



Asian Journal of Plant Sciences

ISSN 1682-3974

science
alert

ANSI*net*
an open access publisher
<http://ansinet.com>

Effect of Alcohol Extracts of the *Ruta graveolens* L. On the Count, Motility and *in vitro* Fertilization Capacity of Rat's Sperm

^{2,3}F. Rahim, ^{1,2,3}G. Saki and ¹M. Bazrafkan

¹Laboratory of Cell Culture, Department of Anatomical Sciences, Faculty of Medicine, Ahwaz Jundishapur University of Medical Sciences, Ahwaz, Iran

²Physiology Research Center, Ahwaz Jundishapur University of Medical Sciences, Ahwaz, Iran

³Apadana Clinical Research Center, Private Hospital of Apadana, Ahwaz, Iran

Abstract: This study is an attempt to elucidate the effect of alcoholic extract of the *Ruta graveolens* L. on the sperm count, motility and *in vitro* fertilization capacity of Wistar rats. Total 24 adult male Wistar rats, 90-day-old and weighing 210±10.6 g were used in this study. All animals were housed individually per cage under a 12 h light/dark cycle, 20±2°C temperature and 60-65% humidity-controlled room with food and water *ad libitum*. All counts were performed at 37°C in T6 media. The sperm motility was assessed and classified as progressive, no progressive. Initial sperm motility was manually assessed by a single individual in duplicate for each sample by evaluating 100 sperms. In every group of this study 8 adult male rats were used. The sperm count was 2798.5±192.40 in group 1, 2801.8±418.67 in group 2 and 1017.4±820.69 in group 3. Therefore, group 3 has a significant lower sperm count in comparison with other groups. Progressive sperm motility was 57.20±2.81 in group 1, 55.25±1.82 in group 2 and 19.26±3.17 in group 3. The analysis shows that rats in group 3 have significant lower sperm motility in comparison with other groups. The fertilization capacity of sperm of rats in group 3 was significantly lower than other groups. As a conclusion, the alcohol extract of *Ruta graveolens* L. can be suggested as agent against male fertility but the exact mechanism of action is not understood yet so more experimental shall be done to reveal its effect as a contraceptive plant.

Key words: *Ruta graveolens* L., sperm motility, sperm count, *in vitro* fertilization

INTRODUCTION

One of the important concerns is the problem of the overpopulation. The population of the world is growing faster than the supplies of food, shelter and fuel. A lot of efforts have made to find a safe and effective method with the least side effects but beyond condom and vasectomy there is no other choices for male contraception (Nieschlag and Henke, 2005). Many hormonal, immunological and chemical substances are being investigated but none of them is completely desirable and without side effects (Jensen, 2002; Rahim *et al.*, 2009). Looking through history, ancient people were also interested in contraception and had their own way of controlling their family size. Medicinal plants were one of those methods. *Ruta graveolens* L. is a member of Rutaceae family. It is a hardy, evergreen shrub of up to one meter tall, with characteristic grayish green color and a sharp unpleasant odor. This plant have different established effects like antimicrobial, cytotoxic

(Ivanova *et al.*, 2005), fungicide (Oliva *et al.*, 2003), herbicide (Hale *et al.*, 2004) and anti-inflammatory (Raghav *et al.*, 2006, 2007).

Ruta graveolens L. is one of the most ancient and effective contraceptive plants (Maurya *et al.*, 2004). Its potent female anti-fertility and abortive effects have been reported from countries like Brazil (De Freitas *et al.*, 2005), Indian (Gandhi *et al.*, 1991), Peru (Gutiérrez-Pajares *et al.*, 2003) and Mexico (Conway and Slocumb, 1979) both according to their traditional usage and in animal studies. In addition to its potent abortive effects, it has been mentioned by folk and traditional medicine to have a male contraceptive effect. There are a few reports about effect of this plant on count, motility and *in vitro* fertilization capacity of sperm (Harat *et al.*, 2008; Khouri and El-Akawi, 2005). The present study is an attempt to evaluate the effect of alcohol extracts of the *Ruta graveolens* L. on the count, motility and *in vitro* fertilization capacity of Wistar rat's sperm.

MATERIALS AND METHODS

Animals: This experimental study was performed in physiology research center of Ahwaz Jondishapour University of Medical Sciences (AJUMS) from March 2008 to August 2009. Total 24 adult male Wistar rats, 90-day-old and weighing 210 ± 10.6 g were used in this study. The animals were purchased from Laboratory Animals Care and Breeding Center of Ahwaz Jondishapour University of Medical Sciences, Ahwaz, Iran. The fertilizing ability of male mice was proved by selecting post first wave of spermatogenesis that mate and observed positive pregnancy at the beginning of experiment. All animals were housed individually per cage under a 12 h light/dark cycle, $20 \pm 2^\circ\text{C}$ temperature and 60-65% humidity-controlled room with food and water *ad libitum*. All procedures were approved by international guidelines and by the Institute Research Ethics and Animal Care and Use Committee of Ahwaz Jondishapour University of Medical Sciences. Every effort was made to minimize the number of animals used and their suffering.

Preparation of extract: *Ruta graveolens* L. aerial parts were collected from an Iranian plants species and dried in a ventilated place at room temperature for 15 days. The aerial parts were grinded in an electric grinder and the powder (72.3 g) was extracted in 1500 mL of 70% ethanol under stirring for 72 h. The extract was filtered, concentrated in a vacuum evaporator at 50°C and then lyophilized. The yield of the lyophilized extract was stored in the dark, at room temperature, in desiccators with silica gel (Harat *et al.*, 2008).

Experimental setup: Twenty-four adult male rats were subdivided randomly to 3 groups: group 1: serve as untreated controls, group 2: received 1 mL day^{-1} of 5% ethanol and group 3: received 1 mL day^{-1} of 5% ethanol extract of *Ruta graveolens* L. (20 mg day^{-1}) for 50 consecutive days by intraperitoneal injection. Twenty-four hours after last treatment, rats in three groups were killed and their epididymis were excised and weighed.

Sperm motility analysis: Sperm motility of three groups was determined using Makler chamber. All counts were performed at 37°C in T6 media. The sperm motility was assessed and classified as progressive, no progressive. Initial sperm motility was manually assessed by a single individual in duplicate for each sample by evaluating 100 sperms. Total motility was defined as any movement of the sperm head and progressive motility was defined as the count of those spermatozoa that moved in a forward direction (Movassaghi *et al.*, 2009).

Measurements of sperm count: Sperms were collected from the epididymis of each rat by flushing with the same volume (about 8 mL) of T6 medium. Collected samples were centrifuged at 100 g for 2 min and the precipitate portion was resuspended in 10 mL of fresh T6 medium. A fraction of suspension (100 μL) was mixed with an equal volume of 1% Trypan blue in the same medium and numbers of sperms were counted in four chambers of hemocytomere slide (Pook *et al.*, 2005). The sperm number was expressed mL^{-1} of suspension.

Oocyte collection: Adult female Wistar rats that were between 10 to 12 weeks old were administered intraperitoneally with 10 IU Pregnant Mare Serum Gonadotropine (PMSG) for superovulation; this was followed 46-48 h later by the intraperitoneal administration of 10 IU Human Chorionic Gonadotropine (HCG). Rats were killed 12-14 h after HCG injection by cervical dislocation method. After disinfection with 70% alcohol and opening the abdomen wall, the Y shaped uterus, ovaries and oviducts were identified. The oviducts were excised as follows: clamping cornua, dissecting the peritoneum and fat between ovary and tube and then cutting the fallopian tube from the proximal end and cumulus- oocyte complexes were collected in T6 medium. The granulosa cells of oocytes were removed by pipetting in T6 medium containing 80 IU mL^{-1} hyaluronidase and mature oocytes obtained and randomly divided into three groups (Hjollund *et al.*, 2004).

In vitro fertilization: *In vitro* fertilization was carried out in drops of T6 medium plus 5 mg mL^{-1} BSA under mineral oil. A pre-incubated capacitated sperm suspension of different groups as mentioned above was gently added to the freshly ovulated ova which divided in three groups to give a final motile sperm concentration on 100000 mL^{-1} . The combined sperm- oocyte suspension was incubated for 4-6 h. The ova were then washed through several changes of medium and finally incubated in drops of T6+5 mg mL^{-1} BSA under mineral oil. Fertilization was assessed by recording the number of 2 cell embryos 24-26 h after completion of fertilization *in vitro* (Ozawa *et al.*, 2002).

Statistical analysis: All the reported values except fertilization rate were reported as Mean \pm Standard Deviation (SD) and percentage. Fertilization rate reported as percentage (%). The statistical significance of difference between the control and experimental groups was determined by the unpaired t-test. Differences between the means were considered to be significant when $p < 0.05$ was achieved.

Table 1: Sperm count in 1 g of epididymis, different type of motility and *in vitro* fertilization capacity of sperm in three groups of study

Study groups	Variable				Fertilization capacity (%)
	Sperm count(10^6)/ 1 g of epididymis	Progress motility	Non-progress motility (Mean \pm SD)	Immotile sperm	
Group 1 (n = 8)	2798.5 \pm 192.40	57.20 \pm 2.81	23.15 \pm 3.19	18.65 \pm 5.61	79.5
Group 2 (n = 8)	2801.8 \pm 418.67	55.25 \pm 1.82	25.42 \pm 5.90	20.33 \pm 3.17	81.3
Group 3 (n = 8)	1017.4 \pm 820.69*	19.26 \pm 3.17*	56.25 \pm 1.66	24.49 \pm 4.23	8.3*

Group 1: Untreated controls, Group 2: The adult male rats treated with the 5% ethanol. Group 3: The adult male rats treated with the extract of *Ruta graveolens* L. n: Number of replications in each group, *The significant lower sperm count, the significant lower sperm motility, the significant lower fertilization capacity ($p < 0.05$)

RESULTS

In every group of this study 8 adult male rats with 12 weeks old were used. As shown in Table 1 the sperm count \pm SD in 1 g of epididymis was 2798.5 \pm 192.40 in group 1, 2801.8 \pm 418.67 in group 2 and 1017.4 \pm 820.69 in group 3. Therefore, group 3 has a significant lower sperm count in comparison with other groups. Sperm with progressive motility was 57.20 \pm 2.81 in group 1, 55.25 \pm 1.82 in group 2 and 19.26 \pm 3.17 in group 3 (Table 1). Statistical analysis show that rats in group 3 have significant lower sperm motility in comparison with other groups. There was no difference between other groups. The fertilization capacity of sperm of rats in group 3 was significantly lower than other groups.

DISCUSSION

The present study clearly shows that intra-peritoneal injection of *Ruta graveolens* L. extract decreased the epididymal sperm count of rats. This finding is in agreement with the previous studies (Harat *et al.*, 2008; Khouri and El-Akawi, 2005). It is well known that the function of accessory reproductive organs and also process of spermatogenesis are androgen dependent (Choudhary and Steinberger, 1975) so the alcohol extract of *Ruta graveolens* L. may act directly or indirectly on the pituitary gland secretory function and cause decrease in the androgen. In this study, we observed that testosterone hormone level in treated rats significantly decreased but the level of Follicular Stimulating (FSH) and Leutinizing Hormone (LH) did not observed due to some limitation. In this study, we observed that the progress motility of sperm in treated rats significantly decreased comparing to other groups. This manifestation may due to an alteration in the microenvironment in the cauda epididymis, which also had a inhibition action on the metabolism of the treated rats as a result of androgen-inhibitory effect of the alcoholic extract of *Ruta graveolens* L. (Khouri and El-Akawi, 2005) It is well known that the production of spermatozoa able to fertilize and develop a normal progeny results from normal sperm maturation in the epididymis. The composition of the

internal epididymal milieu, responsible for sperm maturation, is under androgen control (Almeida *et al.*, 1998). In rats, an androgen-binding protein secreted by sertoli cells into the lumen of seminiferous tubules under follicle-stimulating hormone stimulation is transported to the epididymis, where it accumulates at concentrations higher than those found in the testes. This leads to a high local concentration of androgens, essential for maturation of epididymal spermatozoa (Almeida *et al.*, 2000). The reduced fertilization capacity of treated rats is probably due to a decrease in the number and progress motility of sperm. As a conclusion, the alcohol extract of *Ruta graveolens* L. can be suggested as agent against male fertility but the exact mechanism of action is not understood yet.

ACKNOWLEDGMENTS

This project with grant No. u-22 was financially supported by the research deputy of Ahwaz Jondishapour University of Medical Sciences (AJUMS). We would like to express our great appreciation for their support.

REFERENCES

- Almeida, S.A., S.O. Petenusci, J.A. Anselmo-Franci, A.A. Rosa-e-Silva and T.L. Lamano-Carvalho, 1998. Chronic intermittent immobilization of male rats throughout sexual development: A stress protocol. Braz. J. Med. Biol. Res., 31: 1443-1448.
- Almeida, A.S., W.G. Kempinas and T.L.L. Carvalho, 2000. Sexual behavior and fertility of male rats submitted to prolonged immobilization-induced stress. Braz. J. Med. Biol. Res., 33: 1105-1109.
- Choudhary, A. and E. Steinberger, 1975. Effect of 5 α -reduced androgen on sex accessory organs, initiation and maintenance of spermatogenesis in the rat. Biol. Reprod., 12: 609-617.
- Conway, G.A. and J.C. Slocumb, 1979. Plants used as abortifacients and emmenagogues by Spanish New Mexicans. J. Ethnopharmacol., 1: 241-261.

- De Freitas, T.G., P.M. Augusto and T. Montanari, 2005. Effect of *Ruta graveolens* L. on pregnant mice. *Contraception*, 71: 74-77.
- Gandhi, M., R. Lal, A. Sankaranarayanan and P.L. Sharma, 1991. Post-coital antifertility action of *Ruta graveolens* L. in female rats and hamsters. *J. Ethnopharmacol.*, 34: 49-59.
- Gutiérrez-Pajares, J.L., L. Zúñiga and J. Pino, 2003. *Ruta graveolens* L. aqueous extract retards mouse preimplantation embryo development. *Reprod. Toxicol.*, 17: 667-672.
- Hale, A.L., K.M. Meepagala, O. Anna, A. Giovanni and O.D. Stephen, 2004. Phytotoxins from the leaves of *Ruta graveolens*. *J. Agric. Food Chem.*, 52: 3345-3349.
- Harat, Z.N., M.R. Sadeghi, H.R. Sadeghipour, M. Kamalinejad and M.R. Eshraghian, 2008. Immobilization effect of *Ruta graveolens* L. on human sperm: A new hope for male contraception. *J. Ethnopharmacol.*, 115: 36-41.
- Hjollund, N.H., J.P. Bonde, T.B. Henriksen, A. Giwercman, J. Olsen and Danish First Pregnancy Planner Study Team, 2004. Reproductive effects of male psychologic stress. *Epidemiology*, 15: 21-27.
- Ivanova, A., B. Mikhova, H. Najdenski, I. Tsvetkova and I. Kostova, 2005. Antimicrobial and cytotoxic activity of *Ruta graveolens*. *Fitoterapia*, 76: 344-347.
- Jensen, J.T., 2002. Male contraception. *Curr. Womens Health Rep.*, 2: 338-345.
- Khoury, N.A. and Z. El-Akawi, 2005. Antiandrogenic activity of *Ruta graveolens* L. in male Albino rats with emphasis on sexual and aggressive behavior. *Neuro Endocrinol. Lett.*, 26: 823-829.
- Maurya, R., S. Srivastava, D.K. Kulshreshta and C.M. Gupta, 2004. Traditional remedies for fertility regulation. *Curr. Med. Chem.*, 11: 1431-1450.
- Movassaghi, S., G. Saki, F. Javadnia, M. Panahi, M. Mahmoudi and F. Rhim, 2009. Effects of methyl-beta-cyclodextrin and cholesterol on cryosurvival of spermatozoa from C57BL/6 mouse. *Pak. J. Biol. Sci.*, 12: 19-25.
- Nieschlag, E. and A. Henke, 2005. Hopes for male contraception. *Lancet*, 365: 554-556.
- Oliva, A., K.M. Meepagala, E.W. David, H. Dewayne, L.H. Amber, A. Giovanni and O.D. Stephen, 2003. Natural fungicides from *Ruta graveolens* L. leaves, including a new quinolone alkaloid. *J. Agric. Food Chem.*, 51: 890-896.
- Ozawa, N., N. Goda, N. Makino, T. Yamaguchi, Y. Yoshimura and M. Suematsu, 2002. Leydig cell-derived heme oxygenase-1 regulates apoptosis of premeiotic germ cells in response to stress. *J. Clin. Invest.*, 109: 457-467.
- Pook, M., B. Tuschen-Caffier, J. Kubek, W.B. Schill and W. Krause, 2005. Personality, coping and sperm count. *Andrologia*, 37: 29-35.
- Raghav, S.K., B. Gupta, C. Agrawal, K. Goswami and H.R. Das, 2006. Anti-inflammatory effect of *Ruta graveolens* L. in murine macrophage cells. *J. Ethnopharmacol.*, 104: 234-239.
- Raghav, S.K., B. Gupta, A. Shrivastava and H.R. Das, 2007. Inhibition of lipopolysaccharide-inducible nitric oxide synthase and IL-1beta through suppression of NF-kappaB activation by 3-(1-1-dimethyl-allyl)-6-hydroxy-7-methoxy-coumarin isolated from *Ruta graveolens* L. *Eur. J. Pharmacol.*, 560: 69-80.
- Rahim, F., G. Saki, B. Ghavamizadeh, A. Jafaei and M. Kadkhodae, 2009. The effect of oxamate on fertilization capacity of mouse sperm *in vitro*. *Int. J. Pharmacol.*, 5: 178-180.