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Long and Short Terms Treatment Effect of *Peganum harmala* on Female Albino Rats Fertility and Pregnancy

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This study was conducted to investigate the effects of *Peganum harmala* oral administration on fertility and pregnancy outcome of female albino rats. Exposure to *Peganum harmala* for 4 weeks did not have significant effect on most parameters investigated. However, a slight decrease in the relative ovarian and embryo weights was observed. Administration of *Peganum harmala* for 12 weeks significantly reduced the percentage of pregnancies and the number of implantation sites when compared with controls. In addition, a decrease in ovarian weights and in viable fetuses' number was also observed.

Key Words: *Peganum harmala*, Female rats, Fertility, Pregnancy, Reproductive organs.

INTRODUCTION

Medicinal plants constitute the first source of drugs and this was recognized by ancient cultures as the Chinese, Indians and Arabs. Medicinal plants are believed to contain important biomedical elements with potential therapeutic as well as toxic effects. Thus, in search for new drugs, study of plants used in traditional medicine worldwide should still be seen as a logical search strategy[1-4]. The *Peganum harmala* L. (Syrian rue) is a wild-growing flowering plant that belongs to the Zygophylaceae family where the active principle of this plant seeds is recognized to be the harmaline[5]. It is frequently found in the Middle East and North Africa regions[6,7]. Since ancient times, it has been claimed that this plant has important medicinal effects. Extracted plant's seeds are known to possess hypothermic and hallucinogenic properties[5,8,9]. In addition, within the Middle East and North Africa, it was popular for its traditional usage as an emmenagogue drug and an abortifacient agent[10]. There are several reports in the literature that indicate its diversified pharmacological effects such as antibacterial, antifungal and monoamine oxidase (MAO) inhibition[11]. With emphasis on treatment of some dermatological conditions this plant was also considered in treatment of some hypothermic conditions[12,13] and cancer[14]. This plant was also known to interact with α_2-Adrenoceptor subtypes inducing hallucination[15]. Other reports from literature indicate

diversified unwanted effects of this plant when taken in high dosage such as visual disturbances, loss of coordination agitation and in same cases paralysis when taken in high doses[5].

A large number of plant species have been tested for their effects on fertility regulation beginning *ca.* 50 years ago and were subsequently fortified by national and international agencies[16,17]. The role of diversified plant products in inducing male and female infertility in experimental animals has drawn the attention of researchers[18-20]. Therefore and in the light of these facts the present work was conducted to monitor the effects of *Peganum harmala* on female rat's reproductive system with emphasis on the fertility and pregnancy outcome.

EXPERIMENTAL

Adult female Sprague-Dawley rats (40) weighing 250-300 g were used in this study. Rats were raised in the animal house unit/Jordan University of Science and Technology, Irbid, Jordan (JUST) under a controlled temperature of 21 ± 1.0°C and 12 h light/dark cycle. Animals were feed with regular diet (manufactured by the Faculty of Veterinary Medicine at (JUST), according to standard recipes) and water was provided *ad libitum*. Female rats were randomly divided into two treatments and two corresponding control groups of 10 rats each.

Treatment with *Peganum harmala*: *Peganum harmala* plant extract was dissolved in tap water and treated rats receive this extract through an intra-gastric tube administration at a concentration of 300 mg/kg/body weight as one morning dose.

Treated rats were divided into two groups: Group 1 consists of 10 female rats treated for a period of 4 weeks. Group 2 consist of 10 female rats treated for a period of 12 weeks.

Control groups: Group 3 and 4 consist of 20 female rats receiving no treatment.

All rats were allowed normal diet and access to drinking water for the same time periods.

Fertility test: Routine daily observation of rats exposed to *Peganum harmala* for clinical signs of toxicity was done. In addition, treated rats body weights were measured weekly.

After each treatment time period, treated and control groups of rats were divided randomly into subgroups of two female rats that were caged with a sexually mature male rat for 10 d to allow mating. The effect of *Peganum harmala* ingestion on the occurrence of implantation was estimated in treated and their control counterpart's female rats after the appropriate time of mating exposure. It was estimated that at least two estrous cycles have elapsed during this exposure time[21].

After the estimated mating time, treated and control counterparts female rats were weighted and sacrificed by cervical dislocation under light ether anesthesia. Autopsy was performed and the following parameters in both groups were recorded: the number of implantation sites, the number of viable fetuses and the number of resorption sites. Furthermore, uterus weights, ovary weight in addition to the embryo weights were recorded.

Statistical analysis: Data was expressed as mean ± standard deviation (SD). The differences between *Peganum harmala* treated and controlled groups were analyzed using Student 't' test[22].

RESULTS AND DISCUSSION

Exposure toxicity of *Peganum harmala*: None of the female rats used within the 4 week exposure group (group 1) showed any clinical signs of toxicity. However, one female rat exposed for 12-week treatment period with *Peganum harmala* (group 2) died.

Effects of *Peganum harmala* on fertility: Short term treatment with *Peganum harmala* extract for 4 weeks revealed a slight decrease with no significant reduction in the rate of impregnation, the number of implantation sites, as well as the number of viable fetuses when compared with controls (Table-1a). A slight but not significant elevation in the percentage rate of resorption site was observed in this group when compared with controls. Furthermore, the ratio between the resorption and the total number of implantation was observed to be in a slight elevation (Table-1a).

TABLE-1a
EFFECT OF 4-WEEK EXPOSURE TO *P. harmala* ON FERTILITY OF FEMALE RATS

Treatment	No. of Pregnant females	No. of Implantation	No. of Viable Fetuses	Rats of Resorption sites	No. of Resorption sites /Total No. of Implantation sites
Control	9/10	9.33 ± 2.39	8.77 ± 2.72	4/10 (40%)	5/84 (5.90%)
P. harmala	7/10†	7.65 ± 4.14‡	7.86 ± 0.45‡	5/7 (71.4 %)	24/54 (44.44%)

The effect of 12 weeks exposure to *Peganum harmala* by female rats (group 2) on the fertility indicate that there is a significant decreases in the percentage of impregnated rats in the treatment group when compared with the control counterparts (Table-1b). Table-1b also indicates that the long term exposure to *Peganum harmala* for 12 weeks induces a decrease in both the number of implantation sites as well as the number of viable fetuses to a statistically significant level. It is also observed that the percentage of resorption sites in treated female rats for long term period is

elevated, where the ratio between the resoption sites and the number of implantation was induced greatly (Table-1b).

TABLE-1b
EFFECT OF 12-WEEKS EXPOSURE TO *P. harmala* ON FERTILITY OF FEMALE RATS

Treatment	No. of Pregnant females	No. of Implantation	No. of Viable Fetuses	Rats of Resorption sites	No. of Resorption sites /Total No. of Implantation sites
Control	9/10	9.33 ± 2.39	8.77 ±2.72	4/10 (40%)	5/84 (5.90%)
P. harmala	3/8†	6.13 ± 2.81‡	6.23± 1.85‡	3/5 (60%)	14/18 (29.16%)

Results are expressed as means ± SEM.
†$p < 0.05$: significantly different from the control group (Fisher exact test).
‡$p < 0.05$: significantly different from the control group (Student's *t* test).

Effects *Peganum harmala* on maternal organs weight and embryo weight: Table-2a shows that ingestion of *Peganum harmala* for 4 weeks resulted in a slight but insignificant reduction in female rat's body as well as uterine weights. A statistical significance decrease in the relative ovarian and embryo weights in this group was observed when compared with control counterparts (Table-2a).

TABLE-2a
EFFECT OF 4 WEEKS EXPOSURE TO *P. harmala* ON MATERNAL BODY, ORGAN AND EMBRYO WEIGHTS

Treatment	Final body weight (g)	Ovary weight (g) (mg/100g Bwt)	Uterus weight (g) (mg/100g Bwt)	Embryo weight (g) (mg/100g Bwt)
Control	256 ± 18.67	0.34 ± 0.05	0.51 ± 0.01	0.31 ± 0.04
P. harmala	238 ± 13.56	0.28 ± 0.01*	0.45 ± 0.03	0.26 ± 0.07†

Results are expressed as means ± SEM; *$p < 0.05$, †$p < 0.01$: significantly different from the control group (Student's *t* test).

TABLE-2b
EFFECT OF 12 WEEKS EXPOSURE TO *P. harmala* ON MATERNAL BODY, ORGAN AND EMBRYO WEIGHTS

Treatment	Final body weight (g)	Ovary weight (g) (mg/100g Bwt)	Uterus weight (g) (mg/100g Bwt)	Embryo weight (g) (mg/100g Bwt)
Control	256 ± 18.67	0.32 ± 0.05	0.49 ± 0.01	0.31 ± 0.04
P. harmala	227 ±15.65	0.23 ± 0.03*	0.41 ± 0.08	0.24 ± 0.32†

Results are expressed as means ± SEM; *$p < 0.05$, †$p < 0.01$: significantly different from the control group (Student's *t* test).

On the other hand, the ingestion of *Peganum harmala* for 12 weeks resulted in a significant reduction in both the relative ovarian weight and embryo weight when was compared to controls (Table-2b). No differences were observed in the final body weight or in the uterine weight in rats treated for 12 weeks with *Peganum harmala* when compared with controls, in contrary a slight reduction can be noticed (Table-2b).

Histological effects of treated female rats: All ovarian section of female treated with *Peganum harmala* showed certain structural variation comparable to their control counterparts. The ovarian sections of females treated with 300 mg/kg of *Peganum harmala* for 12 weeks shows beginning of corpus luteum degeneration due to active angiogenetic changes presented by the presence of large number of congested blood vessels. Highly congested blood vessels were found surrounding corpus luteum and in medulla. Some section showed decreased number of developing follicles, while in others normal developing follicles were absent. The number of atretic follicles ranges between low to high. Besides that, large fluid filled cavities were present in most section. However, in the control group thicker corpus besides significant number of developing follicles at primary, secondary and tertiary stages were present.

P. harmala is currently used by Jordanian urban population as an aphrodisiac and as a fertility promoting agent. The animal model in this work has been previously used by several other workers to assess the adverse effects of other extract obtained from medicinal plants on reproductive functions in rat male[17].

This study was conducted to investigate the exposure effect of *Peganum harmala* on the structure, fertility and the pregnancy outcome of adult female Sprague-Dawley rats. The dose of 300 mg/kg. body weight of *Peganum harmala* was selected to obtain broader range of information on the effects of this plant on the reproduction parameters. Two different time period were selected namely 4 and 12 weeks.

No work has been published in the literature that relates the effects of *Peganum harmala* to structure, fertility and pregnancy outcome. It has been postulated however, that administration of this plant to female rats for 30 d in different dosages induces dose-dependent decrease in the size of the offspring with no toxicological effect observed[7]. This is in accordance with present results which showed that the exposure of adult female rats to *Peganum harmala* for 4 weeks had neither toxic, nor significant effects on the rat's fertility parameters or structure of the reproductive system. However, a slight decrease in the relative ovarian weights and a significant decrease in the embryo weight in rats treated for 4 weeks was observed. On the other hand, an increase in the exposure period for 12 weeks using similar dose of this plant extract revealed a significant decrease in both the relative ovarian and embryo weights when compared to controls.

Histological changes in ovaries of females treated with *Peganum harmala* extract provide a clear evidence of reproductive toxicity of *Peganum harmala* extract ingestion. Degeneration of corpus luteum impedes pregnancy maintenance in case of successive implantation, leading to occurrence of early resorptions. Also the arrestment of developing follicles at primary and secondary stages with the presence of decreased number of developing follicles at primary stage may result in reduction in the final number of mature graafian follicles produced. Congested blood vessels in corpus luteum and in medulla may interfere with material exchange and indicate in active resorption. Furthermore, the presence of degenerative areas and or hypercellullarity in medulla may interfere with migration of developing follicles and impede their future development.

Other important findings of this study showed that this plant might promote a decreased in Sprague-Dawley female rats fertility when intragastric administration for long period of time was applied. This was indicated by the decrease in the reproductive organ weights observed in this group of rats. However, the weights of reproductive organs were markedly decreased as shown in Tables 2a and 2b which might be explained by the fact that the reproductive organ weights can be closely regulated by androgen hormones[23]. One can hypotheses that this extract may act on the hypothalamic-pituitary ovarian axis which may lead to a decrease in the main hormones influencing oogenesis and subsequent pregnancy. The decrease in the weight of reproductive organs can be explained by the possible decrease in the level of androgen hormones that could be decreased in the experimental group of rats. The unexplained decrease in the ovarian weights in treated rats needs to be clarified through both hormonal and histological analysis. In addition, the future use of advanced molecular methodologies might elucidate the pathway through which this plant act to decrease the weight of the ovaries observed in this study. These results, therefore, suggest that any disturbance of the reproductive endocrine functions may possibly and can go hand in hand with multiple sites of androgenic toxicity acting along the hypothalamic-pituitary-ovarian-uterine axis.

Other main finding of this current study was the significant reduction in the occurrence of pregnancy in rats exposed to *Peganum harmala* for 12 weeks. This decrease may be due to long dysfunctional period of the endocrine functions that might lead to decreased secretion of progesterone which is needed for endometrial alteration at the time of implantation and is necessary for successful impregnation[24,25]. This is accordance with present results indicating the significant decrease in the number of implantation sites which could lead to the decrease in viable fetus's number. The author is now conducting a research to investigate the effect of *Peganum harmala*

exposure on serum progesterone levels. In conclusion, the results of the current study suggest that ingestion of *Peganum harmala* by adult female rats causes adverse effects on fertility and reproduction.

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