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Evaluation of Maternal Toxicity in Rats Exposed to the Total Extract of the Alkaloids in the seeds of *Peganum harmala* L. during Pregnancy

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ABSTRACT

Peganum harmala L. (Zygophyllaceae) known locally as harmel is a medicinal plant. In traditional medicine, its seeds have long been used for therapeutic purposes because of their richness in β-carboline alkaloids. This study aimed to evaluate the maternal and developmental toxicity during pregnancy by daily IP administration of 7.99 mg/kg/day (1/20 DL_{50}) of total alkaloids extract in P.harmala' seeds. The results summarized in confirmed pregnancy rates were high 90-100%, decreased locomotor activity, paralysis, and hypothermia. Maternal body weight and weight gain changes were statistically significant in all pregnant. Precisely, the relative weight of ovaries was significantly changed in all the groups treated. The ALAT and gamma GT concentrations show a significant change in the group treated for seven days. Significant changes in the total and indirect bilirubin levels were observed in all treated groups. The hormonal analysis showed a significant decrease in FSH levels in a treated group for seven days and two weeks, Progesterone levels were increased significantly in treated groups for seven and three weeks and increased significantly in a treated group for two groups, however, the levels of Estrogen were changed significantly only in the treated group for three. The results show a significant difference in total resorbed litters and the number of fetus deaths in the group treated for three weeks. The fetus weight in the group treated for two weeks was significant. The results show a significant decrease in the number of implantations and an increase in pre-and post-implantation loss rates, and there were no developed live or dead, and no resorbed fetuses in all treated dams, there were only implantation sites in both uterine horns. The total extract of the alkaloids in the seeds of P. harmala has adverse effects on maternotoxicity, embryonic development, and abortion.

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Introduction

Folk medicine has a lengthy history of evolution over time, which is a combination of many different cultures (Uysal et al., 2021; Akgül et al., 2022). Presently, internet access is the most essential and inexhaustible resource of all knowledge about ethnobotanical plants since it is simple to utilize (Kına et al., 2021; Pehlivan et al., 2021). Furthermore, the most significant advantage of herbal medication is its inexpensive cost in comparison to modern pharmaceuticals, as well as the confidence in minimal adverse effects (Pu et al., 2017; Sevindik et al., 2017; Mohammed et al., 2022), which put people at risk of the poisoning by these plant materials intentionally or accidentally (Hamdy et al., 2017; Al-Tohamy et al., 2018). *Peganum harmala* L. (Zygophyllaceae) known locally as harmel is a plant commonly found in Algeria. It occupies the stations of arid hillsides, dry uncultivated fields, and earthy steppes (Jahandiez and Maire, 1932). In traditional medicine, its seeds have long been used as narcotics, anthelmintics, antispasmodics, and in some cases against rheumatism and asthma (Siddiqui et al., 1988; Bellakhdar, 1997). This use of seeds is due to their richness in β-carboline alkaloids, the most important are harmine, harmaline, harmol, and harmalol as has already been reported for the first time. By Goebel, 1841 (Merck Index, 1989) and taken up by many authors. Its pharmacological interest is no longer to be demonstrated: it exhibits antiviral activity (Rashan and Adaay, 1989), abortive in rats (Shapira et al., 1989; Nath et al., 1993; Adaay 1994), and exhibits toxicity even in humans (Ben Salah et al., 1986). In the present paper, we summarized the maternal and developmental toxicity of total extract of the alkaloids in the seeds of P. harmala in experimental animals during Pregnancy. Few studies have reported significant findings regarding the developmental toxicity of alkaloids in humans. Available data from animal experiments may indicate the cause for concern. unless otherwise stated.

Materials and Methods

Plant Collection and Identification

P. harmala seeds were harvested in August from the Touama area in Bordj Bou Arreridj (northeast Algeria), which has a dry, semi-arid environment. The plant and its seeds were identified based on morphological features (Chopra et al., 1960) (Figure 1). Before extraction, the seeds were air dried at ambient temperature (20 to 25°C) for more than a month, then ground individually in a coffee grinder during the extraction.

Figure 1. The seeds of *Peganum harmala* L.

Extraction of the Total Alkaloids

The complete alkaloid extract in the seeds of P. harmala was extracted by using the Soxhlet extraction technique of Bruneton, (1999) (with slight adjustments), and the presence of alkaloids in the extract was confirmed using Thin Layer of Chromatography (TLC) as described by Bouzidi et al., 2011.

Experiment Animals and Husbandry

Fifteen proven fertile adult males and forty virgin or nulliparous healthy young female albinos wistar rats, 10 weeks of age and weighing 170–200 g, were obtained from our colony in the Ferhat abbes university

For husbandry, three females were placed into a cage with a male rat overnight. The next morning successful mating was confirmed by the presence of sperm in the vaginal smear (Jahnke, 1999), and the following 24 hours were designated as day 0 of gestation (GD0). Confirmed-mated females were assigned to treatment groups by stratified randomization (9/group in the screening study), so that mean body weight on GD0 did not differ among treatment groups in the study. Maternal body weights, for confirmed pregnant females used in these studies, ranged from 170 to 200 g on GD0. Confirmed-mated females were individually housed were housed individually and the Pregnancy was verified by weight gain and abdominal palpation.

Dosage and Treatment

Dose formulations were prepared by dissolving 1/20 DL_{50} mg witch equal to 7.99 mg of total extract of alkaloids of the seeds of P. harmala in10 micro-liter of methanol and one milliliter of normal saline. The suspension has subjected to agitation for 3 minutes to obtain a more homogenous and dispersed suspension. The test dose was prepared daily prior to use. In the morning, time-mated rats were administered a dose volume of 7.99 mg/kg/ day of total extract of the alkaloids daily by intraperitoneal injection. The pregnant rats received the treatment from GD0 to GD6 designed to be the group treated for seven days, GD0 to GD15 of treatment a group treated for two weeks, and a group treated for three weeks from GD0 through GD19. Control rats received an equivalent volume of vehicles alone.

Maternal Evaluations

Clinical Signs, Body Weight

Throughout pregnancy, mated females were observed daily for mortality, morbidity, general appearance, behavior, and clinical condition at least once/day on GD0 through 19, females were observed for clinical condition and signs of toxicity at daily dosing, and generally at first to 8 hours thereafter. Maternal body weights (g) were recorded on the mornings of GD0 and GD20, and immediately after the following sacrifice on GD20. On GD20, females were observed for the clinical condition at weighing and again at scheduled termination.

Gross Findings, Organ Weights

On GD 20, all time-mated females were euthanized and sacrificed by an overdose of ether and were exsanguinated via the aorta in the early morning. A complete gross postmortem (Thoracic and abdominal cavities) examination was performed. The absolute and relative weights of the brain, lungs, liver, spleen, kidneys, heart, and ovaries were recorded. In addition, Pregnancy status was confirmed by uterine examination. Uterine contents were examined to determine the number of implantation sites (sum of live pups and dead fetuses), pre/post-implantation, resorptions, dead or resorbed fetuses, and live fetuses. Uteri which observed very well to record any implantation sites which might have undergone very early resorption (Jahnkle, 1999). the reproductive parameters were computed According to Toyin et al, (2014).

Serum Biochemical Examination

Blood samples were taken from their orbital sinus by hematocrit, and were centrifuged at 3000 rpm for 10 minutes within 1 hour of collection. Using a biochemical analysis is important to estimate alterations in organ function. After serum isolation, all the hepatic (aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutaryl-transferase, alkaline phosphatase (PAL), total, direct, and indirect bilirubin), renal parameters (blood urea nitrogen, creatinine, glucose, and monogrammed Na/K/Cl), and hematologic parameters (RBC, Hb, HCT, MPV, MCV, MCHC, MDW, WBC, LYM, PDI, PTC, LPCR, MID, GRA)

Serum Hormonal Examination

The density of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estrogen, and progesterone hormones was measured according to the ELISA in the laboratory.

Statistical Analysis

The unit for statistical measurement was the pregnant female or the litter. For each statistical comparison, the significance was reported as $P<0.05$. Nonparametric tests applied to continuous variables included the Tukey; one-way analysis of variance (ANOVA) by ranks for among-

group differences for a significant $P<0.05$, and two-way analysis was used for all parameters, except that maternal and fetal body weight parameters, were used to identify significant dose-response trends.

Results

Pregnancy Detection

The presence of sperm in the vaginal smear or observation of a vaginal plug indicates the occurrence of mating. On the other hand, detection of sperm in vaginal smear is an excellent predictor of pregnancy in rats. The day that sperm is detected in the vaginal smear is designated as day 1 of gestation. After 10 days of gestation, the fetuses can be palpated, but palpation is more accurate after day twelve. By day thirteen of gestation, the abdominal enlargement is visible, and mammary development and nipple enlargement can be observed on day 14 of gestation. Successful mating was confirmed by the presence of sperm in the vaginal smear (Figure 2) the following morning and this day was considered day 1 of pregnancy. The rate pregnancy rate was high 100% in the both groups; control and mated females.

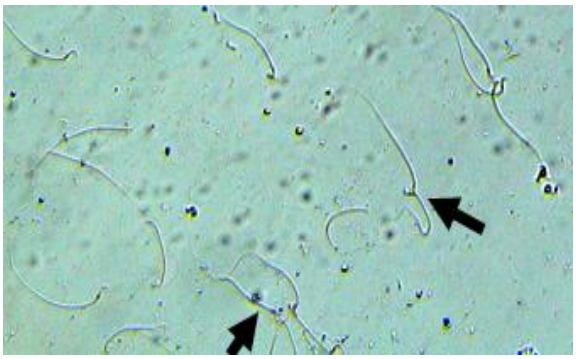

Figure 2. The presence of sperm in the vaginal smear

Maternal toxicity, Abortion

Maternal Evaluations; Clinical Signs and Body Weight

In the maternal and developmental toxicity by repeated intra-peritoneal administration of 1/20 DL_{50} mg/kg/day of total extract of the alkaloids in the seeds of P. harmala, confirmed pregnancy rates were high 90-100% for all groups (Table 1) and no maternal morbidity/mortality was observed in the dams of the control, treated groups for seven days, two weeks, three weeks. On the other hand, decreased locomotor activity, paralysis, dyspnea, depression, and hypothermia were identified in dams of treated groups by total extract in the first two hours after the treatment and only continued in the first seven days of the gestational period.

Upon measuring the maternal body weight and weight gain statistically significant changes were observed in all pregnancy rates during pregnancy whether these changes were an increase or a decrease between the control group and treated groups for seven, two, and three weeks. In addition, there were statistically significant differences in maternal weight gain during pregnancy for the group treated with 1/20 DL_{50} mg/kg/day of total extract (Table 1).

The results show that the relative weights of the liver, heart, and brain were no significant changes in all experimental groups, while the relative weight of kidneys in the groups of females treated for seven days and three weeks by the total extract was significantly increased, but not in the other group in comparison to the control, for the relative weight of lung a the significant increased was observed in the group treated for seven day and two weeks and only in group treated for seven days has a significant increased in spleen relative weight. Precisely the relative weight of ovaries was significantly changed in all the groups treated with the total extract compared to the control group (Figure 3).

Serum Biochemical and Hormonal Analyses

To judge functional abnormalities in the dams, we analyzed serum biochemical values at the end of the gestational period. There were no statistically significant differences in the Aspartate ASAT and PLA concentrations between the treated groups and the control, while the ALAT (Figure 4) and gamma GT concentrations show significant changes in the group treated for seven days only (Figure 5). Significant changes in the total and indirect bilirubin levels were observed in all treated groups in comparison to the control (Figure 6). The renal parameters show a significant change in glucose, blood urea nitrogen and creatinine levels in the group treated for two weeks, the group treated for three weeks and the group treated for seven days respectively (Figure 7). The blood ionogram analysis show no significant changes in the potassium (K), but the levels of sodium (Na) were change significantly in the groups treated for seven days and two weeks, while the levels of chloride (Cl) changed significantly in the group treated for two weeks (Figure 8).

The hormonal analysis shows a significant decrease in FSH levels in the treated group for seven days and two weeks as shown in Figure 9, Progesterone levels increased significantly in treated groups for seven and three weeks and increase significantly in the treated group for two groups, however, the levels of Estrogen were changes significantly only in the treated group for three weeks as shown in Figure 10. There were no changes in the LH levels between the groups and the control.

Hematological Analyses

The blood parameters demonstrated significant changes between the control group and the treated groups in all the parameters as we can see in Table 2.

Embryo/Fetal Evaluations

Table 3 summarizes the reproductive and the findings for the pregnant treated rats with 1/20 DL_{50} mg/kg/day of total extract of the alkaloids on gestational days 1 through 20. The results show a significant difference in total resorbed litters and the number of fetus deaths in the group treated for three weeks only. There were no significant differences between the treated groups related to the number of corpora lutea and between the control and treated groups, the fetus weight in the group treated for two weeks was significant. A remarkable result in all the studies was in the group treated for seven days, Thus the results show a significant decrease in the number of implantations, an increase in pre-and post-implantation loss rates, and there were no developed live or dead, and no resorbed fetuses in all treated dams, there were only implantation sites in both uterine horns.

Table 1. Dam body weight changes of pregnant rats

Parameters	1/20 DL $_{50}$ of total extract (mg/kg/day)			
	Group control	Group treated for seven days	Group treated for two weeks	Group treated for three weeks
Maternal pregnancy status				
No. of rats mated	10	9	9	10
No. of dams	10	8	8	9
Maternal body weight (g)[A,B]				
Gd 1	215±5.137	171.66±5.713	169.88±4,392	178.33±2.205
Gd 7	220±4.249	178.88±8.489*	209.44±4.522	183.88±3.977
Gd 14	224.44±5.429	189.44±5.429*	227.22±4.867*	179.44±5.234
Gd 21	233.88±5.122	196.11±5.122*	252.77±8.544*	198±6.388*

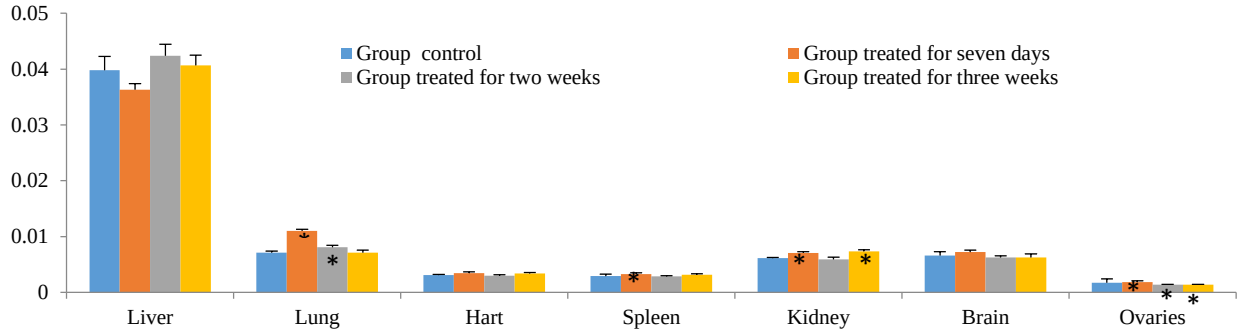

Figure 3. Maternal relative weights of pregnant rats. Values are presented as the means±SEM, * significant differences at P<0.05.

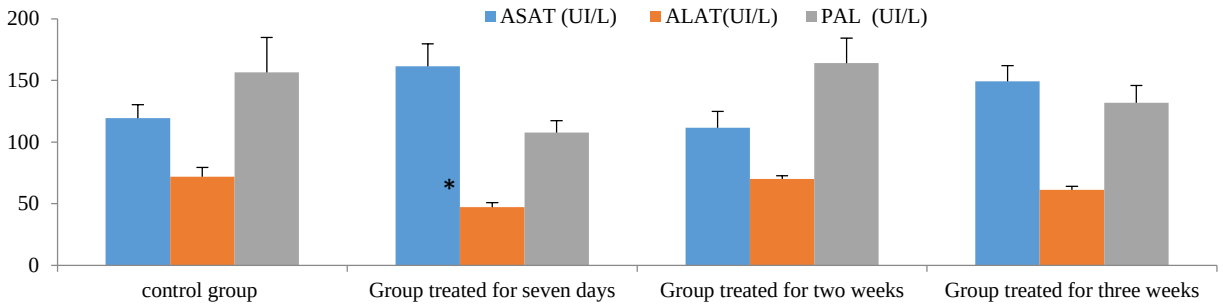

Figure 4. Maternal serum biochemical values of ASAT, ALAT and PAL in of pregnant rats. Values are presented as the Means±SEM, * significant differences at P<0.05.

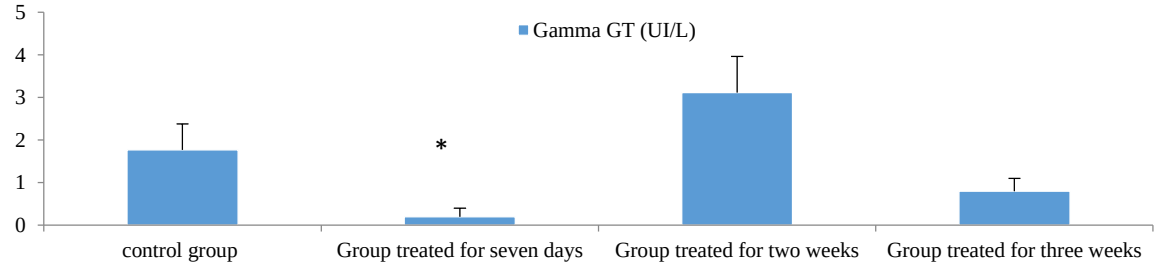

Figure 5. Maternal serum biochemical values of Gamma GT in of pregnant rats. Values are presented as the Means±SEM, * significant differences at P<0.05.

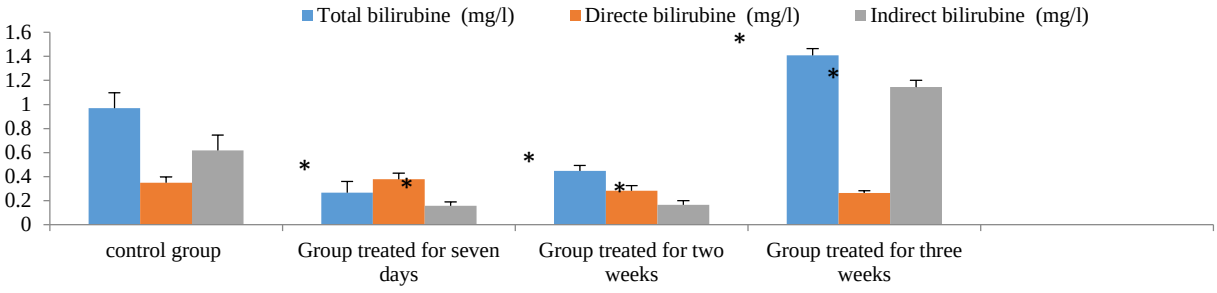

Figure 6. Maternal serum biochemical values of total, direct and indirect bilirubine. Values are presented as the Means±SEM, * significant differences at P<0.05.

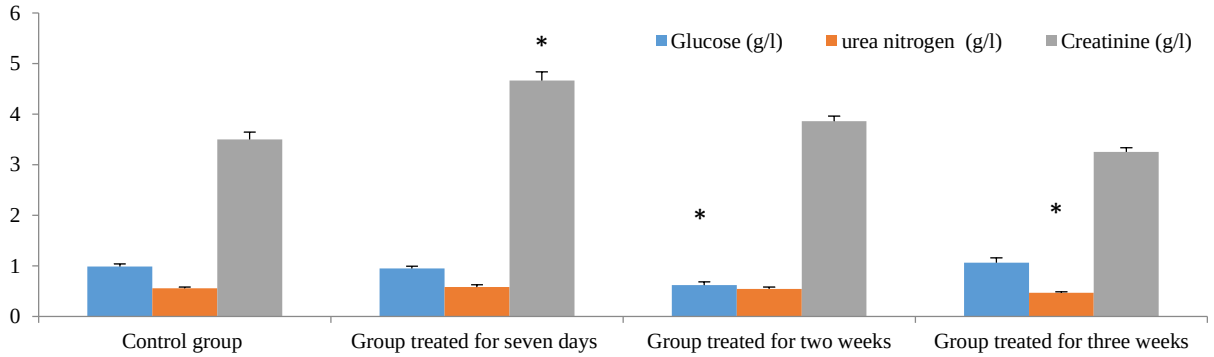

Figure 7. Maternal serum biochemical values of glucose, blood urea nitrogen and creatinine in of pregnant rats. Values are presented as the Means±SEM, * significant differences at P<0.05.

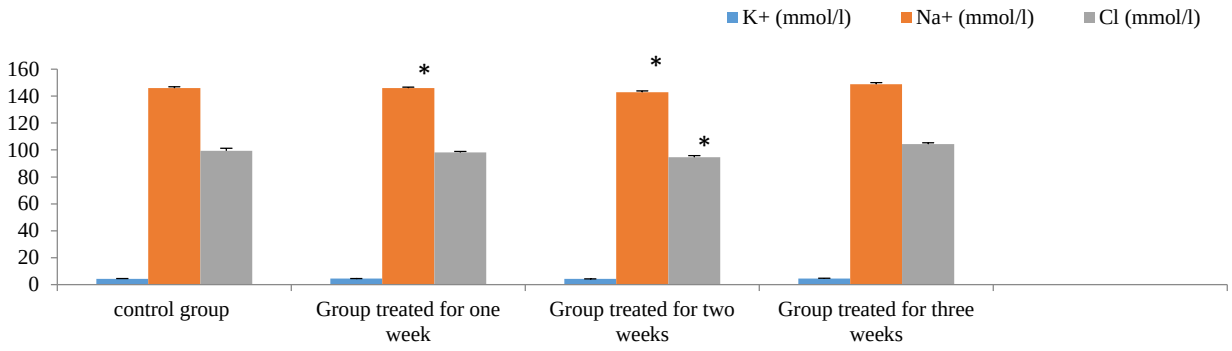

Figure 8. Maternal serum biochemical values of blood ionogramme k+, Na+ and Cl- in pregnant rats. Values are presented as the Means±SEM, * significant differences at P<0.05.

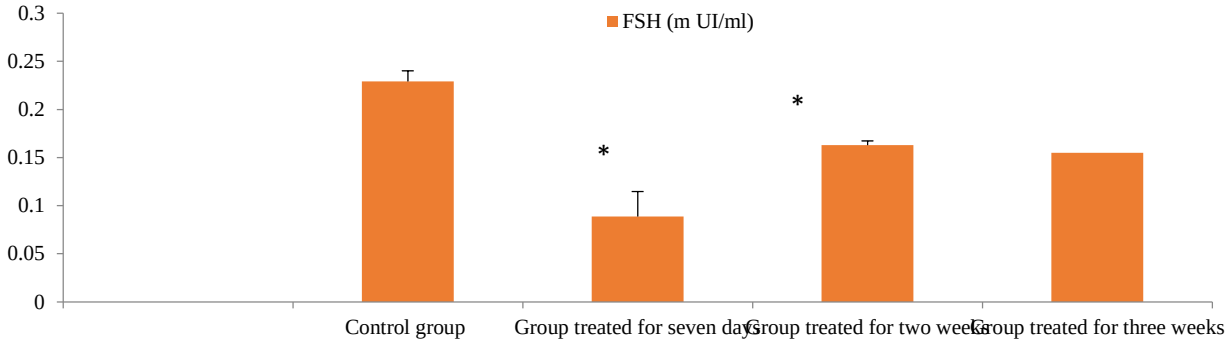

Figure 9. Maternal hormonal values of FSH in of pregnant rats. Values are presented as the Means±SEM, * significant differences at P<0.05.

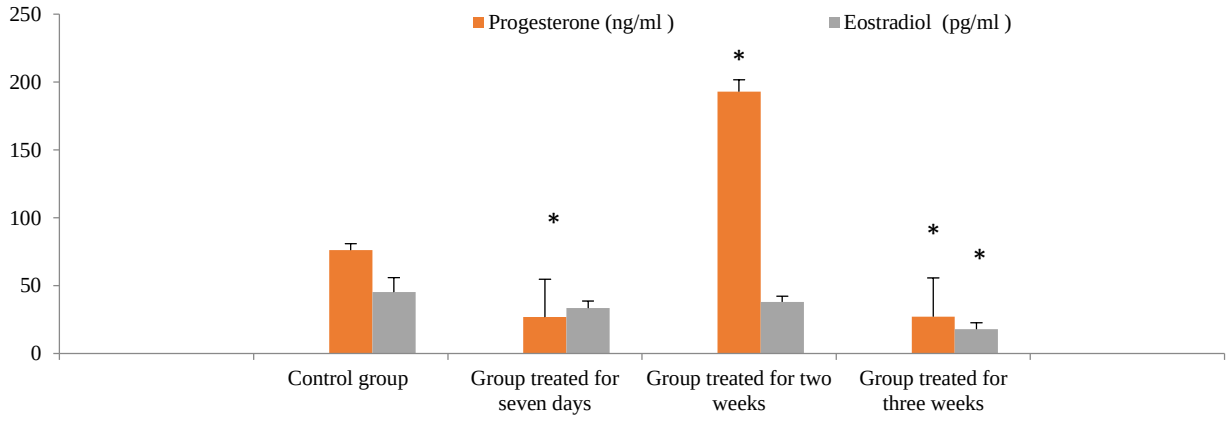

Figure 10. Maternal hormonal values of Progesterone and Estradiol in of pregnant rats. Values are presented as the Means±SEM,*significant differences at P<0.05.

Table 2. Effects of 1/20 DL50 of total extract in the seed of *Peganum harmala* on hematological parameters in female rats.

P	RBC10*6/mm^3	Hb g/dl	HCT %	MPV fl	MCV fl
C	7.004±0.314	12.957±0.495	37.514±1.48	53.743±0.854	5.929±0.115
7D	7.634±0.195	13.344±0.301	42.944±1.034*	56.278±0.376*	7.762±0.127*
2W	5.501±0.588*	10.3±1.034*	29.589±3.12*	54.122±0.548	6.044±0.0818
3W	6.831±0.184	12.613±0.265	36.825±0.929	53.925±0.497	6.188±0.0811
P	MCHC g/dl	RDW	WBC10*3/mm3	Platelet	
C	34.729±0.218	29.257±0.934	5.573±0,642	657.574±112.54	
7D	31.078±0.145*	26.578±0.805*	6.284±0.314	907.903±125.96*	
2W	35.656±0.908	29.733±0.685	5.671±0.837	749.111±73.209	
3W	34.25±0.241	29.325±0.666	6.891±0.955	679.908±98.44	

P: Parameters; C: Control group; 7D: Treated group for seven days; 2W: Treated group for two weeks; 3W: Treated group for three weeks

Table 3. Dam Cesarean section observations and fetal weights

Parameters	1/20 DL $_{50}$ of **total extract** (mg/kg/day)			
	Group control	Group treated for seven days	Group treated for two weeks	Group treated for three weeks
Maternal pregnancy status				
No. corpora lutea/dam	11.222±0.364	11.00±0.840	11.333±0.687	11.778±0.619
Total implant sites				
No. implantation sites/dam	8.5±0.719	3.00±0.00*	9.625±0.498	9.40±0.872
Pre-implantation loss (%)^A	24.25	72.72*	15.07	20.19
Post-implantation loss (%)^B	13.76	100*	14.28	19.14
No. resorptions/dam	0.778±0.434	0.429±0.429*	1.222±0.401	4.333±1.453*
No. fetal life/dam	7.33±0.989	00±00*	8.25±0.701	7.6±0.872
No. fetal death/dam	0.778±0.434	0.429±0.429*	1.222±0.401	4.333±1.453*
Fetal weight (g)	1.074±0.167	-	1.555±0.054*	1.76±0.442
No. stunted fetuses				

A: Values are presented as means ± SD.,*significant differences at P<0.05; B: Pre-implantation loss (%) = [(No. of corpora lutea - No. of implantation sites)/ No. of corpora lutea] ×100; C: Post-implantation loss (%) = [(No. of implantation sites - No. of live embryos)/ No. of implantation sites] ×100.

Discussion

A toxicant usually induces more than one type of effect in a dose-dependent manner. For example, induction of malformation is almost invariably associated with increased embryonic death and an increased incidence of less severe structural changes. Given an effect on one endpoint, secondary investigations for possible associations should be considered, (the nature, scope, and origins of the substance's toxicity should be characterized). The characterization should also include the identification of dose-response relationships to facilitate risk assessment; this is different from the situation in first-pass tests where the presence or absence of a dose-response assists discrimination between treatment-related and coincidental differences (Health Canada Detection of Toxicity to Reproduction for Medicinal Products, Guidance for Industry ICH Topic S5A, 1996). An Embryotoxicity study was conducted to investigate potential adverse effects of total extract of the alkaloids of P. harmala. on pregnant dams or maternotoxicity, embryonic development, and the abortion by inter-peritoneal repeated dosing of 1/20 DL_{50} mg/kg/day on three stages of gestation from GD1 to GD7, GD1 to GD14 and through 21 days.

The results obtained showed no mortality of dams whether the toxicity signs were observed as a daily decrease in locomotor activity, paralysis, depression, and hypothermia first two hours and continued for the first few days of the treatment period only. The clinical presentation of this study is nearly similar to the case report of Azizi et al., (1989), in which Harmaline and Harmine treatment by different inter-peritoneal concentrations in pregnant rats, resulted in toxic symptoms including decreased daily activity and mild tremor were seen that progressed to restlessness, severe tremor (Azizi et al., 1989). particularly when P. harmala co-ingested with other drugs or foods such as the case reported by Yuruktumen et al. (2008) toxic effects of other cases presented 3-4 hours after ingestion of the plant. In human cases of P. harmala intoxication precisely in women (Frison et al., 2008; Yuruktumen et al., 2008) and animals (Marwat et al., 2011) most signs were hypotension. This symptom is related to beta–carbolines, especially Harman. in another hand, the MAO inhibitory by harmaline can induce hypertension crisis in high doses. These intoxications are presented with nervous and digestive system symptoms. Nervous system presentations commence with excitability and progress to muscle trembling and stiffness (Mahmoudian et al., 2002). Animals also have hypothermia, dyspnea, and mydriasis. Neurological presentations are prominent in all cases of harmal toxicity. Our case had tremors as others. It seems that these are related to harmaline effect (Moshiri et al., 2012). Maternal body weight during the gestation period was used to assess maternal toxicity. The change in maternal body weight and weight gain in our study is contrary to the study of treated dams with methanol and acetone extracts of the epigeal parts of P. harmala administered orally for 30 days which suggests that the absence of weight change is not due to malnutrition and/or to toxification (Shapira et al., 1989). But our results are nearly similar to the report of Adaay, 2014, the changes in the body weight of dams treated with 5mg/kg of harmine

and harmaline were negligible, whereas dams treated with 10mg/kg of both alkaloids showed an increase in their body weight. None of the two dosages of harmine and harmaline used in this investigation was lethal to the dams. The daily food intake of dams given was not affected. On the other hand, animals treated with 10mg/kg of harmine and harmaline consumed more food than did the controls. The results revealed that the total extract of the alkaloids during pregnancy is minimally maternotoxic. To judge the functional abnormalities in the dams, we analyzed serum biochemical values at the end of the gestational period. Briefly, ALAT, gamma GT concentrations, and indirect Bilirubin levels show significant changes. The renal parameters show a significant change in glucose, Urea Nitrogen, creatinine, Na, and Cl were changed significantly, The hormonal analysis shows a significant decrease in FSH, Progesterone, and Estrogen. In contrast to the case report by Pranzatelli and Snodgrass, 1987, rats treated with P. harmala showed tremors and convulsions with normal biochemical lab tests (Pranzatelli and Snodgrass, 1987). However, chronic oral administration of aqueous extract of P. harmala for 3 months to rats increased transaminase levels according to Marwat et al., 2011 and Moshiri et al., 2012. According to the study by Komeili et al., 2016 about the effect of the hydroalcoholic extract of P. harmala seeds for 4 weeks in the Streptozotocin-induced diabetic (Zitch) rats which showed an increase in blood glucose as well as changes in lipid profile in comparison with normal rats, while the treatment by hydroalcoholic extract showed a significantly decreased in the levels of glucose, triglyceride, cholesterol, and LDL-c and increased the level of HDL-c in diabetic rats Zitch. These findings are similar to the issue of Singh et al., 2008) which showed that ethanolic extract of P. harmala seed significantly decreased blood glucose levels in normal and diabetic rats at doses of 150 and 250 mg/kg of body weight. P. harmala has been traditionally used to treat diabetes in folk medicine in some parts of the world (Moloudizargari et al., 2013). In mammals, embryo/fetal development occurs within the maternal organism, where mothers receive the initial exposure to chemicals while embryos and fetuses are exposed secondarily through the placenta, a maternal-fetal unit. Moreover, if chemicals undergo biotransformation after being absorbed, the embryos and fetuses are exposed not only to the chemical itself but also to metabolites produced in the maternal organism (Paumgartten, 2010). Our results show a significant difference in totally resorbed litters, the number of fetus death, and a decrease in the fetus weight with a remarkable decrease in the number of implantations, the increase of pre-and post-implantation loss rates, and undeveloped dead fetuses. These are nearly similar to the result of the effect of methanol and acetone extracts of the epigeal parts of P. harmala administered orally for 30 days, The methanol extracts at doses used appeared to produce a dose-dependent significant decrease in body weight in fetuses size. No change in the physical and nutritional status of the animals and no adverse toxicological effects were observed (Shapira et al., 1989). According to the report of Merzouki and his collaborators, the infusion of mixed the Leaves and fruits of P. harmala and administered orally provoke an abortion, mentioned also that midwives of Casablanca in morocco used P. harmala seeds are toxic, and their abortive power by a decoction of a handful of the seed is taken orally to provoke abortion (Marzouki et al., 2000; Bellakhdar, 1997). Here we can suggest that the total extract of the alkaloids of P. harmala can be considered an anti-fertility, anti-implantation, and abortion agent according to our results and the other reports. It was important to mention in the report of Shapira et al., 1987, that the absence of adverse histopathological findings suggests that the effect of methanol extracts on the estrous cycle, as well as on reproduction rate, can be attributed to a direct effect on targets associated within the reproductive system, and are not due to malnutrition and/or to toxification. Chemically, the epigeal parts of P. harmala are known to contain various β-carbolines such as harmaline, harmine, quinazoline alkaloids, vasicine, and vasicinol (Kashimov et al., 1971) with a wide spectrum of biological activities. The effects attributed to harmine and harmaline are the inhibition of sodium-dependent transport (Sepulveda and Robinson, 1974), elevation in the production of hypothalamic norepinephrine and serotonin (Queshi et al., 1979), and the abolition of inhibitory synaptic transmission (Sokolove and Roth, 1978). Abortifacient activity has been reported only for vasicine alkaloids, which have been found to have a uterine stimulatory effect, apparently through the release of prostaglandins (Gupta et al., 1978; Zutshi et al., 1980). Currently, we are in the process of isolating vasicine, vasicinone, deoxivasicinon, harmine, and harmaline, after which it will be possible to evaluate separately the specific effects of each of these alkaloids on the reproductive system. These findings are nearly similar to the case report of Shapira et al., 1989, have reported that The methanolic extract reduced the number of living pups and increased the number of resorption. This extract at doses used produced a decrease in litter size. It is believed that quinazoline alkaloids (vasicine and vasicinone) are responsible for the abortifacient activity of P. harmala extracts. It has been reported that these chemicals have a uterine stimulatory effect, apparently through the release of prostaglandins (Gupta et al., 1978; Zutshi et al.1980; Mahmoudian et al., 2002; Kuete, 2013), but on another side, the study of Fathiazada et al, (2006) revealed that the effects of hydro-alcoholic extract of P. harmala seeds on spontaneous rhythmic contractions of isolated rat uterine were tested on the isolated uterus and endometrial free preparations. The extract of P. harmala seeds was found to exhibit significant spontaneous contractions of the uterus and stripped myometrium relative to the solvent control. After recording the pattern of uterus tissue spontaneous motility, in order to determine the mechanism of the extract of P. harmala seed's pharmacological effects, atropine, indomethacin, or prazosin was added to the organ baths. Pretreatment with atropine in both the whole uterus and in the stripped myometrium preparations had no effects on the response to cumulative dosage of extract of P. harmala' seeds. Calcium-free solution decreased uterus contractions. In calcium dose-response curves, extract of P. harmala seeds in some concentrations produced a uterotonic effect in calcium-free solution in the presence of KCl. This finding showed that EPS may increase calcium influx through voltage-dependant calcium channels and the effects of P. harmala on rat uterus are not dependent on prostaglandins, these contractions are related to external calcium (Fathiazada et al., 2006). Reduced litter size at

birth may be due to a reduced ovulation rate (corpora lutea count), higher rate of pre-implantation deaths, higher rate of post-implantation deaths, or immediate post-natal deaths. In turn, these deaths may be the consequence of an earlier physical malformation that can no longer be observed due to subsequent secondary changes, and so on. Particularly for effects with a natural low frequency among controls, discrimination between treatment-induced and coincidental occurrence is dependent upon association with other types of effects (Shapira et al., 1989). Contrary to the result of the report of (Azizi et al., 1989), where Harmaline and Harmine were injected intraperitoneally into pregnant rats, injections on different days of pregnancy from 1st – 23rd days showed no different toxic symptoms in treated rats. They observed no abortion in rats that received l-80 µg/Kg of alkaloids and all rats delivered live births on time with no obvious abnormalities. Therefore, it is concluded that, because of observed toxicity which can be lethal, pregnant women should not use seeds of espand (Azizi et al., 1989). But the embryotoxicity study of Adaay, 2013 on the effects of harmaline and harmol given intraperitoneally to pregnant rats on days 1st-14th of gestation revealed that the average number of implantation sites and live fetuses per dam was within the control range in the different dose levels of the two compounds. Fetal death or resorption was increased in the treated groups in comparison with the control group, this increase was statistically significant in the groups treated with 10mg/kg of harmaline and harmol. A decrease in fetal body weight was evident in the 5 and 10mg/kg groups of harmaline and harmol in comparison with the control group. Two fetuses from the group treated with 5mg/kg of harmol were abnormal, in one of them the toes of the left leg were missing, and two toes emerged from an abnormal position at the leg, the other fetus showed missing toes and femur of the right hand. The fetal mortality and decrease in their body weight indicate that harmaline and harmol or their metabolites cross the placenta at a fast enough rate to reach concentrations toxic to the embryo. It has been suggested that some harmala alkaloids harmaline and harmol or their metabolites may induce embryo-lethality which varies with the stage of gestation. (Adaay, 2013)

Conclusion

In the present study, The total extract of the alkaloids in the seeds of P. harmala has adverse effects on maternal toxicity, embryonic development, and abortion.

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