	diameter	Caput	Cauda	Seminal vesicle	nmol/l	IU/L
				µm						
Control group	4.75±0.47	56.0±1.94	74.1±1.94	290.6±3.2	6.45±0.96	38.8±0.4	26.08±0.32	17.32±0.17	14.4±2.53	21.87±0.44
Peganum harmala	2.66±0.36	26.17±2.08	44.88±1.06	206.13±1.66	3.4±0.87	31.87±1.33	19.8±1.89	12.33±0.14	6.95±2.33	15.21±0.66

Results are expressed as mean ± S.D.; Ten rats were included per group.
*p<0.05, **p<0.01 **p<0.001 - significantly different from control group (Student's "t" test).

Table 3. Testicular cell population dynamics of *Peganum harmala* fed intact male albino rats.

Condition	Germinal cell types				Interstitial cell type			
	Spermatogonia	Spermatocyte (primary)	Spermatocyte (secondary)	Spermatids	Fibroblast	Immature Leydig cell	Mature Leydig cell	Degenerating cell
Control group	23.99±0.93	18.85±0.80	64.126±3.51	147.71±4.87	63.83±1.64	65.195±3.47	70.64±1.03	18.34±1.67
Peganum harmala	16.88*±3.77	11.56±2.95 **	15.67±4.18 ***	9.17±5.36 ***	34.44±2.39 **	38.37±2.06 **	43.88±1.09 **	73.55±1.23 ***

Results are expressed as mean ± S.D.; Ten rats were included per group.
*p<0.05, **p<0.01 ***p<0.001 - significantly different from control group (Student's "t" test).

Table 4. Effect of of Ruta graveolens and *Peganum harmala* fed intact on male rats fertility.

Condition	No. of male	No. of female	No. of pregnant females	No. of implantation sites	No. of viable fetuses	Total No. of resorption	No.of resorption/total no.of implantation
Control group	10	20	18/20 (85%)	9.62±2.66	9.37±1.16	8	8/173 (5%)
Peganum harmala	10	20	12/20 (60%)	8.45±2.01	8.09±1.29	24	24/101 (23%)

Results are expressed as mean ± S.D.; Ten rats were included per group.
*p<0.05, **p<0.01 ***p<0.001 - significantly different from control group (Student's "t" test).

obtainned from medicinal plants on reproductive functions in male [15].

In rats the whole spermatogenic process requires 53 days to develop and out of which spermatozoa spends the last 6 to 7 days in the final transit through epididymides [27]. The *P. harmala* was administrated for one complete spermatogenic cycle

The present investigation shows that oral administraion of *Peganum harmala* promoted decreased fertility in male albino rats. The weights of reproductive organs were markedly decreased (Table 1). The weight, size and secretory function of testes, epididymes, seminal vesicles, ventral prostate and vasa deferentia are closly regulated by androgen hormone levels [28–29]. The *Peganum harmala*

may act on pituitary gland and decrease main hormone of spermatogenesis It is well established fact that weights, size, histological appearance and secretary functions of the epididymides, seminal vesicle and ventral prostate are closely regulated by the androgens. Changes taking place in these organs after castration can be counteracted by administration of testicular hormones[28–29].

The process of spermatogenesis and accessory reproductive organs function are androgen dependent. In the present study the number of degenerating Leydig cells were significantly increased, it reflect the decrease of androgen level. It is further confirmed by decreased number of spermatocytes (primary and secondary) and spermatids as these stages are completely androgen dependent [30].The decrease weight and histometry of reproductive organs further confirmed androgen decrease.Significant decrease in the sperm motility of cauda epididymis was observed in treatment group. This may be due to activity effects of *Peganum harmala* on the enzymes of oxidative phosphorylation [31].

The results presented in this paper also show that the ingestion of *Peganum harmala* by adult male rats decreased the number of females impregnated by the treated males (Table 5). However, the number of implantations and the number of viable fetuses were decreased. This decrease appear to this effect may be due to decrease in sperm motility and sperm density.

The results presented in this work also show that the accessory organ weights were reduced in adult male rats ingested *P. harmala* (Table 1). This reduction in the accessory glands weights might suggest an alteration in the pattern of testosterane secretion.

In conclusion, these results confirm that the long-term *P. harmala* ingestion produce adverse effects on fertility and reproductive system in adult male rat. However the exact mode of action requires further studies. Moreover, these findings underline the importance of examining a number of parameters concerning the fertility and etiology to monitor the toxic potentials of various xenobiotics.

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