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Embryotoxicity of Harmaline and harmalol Hydrochloride on Pregnant Rats

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Abstract
Peganum harmala alkaloiods are biologically active compounds. They were used as spices and hallucinogens. Harmaline and harmalol hydrochloride were tested for their possible embryolethal effects on Wistar pregnant rats. One hundred and twelve mature rats of both sexes were used in this study. In the first experiment, females were given ip injection of 5 and 10 mg/kg of the two compounds for 14 days starting from the first day of gestation. In the second experiment, males were given ip injection of 10 mg/kg of the two compounds for 21 days and mated with untreated females for three weeks. Numbers of live, dead and resorbed fetuses in addition to skeletal variations were recorded. A remarkable increase in maternal body weight and food intake was noticed in the higher dosages of the first experiment. Increase in the average number of resorbed or dead fetuses was found in the first experiment and the first week mating of the second experiment. A significant decrease in fetal body weight was shown in all dose levels of the two experiments. Skeletal variations were shown in all groups of the two experiments. The study demonstrated that harmala alkaloids are toxic to rat embryos resulted in fetal mortality, decreasing fetal body weight and enhancing skeletal anomalies.

Keywords: Harmaline, Harmalol, Embryotoxicity, Rats.

Introduction
It has long been known that the harmala alkaloids namely, harmaline, harmine, harmalol and harmol are biologically active compounds [1, 2, 3]. Harmaline is almost twice as toxic as harmine and in moderate doses causes tremors and clonic convulsions [4]. These compounds which are extracted from *Peganum harmala* were used as spices and hallucinogens in the Middle East, Turkey, Spain and along the African coast of the Mediterranean sea [2]. Tremor and convulsion were observed in rats treated with aqueous extract of *Peganum harmala* and these toxic signs were postulated to be due to the alkaloids content of the extract which have a central nervous system stimulant effect [(5] and cytotoxic effect [6]. *Peganum harmala* seeds extract showed significant *in vitro* and *in vivo* antileishmanial activities [7]. Shapira et al., 1989 [8] have reported that methanolic extract of *Peganum harmala* reduced the number of living pups, increased the number of resorption and produced a dose dependent decrease in litter size of rats. Abortion is frequent in animals that digest this plant in a dry year [9]. Since *Peganum harmala* seeds are still utilized by local people in many areas for the treatment of tooth aches and in aches related to the vertebral column and since the seeds contain total alkaloids in a percentage of 4-5% [10], the present study was conducted to evaluate the possible embryotoxicity of harmaline and harmalol in pregnant rats.

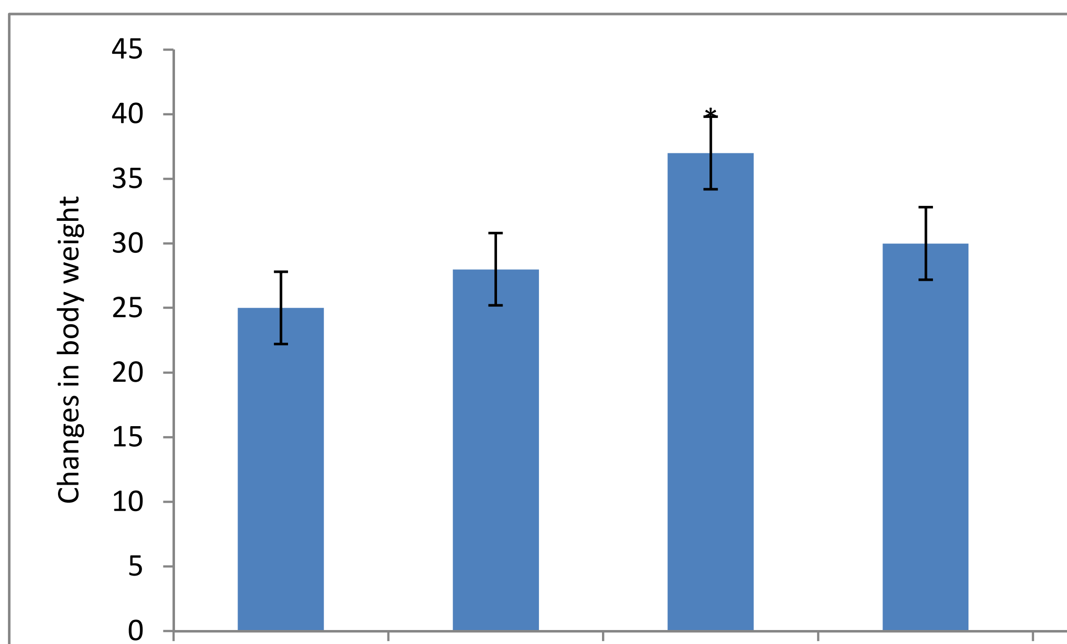
2. Materials and Methods
Albino Wistar rats of both sexes (10-12 Weeks of age and weighing 250-350 gms) which were bred and reared in the High Institute for Infertility Diagnosis & ART/ Al-Nahrain University were used throughout this study. The animals were housed in wire meshed cages and given standard diet and water *ad libitum*. Timed pregnancies were obtained with the day of mating considered as day 1 of pregnancy. Harmaline hydrochloride (I) and harmalol hydrochloride (II) were obtained from Fluka AG. Solutions of the two compounds were prepared immediately before use. Rats were treated intraperitoneally with the test compounds since this is an accepted route for administration in animals [11]. Female rats were treated for 14 days starting from the first day of pregnancy whereas male rats (10 weeks old) were treated for 21 days. Two experiments were carried out:
In the first experiment, four groups of pregnant rats were given 5 and 10 mg/kg body weight from each of the two compounds and one control group received distilled water only. A record of body weight and food intake

was conducted daily throughout the experiment in order to monitor maternal toxicity. Dams were sacrificed on day 20 of pregnancy by cervical dislocation. The uterus was obtained and the number of live, dead and resorbed fetuses was recorded. After that, the whole uterus was placed in ammonium sulfide for 10 minutes in order to stain implantation sites not visible to the naked eye [12]. In the second experiment, two groups of three males each received 10 mg/kg body weight of compounds I and II respectively for a period of 21 days and the third group received distilled water only. All males were mated by the end of the treatment individually to two untreated females for one week and another two females for each of the following two weeks. Pregnant females were sacrificed on day 20 of pregnancy and the same above parameters were similarly recorded.
Live fetuses were removed, weighed to the nearest mg and externally examined under the dissecting microscope for any deformities. Fifty percent of the fetuses from each group were fixed in 95% ethanol, cleared in 1% potassium hydroxide and stained with alizarin res –S for skeletal examination [13]. The remaining 50% of the fetuses from each group were placed in Bouin's fluid for detection of visceral anomalies by the razor section technique [14]. Changes in maternal body weight were evaluated by subtracting the dam's body weight on day one plus the total litter weight from their weight on day twenty of the experiment. Food intake was recorded daily from the first day of gestation until day twenty. Student's t-test was employed for calculation of the differences in the average body weight, food intake, number of implantation sites, live and dead fetuses and fetal body weight [15]. Results are expressed as mean±SEM and statistical significance was considered at p value <0.05.

3. Results and Discussion

Experiment1:

Maternal toxicity: Maternal body weight during the gestation period was used to assess the maternal toxicity of I and II. Changes in the body weight of dams treated with 5mg/kg of both compounds were negligible as compared with the control group, whereas dams treated with 10mg/kg of I and II showed a significant (P≤ 0.05) increase in their body weight (Figure, 1). None of the two dosages of I and II used in this investigation was lethal to the dams. The daily food intake of dams given the lowest doses of I and II was not affected in comparison with the controls. On the other hand, animals treated with 10mg/kg of both compounds consumed more food than did the controls.



*P≤ 0.05 in comparison with the control.

Figure 1: Effect of harmaline and harmalol on the average maternal body weight by the end of gestational period in rats.

Embryotoxicity: Table 1 summarizes the effects of I and II given intraperitoneally to pregnant rats on day 1-14 of gestation. From these results it can be seen 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. The average frequency of

fetal death or resorption was increased in the treated groups in comparison with the control group, this increase was statistically significant (P≤ 0.05) in the groups treated with 10mg/kg of I and II.
A significant (P≤ 0.05) decrease in fetal body weight was evident in the 5 and 10mg/kg groups of I and II in comparison with the control group. Two fetuses from the group treated with 5mg/kg of II were abnormal, one of them the toes of the left leg were missing and two toes emerged from 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 I and II / 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 may interact with DNA [16], therefore, it is possible that I and II / or their metabolites may induce embryolethality which varies with the stage of gestation. Nrubert *et al*., 1980 [17] reported that the rate of DNA replication in the embryo increase 1000 fold during 2 or 3 days of early organogenesis around day 13 of gestation in rats.

Table 1: Effects of harmaline and harmalol given to pregnant rats on days 1-14 of gestation.

Group / Parameters	control	5mg/kg harmaline	10mg/kg harmaline	5mg/kg harmalol	10mg/kg harmalol
No. of Dams	9	11	10	7	10
Total implant. Sites	73	75	82	53	78
Average No. implants./dam±SEM	8.1±1.09	6.8±1.06	8.2±0.97	7.6±1.21	7.8±1.03
Total No. fetal mortality	4	12	15	10	18
Average fetal mortality/dam±SEM	0.4±0.2	1.1±0.5	1.5±0.6*	1.4±0.6	1.8±0.6*
Percentage fetal mortality	5.5	16	18.3	18.9	23
Total live fetuses	69	63	67	43	60
Average live fetuses/dam±SEM	7.7±1.6	5.7±1.4	6.7±1.6	6.1±1.5	6.0±1.4
Percentage live fetuses	94.5	84.0	81.7	81.1	77.0
Dams with resorption and live fetuses/total dams	4/9	6/11	7/10	3/7	7/10
Average fetal body weight gm± SEM	2.47±0.05	2.03±0.08*	1.99±0.08*	2.19±0.04*	2.04±0.21*
No. stunted fetuses	0	0	0	2	0

* P≤ 0.05 in comparison with the control group.

Experiment 2:
Embryotoxicity: The results of the three weeks paternal mating of this experiment are shown in tables 2, 3 and 4 for the first, second and third week respectively. It is obvious from these results that the average number of implantation sites and live fetuses were not affected during the three weeks of the experiment. The average number of fetal death and resorption was significantly (P≤ 0.05) increased during the first week in the groups treated with the two compounds in comparison with the control groups but this effect was not appeared during the second and third weeks of the experiment in any of the two compounds treated groups. A significant (P≤ 0.05) decrease in fetal body weight was found during the first week and a highly significant (P≤0.01) decrease was exhibited during the second and third weeks in the groups treated with I and II as compared to the control groups. In case of the paternal treatment, the effects were very clear on the embryo resulted from the mother mated during the first week after the cease of treatment in the males. These effects represented by the average number of dead or resorbed fetuses since it was significantly increased in the groups treated with both compounds. 4/5 and 3/5 of the dams showed resorption and live fetuses in the harmaline and harmalol treated groups respectively in comparison with 1/5 in the control group. Average fetal body weight was significantly affected in the three weeks mating after the end of male dosing.

The present results may suggest that the two compounds increase the frequency of dominant lethal mutation in the sperm and that the post implantation loss could be attributed to the increase in the frequency of lethal mutations.These results are in accordance with that reported by Murasaki *et al*. [18] after treatment of some

harmala alkaloids to male rats which resulted in a decreased spermatogenic activity in the testes and germinal epithelium after day 7 of treatment. El-Dwairi and Banihani [19] reported that the aqueous extracts of *Peganum harmala* might have adverse effects on the processes of spermatogenesis due to direct or indirect effects on seminiferous tubules and or the pituitary testicular axis. No visceral anomalies could be detected in experiment 1 and 2 by using the razor section technique after examination of the sections by the dissecting microscope.
Table (5) shows skeletal variations in all groups of both experiments including the control group, but the incidence in the experimental groups was more than in the control group. These variations including rudimentary 13th rib, extra 14th rib and in some cases fused ribs. The embryonic defects observed in some embryo are probably not due directly to treatment with harmaline and harmalol since most of those defects occurred in the control embryos as well, so it is possible that the two compounds enhance the spontaneous occurrence of such anomalies.

Table 2: Effects of Harmaline and Harmalol on Fetuses Resulted From the First Week Paternal Treatment.

Group / parameters	control	10mg/kg harmaline	10mg/kg harmalol
No. of Dams	5	5	5
Total implant. Sites	40	44	38
Average No.	8.0±0.63	8.8±0.96	7.6±1.12
Total No. fetal mortality	1	7	8
Average fetal	0.2±0.1	1.6±0.5*	1.8±0.6*
Percentage fetal mortality	2.5	13.6	18.4
Total live fetuses	39	37	30
Average live fetuses/dam±Se	7.8±0.5	7.4±1.1	6.0±0.5
Percentage live fetuses	97.5	86.4	81.6
Dams with resorption and	1/5	4/5	3/5
Average fetal body weight	2.58±0.02	2.29±0.08*	2.13±0.13*
No. stunted fetuses	0	0	0

* $P \leq 0.05$ in comparison with the control group.

Table 3: Effects of Harmaline and Harmalol on Fetuses Resulted From the second Week Paternal Treatment.

Group / parameters	control	10mg/kg harmaline	10mg/kg harmalol
No. of Dams	5	5	4
Total implant. Sites	45	51	40
Average No. implants./dam±Se	9.0±0.44	10.2±1.11	10.0±1.47
Total No. fetal mortality	1	4	1
Average fetal mortality/dam±Se	0.2±0.2	0.8±0.48	0.3±0.25
Percentage fetal mortality	2.2	7.8	2.5
Total live fetuses	44	47	39
Average live fetuses/dam±Se	8.8±0.37	9.4±1.32	9.7±1.31
Percentage live fetuses	97.8	92.2	97.5
Dams with resorption and live fetuses/total dams	1/5	2/5	1/5
Average fetal body weight gm±Se	2.62±0.03	2.10±0.17**	2.06±0.15**
No. stunted fetuses	0	0	0

** $P \leq 0.01$ in comparison with the control group.

Table 4: Effects of Harmaline and Harmalol on Fetuses Resulted From the third Week Paternal Treatment.

Group / parameters	control	10mg/kg harmaline	10mg/kg harmalol
No. of Dams	5	5	6
Total implant. Sites	45	52	56
Average No. implants./dam±Se	9.0±0.31	10.4±0.67	9.5±1.94
Total No. fetal mortality	2	4	5
Average fetal mortality/dam±Se	0.4±0.24	0.8±0.58	0.8±0.40
Percentage fetal mortality	4.4	7.7	9.0
Total live fetuses	43	48	51
Average live fetuses/dam±Se	8.6±0.39	9.6±1.16	8.6±1.70
Percentage live fetuses	95.6	92.3	91.0
Dams with resorption and live fetuses/total dams	2/5	2/5	3/6
Average fetal body weight gm±Se	2.51±0.03	2.02±0.08**	1.95±0.61**
No. stunted fetuses	0	0	0

**P≤ 0.01 in comparison with the control group.

Table 5: Skeletal variations in rat fetuses after maternal and paternal treatment with harmaline and harmalol.

Fetuses / reatmenTt	No. Fetuses Examined	Percent * Affected Fetuses
Maternal treatment control	34	11.8
5mg/kg Harmaline	32	15.6
10mg/kg Harmaline	33	39.5
5mg/kg Harmalol	22	18.0
10mg/kg Harmalol	30	43.3
Paternal treatment first week mating control	20	10.0
10mg/kg Harmaline	19	37.0
10mg/kg Harmalol	16	37.5
Second week mating **c**ontrol	22	13.6
10mg/kg Harmaline	24	25.0
10mg/kg Harmalol	20	20.0
Third week mating control	22	13.6
10mg/kg Harmaline	24	21.0
10mg/kg Harmalol	26	15.4

*Skeletal variations include rudimentary 13th ribs, extra 14th ribs and some fused ribs.

β-Carboline alkaloids are a large group of natural and synthetic indole alkaloids with different degrees of aromaticity, some of which are widely distributed in nature, including various plants. These compounds are of great interest due to their diverse biological activities. Particularly, these compounds have been shown to intercalate into DNA, to inhibit cyclin-dependent kinase (CDK), Topisomerase, and monoamine oxidase, and to interact with benzodiazepine receptors and 5-hydroxy serotonin receptorset [16]. Some derivatives of this group are the harmala alkaloids, namely, harmaline, harmine, harmalol, harmol and tetrahydroharmine which have

been extracted from *Peganumh armala* [20, 21]. These harmala alkaloids are potent reversible inhibitors of monoamine oxidase tested *in vitro* or *in vivo* in animals and human [21-23]. Harmaline was found to be the main effective antinociceptive agent of *Peganum harmala* alkaloids extract [24]. Slotkin and Distefano [25], reported the conversion in the rat of harmaline to harmalol, harmine and several other compounds. Although the conversion of the harmaline series to the harmine series of compounds took place *in vivo*, the opposite conversion did not occur.

Conclusion

In conclusion, the results of the present study demonstrated clearly that harmala alkaloids used in this study are toxic to rat embryos in the used doses resulted in fetal mortality, decreasing fetal body weight and enhancing skeletal anomalies.

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