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# Anticonceptive and antifertility activities of various *Ruta graveolens* extracts in female rats

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## Abstract

The anti-conceptive, anti-fertility and reproductive effects of various *R. graveolens* extracts were tested in Sprague-Dawley adult female rats. Intragastric administration of 800 mg/kg of aqueous 1, aqueous 2, methanol, ethanol, hexane, ether or dichloromethane extracts of the *R. graveolens* aerial parts from day 1 to day 6 post-coitum had no significant anti-implantation activity in rats. The ether extract administered at 800 mg/kg exhibited severe toxicity and was inactive as anti-implantation agent when administered at 400 mg/kg into the rat. Administration of aqueous1, aqueous2, ethanol or hexane preparations significantly increased the number of resorbed embryos. None of the extracts had significant effect on maternal body weight gain. However, aqueous 2, methanol, ethanol or dichloromethane extracts showed a significant reduction in fetal body weight. Placental weight was significantly reduced in female's ingested methanol, ethanol, ether or dichloromethane extracts. On the other hand, administration of hexane extract (400 mg/kg) of *R. graveolens* on days 6-15 post-coitum significantly decreased the number of females with born fetuses and increased the mortality rate among the borne fetuses. Prenatal exposure (days 6-15 of gestation) of male and female rat offspring to 400 mg/kg hexane extract of *R. graveolens* caused a significant delay on the timing of testicular descent and vaginal opening, respectively. Likewise, administration of hexane extract (400 mg/kg) of *R. graveolens* for 30 consecutive days had no significant effect on the occurrence of pregnancy in female rats.

The results strongly suggest that *R. graveolens* extracts had adverse effects on female rat reproduction.

**Key words:** *Ruta graveolens*; fertility; reproduction; rats.

## Introduction

Many plants have been found to possess anti-ovulatory, interceptory, abortifacient, and emmenagogue activities in laboratory animals (Farnsworth *et al.*, 1975a, 1975b; Conway *et al.*, 1979; Prakash *et al.*, 1985; Bhargava, 1988; Kamboji, 1988; Desta, 1994). *Ruta graveolens* L. (Rutaceae) seems to be used most widely. *R. graveolens* is a branching shrub with green-blue compoundly pinnate leaves and greenish-yellow flowers. The plant is an endemic of the Mediterranean region. The plant has been valued for many centuries. In the Middle Ages it was credited with anti-magical powers, considered a cure for countless illnesses and often called

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herb of Grace (Greenwood, 1982). It has been used as an abortifacient or emmenagogue in many parts of the world (Benzanger-Beauquesne *et al.*, 1980; Culpeper, 1950; Watt *et al.*, 1962; Burlage, 1968). The reputed anti-fertility activity of the plant is as universally acknowledged as it is contradictory (Kong *et al.*, 1989). Several constituents have been isolated from *R. graveolens* including alkaloids, essential oil, flavonoids, coumarins and limonoids (Watt *et al.*, 1962; Nieschulz *et al.*, 1965; Hegnauer, 1982; Reynolds *et al.*, 1982; Middleton, 1984; Zobel *et al.*, 1988).

In Jordan, *R. graveolens* is widely used in the folkloric medicine as an antispasmodic, diuretic, sedative, analgesic and externally antirheumatic (Kareem *et al.*, 1986). Oral administration of the hot water extract or the 95% ethanol extract at 1 mg/kg and 40-80 mg/kg, respectively, revealed significant anti-implantation effects in rats (Guerra *et al.*, 1978). Administration of chloroform extracts of the root, stem and leaf of *R. graveolens* into the female rats from day 1 to day 10 post-coitum showed significant anti-fertility activity (Kong *et al.*, 1989). In their experiments, chalpensin was found to be the active component with some toxicity. In another study, the powdered root, aerial parts and the aqueous extract of aerial parts showed potential anti-conceptive activity in female rats when administered on days 1-10 post-coitum (Gandhi *et al.*, 1991). In the same study, sequentially prepared petroleum ether and methanol extracts of the plant aerial parts were active as anti-conceptive, while the benzene and chloroform extracts were toxic and inactive. Recently, we found that administration of the aqueous leaf extract of *R. graveolens* for 25 days at a dose of 50 mg/mice/day caused a significant reduction in mice fertility, number of implantation sites and number of viable fetuses (Alkofahi *et al.*, 1996).

Despite the large volume of ethnomedical studies demonstrating the anti-fertility or abortifacient effect of the common rue, there is no clear-cut results in human or animal studies indicating a definite preparation or compound as the active principle (Kong *et al.*, 1989). As part of our well-established and on-going research directed toward evaluation of the anti-fertility activity of various plants used in the folkloric medicine in Jordan (Alkofahi *et al.*, 1996; Elbetieha *et al.*, 1996; Elbetieha *et al.*, 1998; Elbetieha *et al.*, 2000), this work was undertaken to investigate the anti-fertility effects of various *R. graveolens* extracts in the rat.

## Material and Methods

*Animals:* Adult male and female Sprague-Dawley rats (200-250g) were raised in the animal house unit at Jordan University of Science and Technology. Rats were maintained under controlled temperature of (21±1)° C in 12 h light : 12 h darkness schedule. Food and tap water were available ad libitum.

*Plant materials:* The plant was grown in a house garden at Essarih town, Irbid, Jordan, identified by Professor Ahmed Al-Oglah, Department of Biology, Faculty of Science, Yarmouk University, Irbid, Jordan. Around 6.5 kg of the aerial parts (leaves, stems and flowers) were collected during the months of April, May and June 1999. A voucher specimen (number 156) of the plant has been deposited at the herbarium in the Department of Medical Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology. Samples were air dried, milled to coarse powder and weighed before processing.

*Extract preparation:* A series of sequentially extracted organic extracts by Soxhlet (increasing order of polarity) were obtained as follows: Each 500 g of dried and ground *R. graveolens* were extracted with n-hexane (2L) in a soxhlet apparatus at 75-80° C for 72 h. The extract was filtered and concentrated under reduced pressure at low temperature using rotary evaporator. The plant residue obtained after n-hexane extract, was then macerated and refluxed in more

polar solvent (2L) diethylether at 35° C for 72 h. The mixture was filtered and the diethylether solution was concentrated under reduced pressure at low temperature as before. The residue was macerated and refluxed in methylene chloride (2L) at 40° C for 72 h. The filtered methylene chloride extract was concentrated using the previous procedure. The residue after methylene chloride was macerated and refluxed in methanol (2L) at 60-65° C for 72 h, filtered and concentrated under reduced pressure. Finally, the residue after methanol extract was macerated in distilled water (2L) for 72 h, filtered and concentrated over the water bath, this extract was referred to as aqueous-2. All crude extracts of the five different solvents were collected, weighed, and stored at –20° C. The yield of the five solvent extracts was calculated (Table 1).

**Table 1. Yield of various *R. graveolens* aerial part fractions**

| Solvent | Yield (g/kg) |
|---|---|
| Hexane | 21.76 |
| Ether | 5.64 |
| Dichloromethane | 3.44 |
| Methanol | 48 |
| Aqueous 1 | 130 |
| Volatile oil | 1.5 |
| Ethanol | 43 |
| Aqueous 2 | 102.5 |

*preparation of ethanol and aqueous 1 extracts:* After volatile oil collection, the distilled water remained in Clavenger's apparatus was filtered and dried over the water bath, the residue was then macerated with ethanol for 2 days, filtered, and then concentrated under reduced pressure using rotary evaporator. The weight of volatile oil, ethanol and distilled water extracts was recorded, and the yield for each extract was estimated. The extract prepared by using distilled water was referred to as aqueous.

## Bioassay Methods

*Anti-implantation experiments:* Virgin female rats on the day of proestrus were left one night with male rats of proven fertility (50 weeks old). Each male was caged with three virgin females at around 16:00 h. The presence of copulation plug or sperms in the vaginal smears on the following morning was regarded as day one of pregnancy. The various extract of *R. graveolens* were prepared in 800 mg/kg or specified otherwise in a total volume of 1.0 ml. Administration of *R. graveolens* extracts was conducted in three experiments according to the vehicle used: in one experiment the pregnant females were randomly divided into five groups of 10 animals each. The first group served as the control and was administered normal saline. The other four groups were administered either aqueous 1, aqueous 2, methanol or ethanol extracts. In the second experiment the mated rats were divided into four groups of 10 animals each. The first group served as the control and was administered olive oil. The other three groups were given either 800 mg/kg hexane, 400 mg/kg ether or 800 mg/kg ether extracts. In

the third experiment mated animals were divided into two groups. The first group was administered the vehicle (80% olive oil, 10% ethanol and 10% benzene) and the second group was administered dichloromethane extract. The extract preparations and the vehicle were administered intragastrically from day 1 to day 6 of pregnancy using animal intubation needles (Propper and Sons, New York). All animals were sacrificed on day 20 of gestation under light ether anaesthesia. Before autopsy, body weights of the animals was recoded and during autopsy, the following measurements were recorded: number of pregnant females, number of implantation sites, number of viable fetuses, number of resorptions along the uterine horns, gravid uterus weight, fetal body weight, and placental weight.

*Vaginal opening and testicular descent determination:* Because hexane extract caused the highest level of resorptions and some but insignificant degree of antiimplantation activity (Table 2), it was chosen to further investigate its teratological effects and effects on sexual maturation of male and female rat offspring. Inseminated females were assigned into two different groups each contains 9 animals. One group was administered hexane extract (400 mg/kg) on days 6-15 of gestation and the other group was administered olive oil as a vehicle. The length of administration corresponds to the period of organogenesis in the rat. Treated females and their control counterparts were left to the time of delivery. At the day of parturition, the number of females that successfully delivered fetuses and the litter size were recorded. The day of parturition was designed as day one of postnatal life. On the first day of life, litters were culled to eight neonates. In cases where fewer than eight pups were delivered, additional neonates were fostered from other litters. Offspring were daily checked for any signs of illness or mortality. Offspring were weaned on day 21 of postnatal life and housed in single sex sibling group of 8-10 animals per cage. From day 20 of age, the day of vaginal opening in females and testicular descent in male offspring were inspected on daily basis to monitor the latency of sexual maturity in both control and hexane crude extract exposed groups.

*Fertility test and examination of fetuses:* Fertility was estimated in adult female rats administered hexane extract (400 mg/kg) for 30 consecutive days, and for their control counterparts. After intra-gastric administration of hexane extract of *R. graveolens* for 30 days, treated females and their control counterparts were divided randomly into groups of two animals each, and housed in an individual cage with a sexually mature untreated male of proven fertility for 10 days. During this period, at least two estrous cycles should have elapsed (Rugh, 1968). Then males were removed, and one 10 days after their removal, the treated females as well as their control counterparts were killed under light ether anaesthesia and their uteri were examined. During autopsy, the following measurements were recorded: number of pregnant females, number of viable fetuses, number of resorptions, and number of implantation sites.

*Statistical analysis:* Data are expressed as mean ± S.D. Differences between control and *R. graveolens* fractions administered groups were analyzed using Fisher Exact test, Chi-square test and Mann-Whitney test. A p value less than 0.05 was considered significant.

## Results

*Effect of R. graveolens extracts on implantation and maternal body weight gain in pregnant female rats:* The aqueous 1, aqueous 2, methanol, ethanol and dichloromethane extracts of the aerial parts of *R. graveolens* dosed at 800 mg/kg body weight from day 1 to day 6 post-coitum had no significant antiimplantation effects (Table 2). Ether extract administered at 800 mg/kg exhibited sever toxicity in that 7 animals out of 9 did not survive the duration of the

experiment. After the dose of ether extract was lowered to 400 mg/kg, only 1 animal out of 12 died at early stages of the experiments suggesting that ether extract had the highest maternal toxicity effect.

The data presented in Table 2 shows that intragastric administration of aqueous 1 ($p<0.0001$), aqueous 2 ($p<0.0005$), ethanol ($p<0.005$) and hexane ($p<0.0005$) extracts of *R. graveolens* at a dose of 800 mg/kg caused a significant increase in the number of resorbed embryos.

**Table 2. Effect of intragasric administration of various *R. graveolens* extracts on implantation in female rat**

| Treatment | Dose(mg/kg B.wt.) | No.of dead animals | No. of pregnant/no. tested (%) | No. of resorptions/no. of implantations (%) | Antifertility rate (%) |
|---|---|---|---|---|---|
| **Expt. 1** | | | | | |
| Normal saline (control) | | - | 9/10 (90) | 5.9 | 10 |
| Aqueous 1 | 800 | - | 9/10 (90) | 48.1*** | 10 |
| Aqueous 2 | 800 | - | 8/11 (73) | 27.8** | 27 |
| Methanol | 800 | - | 5/7 (71) | 8 | 29 |
| Ethanol | 800 | - | 8/10 (80) | 23.7* | 20 |
| **Expt. 2** | | | | | |
| Olive oil (vehicle) | - | - | 8/10 (80) | 13 | 20 |
| Hexane | 800 | - | 5/9 (55.6) | 56** | 44.4 |
| Ether | 800 | 7/9 | - | - | - |
| Ether | 400 | 1/12 | 5/11 (45.4) | 25 | 54.6 |
| **Expt. 3** | | | | | |
| Vehicle[a] | - | - | 8/9 (88) | 13 | 12 |
| Dichloro-methane | 800 | - | 5/9 (55.6) | 15.7 | 44.4 |

[a]Vehicle: 80% olive oil, 10% ethanol and 10% benzene

*$P<0.005$, **$P<0.0005$, ***$P<0.0001$ significantly different from the control group (Fisher Exact Test)

Table 3 shows that none of the extracts administered between day 1 and day 6 of post-coitom had a significant effect on maternal body weight gain. However, aqueous 2, methanol, ethanol ($p<0.001$) or dichloromethane ($p<0.005$) extracts caused a significant reduction in the fetal body weight. On the other hand, placental weight was significantly reduced after administration of methanol ($p<0.005$), ethanol, dichloromethane ($p<0.0001$), or ether ($p<0.001$) extracts.

*Effect of R. graveolens hexane extract (400 mg/kg) on the pregnancy outcomes and sexual maturation:* Administration of hexane extract at a dose of 400 mg/kg on days 6-15 post-coitum significantly ($p<0.05$) reduced the number of pregnant females with born fetuses and increased ($p<0.005$) the early mortality rate among the born fetuses (Table 4). Furthermore, the prenatal exposure of male and female rat offspring during the period of organogenesis to hexane extract (400 mg/kg) caused a significant ($p<0.0001$) delay on the timing of testicular descent in males offspring and the timing of vaginal opening in females offspring (Table 5).

*Effect of R. graveolens hexane extract on fertility of adult female rats:* The data presented in Table 6 demonstrate that intragastric administration of hexane extract at a dose of

**Table 3. Effect of *R. graveolens* fractions on maternal and fetal body weights**

Results are expressed as mean ±S.D.

| Parameter | Normal saline | Aqueous 1 | Aqueous 2 | Methanol | Ethanol | Olive oil | Hexane | Ether | Vehicle [a] | Dichloromethane |
|---|---|---|---|---|---|---|---|---|---|---|
| Initial B.wt (g) | 226.75±28.32 | 236.60±19.24 | 241.18±27.33 | 235.14±18.88 | 241.05±18.96 | 222.9±15.43 | 227±17.95 | 219±13.84 | 211.50±7.86 | 223.38±12.38 |
| Final B.wt (g) | 269.80±23.58 | 266.90±35.18 | 277.72±46.41 | 263.64±17.16 | 277.15±31.57 | 268±18.67 | 256.83±38.20 | 253.40±19.98 | 259±20.33 | 250.61±19.04 |
| Gravid uterus weight (g) | 34.78±10.27 | 26.84±12.67 | 24.69±13.09 | 28.34±8.64 | 26.50±7.04 | 17.78±5.85 | 15.67±7.39 | 16.64±8.69 | 13.21±5.98 | 15.82±5.30 |
| Body weight difference (g)[a] | 43.05±16.43 | 30.30±32.45 | 27.45±31.64 | 28.50±22.74 | 36.10±24.74 | 45.10±17.82 | 29.11±29.61 | 34.40±17.71 | 45.33±22.24 | 27.22±19.25 |
| Corrected B. wt. (g) | 11.73±9.46 | 14.18±9.46 | 9.49±18.32 | 8.25±11.06 | 17.54±12.83 | 32.65±11.28 | 23.88±22.35 | 26.83±10.92 | 35.04±18.94 | 18.42±16.92 |
| Fetal B.wt. (g) | 2.11±0.29 | 2.00±0.49 | 1.41±0.17*** | 1.41±0.12*** | 1.36±0.15*** | 1.32±0.20 | 1.31±0.14 | 1.53±0.36 | 1.23±0.27 | 0.88±0.43* |
| Placental weight (g) | 0.44±0.08 | 0.48±0.12 | 0.44±0.16 | 0.40±0.04* | 0.38±0.07*** | 0.53±0.12 | 0.60±15 | 0.43±0.08** | 0.55±0.14 | 0.35±0.08*** |

[a]Body weight difference = final body weight – initial body weight.
Corrected body weight difference = body weight difference – gravid uterus weight.
*$P<0.005$, **$P<0.001$, ***$P<0.0001$ significantly different as compared to control groups (Mann-Whitney test).