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Reproductive Toxic Effects of *Artemisia herba alba* Ingestion in Female Spague-dawley Rats

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Abstract: The objectives of this study is to investigate the toxic effects of *Artemisia herba Alba* (300 mg/kg/ body wight) on the reproductive system after administration to female Sprague-Dawley rats weighting 250-300 g for two time periods 4 and 12 weeks. Twenty adult female rats were divided into two groups and exposed to Topiramate diet at a concentration of 300 mg/kg/body weight for two periods of time. First group containing 10 rats received treatment for 4 weeks and a second group of 10 rats received the same dose of treatment for a period of 12 weeks and compared with twenty non-exposed female rats received vehicle treatment. Female rats were allowed mating with males after 10 days prior to the last administration dose. Animals were autopsied under light anesthesia after mating and several parameters were determined including: Number of pregnant rats, body and reproductive organ weight, number of implantation sites, viable fetuses and resorption sites. Assessment of pregnancies in females was measured and the significance of these results was calculated using student's t and Chi-square tests. The effect of *Artemisia herba alba* exposure on fertility was assessed in terms of pregnant rats number, implantation sites, viable fetuses and resorption sites. Exposure to *Artemisia herba alba* for 4 weeks did not have much effect on fertility. Significant decrease in the relative ovarian weights and embryo weights in rats exposed to *Artemisia herba alba* were observed. Exposure to *Artemisia herba alba* for a 12 weeks resulted in a reduction in the percentage of pregnancies and in the number of implantation sites when compared with controls in both treatment periods. Rats receiving 12 weeks treatment showed an increase in ovarian weights and a decrease in viable fetus's number. These results indicate that long-term exposure of female rats to *Artemisia herba Alba* causes adverse effects on the reproductive system and fertility. The results of the current study suggest that ingestion of *Artemisia herba alba* by adult female rats causes adverse effects on fertility and reproduction.

Key words: *Artemisia herba alba*, female rats, fertility, pregnancy, reproductive organs

INTRODUCTION

Since ancient time phytotherapy has been used as folk medicine to treat various diseases including fertility regulation, a fact that has been reported in the ancient literature of indigenous systems of medicine. 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 (Kamboj and Dhawan, 1982; Purohit and Daradka, 1999; Khouri and El-Akawi, 2005).

Artemisia herba alba (Composate) is commonly known by the Arabic name sheh and is a popular folk remedy for the treatment of falling hair (crushed and applied to hair), chest, stomach, muscular pains (fumigations), cough, diarrhea, fever, poisoning (to drink and for irrigation), vomit, lungs, flatulence. For this purpose, the native use a hot water decoction made from the fresh leaves and branch lets. *A. herba alba* used by local population of some middle east countries as an antidiabetic activity (Iriadam, 2006), *A. herba alba* also used as an antihelminitic (Khafagy *et al*., 1971). A literature survey revealed that certain other species of Artemisia also shown antimalarial (Haynes; 2006), antibacterial (Kordali *et al*., 2005) and insecticidal (Saadali *et al*., 2001).

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Phytochemical investigation of *A. herba alba* have shown that it contains santonin (Khafagy *et al*., 1971), sequiterpene lactones (Foglio *et al*., 2002) and flavonoids (stermitz *et al*., 2002). Components of the essential oil have also been investigated (Saleh *et al*., 2006).

Therefore in the light of these facts present study was conducted to monitor the effects of *Artemisia herba-albaa* on female rat's reproductive system with emphasis on the fertility and pregnancy outcome.

MATERIALS AND METHODS

Animals: Adult female Sprague-Dawley rats (40) weighing 250-300 g were used in this study. Rats were raised in the animal house unit/Jerash National University, Jerash, Jordan, 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 artemisia herba-albaa: *Artemisia herba-albaa* 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 Artemisia herba-albaa 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 ten days to allow mating. The effect of Artemisia herba-albaa 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 (Lane-Petterand Pearson, 1971).

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±and Standard Deviation (SD). The differences between Artemisia herba-albaa treated and controlled groups were analyzed using Student t-test (Dixon and Massey, 1957).

RESULTS

Exposure toxicity of artemisia herba-albaa: 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 Artemisia herba-albaa (group 2) died.

The effects of Artemisia herba-albaa on fertility: Short term treatment with Artemisia herba-albaa 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).

The effect of 12 weeks exposure to *Artemisia herba-albaa* 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). Moreover, Table 1b also indicates that the long term exposure to Artemisia herba-albaa 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 resorption sites and the number of implantation was induced greatly (Table 1b).

Table 1a: The effect of 4 week exposure to *Artemisia herba alba* on fertility of female rats

Treatments	No. of pregnant females	No. of implantation	No. of viable fetuses	Rats with resorption	Resorption/total No. of implantation
Control	9/10	9.33±2.39	8.77 ±2.72	4/10(40%)	5/84 (5.9%)
Artemisia herba alba	6/10	8.04±4.14	8.25±0.45	5/6(83.3)	17/48 (36%)

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

Table 1b: The effect of 12 week exposure to *Artemisia herba alba* on fertility of female rats

Treatments	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 (5.9%)
Artemisia herba alba	4/10†	6.98±2.81*	6.83±1.85*	4/5(80%)	14/28(50%)

Results are expressed as means±SEM, *p<0.05: Significantly different from the control group (Student's t-test), † p<0.05: Significantly different from the control group (Fisher exact test)

Table 2a: The effect of 4 weeks exposure to *Artemisia herba alba* on maternal body, organ and embryo weights

Treatments	Final body weight (g)	Ovary weight (g) (mg/100 g Bwt)	Uterus weight (g) (mg/100 g Bwt)	Embryo weight (g) (mg/100 g Bwt)
Control	268±18.67	0.37±0.05	0.53±0.01	0.34±0.04
Artemisia herba alba	256±11.56	0.33 ±0.05*	0.49±0.04	0.30±0.06†

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

Table 2b: The effect of 12 weeks exposure to *Artemisia herba alba* on maternal body, organ and embryo weights

Treatments	Final body weight (g)	Ovary weight (g) (mg/100 g Bwt)	Uterus weight (g) (mg/100 g Bwt)	Embryo weight (g) (mg/100 g Bwt)
Control	268±18.67	0.37±0.05	0.53±0.01	0.34±0.04
Artemisia herba alba	249±13.65	0.32±0.06*	0.47±0.08	0.28±0.31†

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

The effects artemisia herba-albaa on maternal organs weight and embryo weight: Table 2a shows that ingestion of *Artemisia herba-albaa* 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).

In contrary to this, the ingestion of *Artemisia herba-albaa* 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 weigh or in the uterine weight in rats treated for 12 weeks with Artemisia herba-albaa when compared with controls, in contrary a slight reduction can be noticed (Table 2b).

DISCUSSION

The *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 (Khouri and El-Akawi, 2005).

This study were conducted to investigate the exposure effect of Artemisia herba-albaa on the structure, fertility and the pregnancy outcome of adult female Sprague-Dawley rats. The dose of 300 mg kg^{-1}. body weight of *Artemisia herba-albaa* 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.

It is worthwhile to mention that to our knowledge, no work has been published in the literature that relates the effects of Artemisia herba-albaa to structure, fertility and pregnancy outcome. It has been postulated however, that administration of this plant to female rats for 30 days in different dosages induces dose-dependent decrease in the size of the offspring with no toxicological effect observed (Shapira *et al*., 1989). This is in accordance with our results which showed that the exposure of adult female rats to Artemisia herba-albaa 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 were 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.

Other important findings of this study showed that this plant might promote a decreased in Sprague-Dawley female rats fertility when intra-gastric 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 Table 2a and b which might be explained by the fact that the reproductive organ weights can be closely regulated by

androgen hormones (Richard *et al*., 2000). If so, we 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 acts 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 Artemisia herba-albaa 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 (Choudhary and Steinberger, 1975; Agrawal, 1986).

This may go hand in hand with our results indicating the significant decrease in the number of implantation sites which could lead to the decrease in viable fetus's number. We are now conducting a research to investigate the effect of *Artemisia herba-albaa* exposure on serum progesterone levels. In conclusion, the results of the current study suggest that ingestion of *Artemisia herba-albaa* by adult female rats causes adverse effects on fertility and reproduction.

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