Asian Journal of Chemistry Vol. 18, No. 3 (2006), 2280–2284

Antiandrogenic Activity of *Ruta graveolens* L. in Female Albino Rats

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The objective of this study is to investigate the toxic effects of *Ruta graveolens* (250 mg/kg body weight) on the reproductive system after administration to female Sprague-Dawley rats weighing 250–300 g for two time periods of 4 and 12 weeks. Twenty adult female rats were divided into two groups and exposed to *Ruta graveolens* diet at a concentration of 100 mg/kg/body weight for two periods of time. The first group containing 10 rats received treatment for 4 weeks and the 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 that received vehicle treatment. Female rats were allowed mating with males after 10 d prior to the last administration of 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 *Ruta graveolens* exposure on fertility was assessed in terms of pregnant rats number, implantation sites, viable fetuses and resorption sites. Exposure to *Ruta graveolens* for 4 weeks did not have much effect on fertility. Significant decrease in the relative ovarian weights and embryo weights in rats exposed to *Ruta graveolens* was observed. Exposure to *Ruta graveolens* for 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 numbers. These results indicate that long-term exposure of female rats to *Ruta graveolens* causes adverse effects on the reproductive system and fertility.

Key Words: *Ruta graveolens* L., Female rats, Fertility, Reproductive effects, Reproductive organs.

INTRODUCTION

Plant preparations play an important role in 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[1,2].

Ethanobotanical knowledge provides very useful basic clues not only in the problem relating to nomenclature identification of crude drug extracts but also in

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the discovery and the use of medicinal plants. *Ruta graveolens* L. is currently used by the Jordanian population systemically for its antispasmodic, diuretic, sedative and analgesic effects and externally for its anti-rheumatic effect.

The role of this indigenous plant product in the induction of male and female fertility in experimental animals has drawn the attention of researchers over the turn of the century[3–5]. Several authors have reported the anti-fertility outcome activity of this plant[6–8]. Many other researchers also focussed on the study of the fertility regulations by this plant in different other countries such as Japan[9–11], China[12], India[2, 4], Brazil[13], as well as in Ethiopia[8].

Our aim is to study and evaluate the effect of *Ruta graveolens* on pregnancy outcome and on the fetal intrauterine development using female Sprague-Dawly rats treated with one dose of 250 mg/kg body weight administrated orally for two different treatment periods.

EXPERIMENTAL

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

Administration of *Ruta graveolens*

20 rats were exposed to treatment with *Ruta graveolens* through an intragastric tube at concentrations of 250 mg/kg body weight, dissolved in tap water for two periods of time namely 4 weeks (10 female rats representing group 1) and 12 weeks (10 female rats representing group 2). The two groups of rats were allowed drinking water and normal diet *ad libitum*; in addition, rats were receiving a dose of 250 mg/kg/rat body wight/d in the form of tablets of *Ruta graveolens* that were dissolved in distilled water through intragastric intubations. The two remaining groups (group 3 and 4) receiving normal diet were considered as control and were allowed access to normal diet and drinking water *ad libitum*.

Fertility test

Animals were observed daily from the first day of exposure to *Ruta graveolens* for clinical signs of toxicity and their body weights were measured weekly. After the two exposure times, treated and untreated control counterpart rats were divided randomly into groups of two rats each and housed with a sexually mature untreated male of proven fertility for 10 d to allow mating. The effect of *Ruta graveolens* ingestion on the occurrence of implantation was estimated in the rats and in their control counterparts after the appropriate time of exposure. During this exposure time, namely 10 d, at least two estrous cycles should have elapsed[14]. The untreated male rats were removed from cages and the treated female rats and their control counterparts were killed by cervical dislocation under light ether anesthesia. Autopsy was performed afterwards and the following parameters were recorded: the number of implantation sites, the number of viable fetuses and the number of resorption sites. Furthermore, maternal body weight, uterus weight, ovary weight in addition to the embryo weights were also recorded.

Statistical analysis

Data was expressed as mean ± standard deviation (SD). The differences between *Ruta graveolens* treated and control groups were analyzed using Student 't' test[15].

RESULTS AND DISCUSSION

Exposure levels and toxicity of *Ruta graveolens*: None of the animals within the 4 week exposure group (group 1) showed any clinical signs of toxicity. However, for the 12 week exposure group (group 2), one animal out of 10 died due to the exposure to *Ruta graveolens* at concentrations 100 mg/kg body weight, respectively.

Effect of *Ruta graveolens* on fertility: Table-1a shows the effect of *Ruta graveolens* ingestion for 4 weeks (group 1) on the fertility of the treated female rats. In what concerns the number of treated females impregnated by control untreated male rats, a non-significant reduction was observed. In addition, the number of implantation sites in the treated rats was observed to be slightly decreased with no significant difference between the control and the *Ruta graveolens* treated rats in this group. The number of viable fetuses was equal in the treated female rats when compared with the controls. The percentage of resorption was elevated where the ratio between the resorption and the total number of implantation was observed to be elevated.

TABLE-1a
EFFECT OF FOUR-WEEK EXPOSURE TO *RUTA GRAVEOLENS* ON FERTILITY OF FEMALE RATS

Treatment	No. of pregnant females	No. of implantation	No. of viable fetuses	Rats with resorptions	Resorptions/ total no. of implanations
Control	9/10	9.33 ± 2.39	8.77 ± 2.72	4/10 (40%)	5/84 (5.9%)
Ruta graveolens	7/10†	8.05 ± 4.14*	8.13 ± 0.45*	5/7 (71.4%)	27/65 (41.53%)

Results are expressed as mean ± 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).
‡$p < 0.001$.

Table-1b indicates the effect of ingestion of *Ruta graveolens* for 12 weeks period (group 2) on the fertility of female treated rats.

TABLE-1b
EFFECT OF TWELVE-WEEK EXPOSURE TO *RUTA GRAVEOLENS* ON FERTILITY OF FEMALE RATS

Treatment	No. of pregnant females	No. of implantations	No. of viable fetuses	Rats with resorptions	Resorptions/ total no. of implanations
Control	9/10	9.33 ± 2.39	8.77 ± 2.72	4/10 (40%)	5/84 (5.9%)
Ruta graveolens	5/9†	7.78 ± 2.81*	6.75 ± 1.85*	3/5 (60%)	14/48 (29.16%)

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).
‡$p < 0.001$.

There were significant decreases in the percentage of pregnant rats in the *Ruta graveolens* treated group ($p < 0.05$) when compared with control counterparts. Furthermore, exposure to *Ruta graveolens* resulted in a decrease in the number of implantation sites as well as the number of viable fetuses and this group in a statistically significant ($p < 0.05$) manner. The percentage of resorption sites in treated rats was more increased in this group whereas the ratio between the resoption and the number of implantations was greatly increased.

Effect of *Ruta graveolens* on maternal organ weights and embryo weights: Table-2a shows that ingestion of *Ruta graveolens* for 4 weeks resulted in a non-significant reduction in rats' body weight where a statistical decrease in the relative ovary weight ($p < 0.05$) was demonstrated. A significant decrease in the embryo weights in this group ($p < 0.05$) was observed when compared with rats from control counterparts. There were no significant differences in the uterine weights obtained from female treated rats when compared to the controls.

TABLE-2a
EFFECT OF FOUR-WEEKS EXPOSURE TO *RUTA GRAVEOLENS* ON MATERNAL BODY, ORGAN AND EMBRYO WEIGHTS

Treatment	Final body weight[a] (g)	Ovary weight (g) (mg/100 g bwt)	Uterus weight (g) (mg/100 g bwt)	Embryo weight (g) (mg/100 g bwt)
Control	256 ± 18.67	0.34 ± 0.05	0.51 ± 0.01	0.31 ± 0.04
Ruta graveolens	238 ± 13.56	0.29 ± 0.01*	0.46 ± 0.03	0.27 ± 0.07†

[a]Relative weights. Results are expressed as mean ± SEM.
*$p < 0.05$,
† $p < 0.01$ significantly different from the control group (Student's t test).

Table-2b shows that the ingestion of *Ruta graveolens* for 12 weeks resulted in a significant reduction in the relative ovarian weight ($p < 0.05$) when compared to controls. Animals exposed to *Ruta graveolens* treatment showed also a more significant decrease in the embryo weight ($p < 0.05$) when compared to controls. No significant differences were observed in the final body weight nor in the uterine weight in the animals treated with *Ruta graveolens* when compared to controls; however, a slight reduction can be noted.

TABLE-2b
EFFECT OF TWELVE-WEEKS EXPOSURE TO *RUTA GRAVEOLENS* ON MATERNAL BODY, ORGAN AND EMBRYO WEIGHTS

Treatment	Final body weight[a] (g)	Ovary weight (g) (mg/100 g bwt)	Uterus weight (g) (mg/100 g bwt)	Embryo weight (g) (mg/100 g bwt)
Control	256 ± 18.67	0.34 ± 0.05	0.49 ± 0.01	0.31 ± 0.04
Ruta graveolens	237 ± 15.65	0.26 ± 0.03*	0.43 ± 0.08	0.28 ± 0.32†

[a]Relative weights. Results are expressed as mean ± SEM.
*$p < 0.05$,
† $p < 0.01$ significantly different from the control group (Student's t test).

The present investigation shows that intragastric administration of *Ruta graveolens* promotes a decreased in fertility in female Sprague-Dawley rats. The weights of reproductive organs were markedly decreased as shown in Tables 2a and

2b. It has been postulated that the reproductive organ weights can be closely regulated by androgen hormones[16]. This drug may act on pituitary gland and may lead to a decrease in the main hormones influencing pregnancy. The decrease in weight of reproductive organs further confirms androgen underbalnce. However, this decrease in the ovarian weights in treated rats is unexplained and needs to be clarified through both hormonal and histological studies. This will help to elucidate whether the weight decrease in the ovaries observed in this study is due to hyperplasia and/or hypertrophy of the tissue component of this organ.

Any decrease in the weight of reproductive organs is under hormonal control. These results, therefore, suggest that any disturbance of the reproductive endocrine functions may possibly go hand in hand with multiple sites of toxicity acting along the hypothalamic-pituitary-ovarian-uterine axis.

The main finding of the current study was the significant reduction in the occurrence of pregnancy in rats exposed to *Ruta graveolens* for 12 weeks. This decrease may be due to alteration of the reproductive endocrine functions leading to decreased secretion of progesterone which is needed for endometrial alteration at the time of implantation and is necessary for successful impregnation[17, 18]. This may also explain the significant decrease in the number of implantation sites leading to decrease in the number of viable fetuses. We are now investigating the effect of *Ruta graveolens* exposure on serum progesterone levels.

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(*Received*: 15 November 2005; *Accepted*: 3 March 2006) AJC-4713