 ISSN 0172–780X **www.nel.edu**

ORIGINAL ARTICLE

Antiandrogenic activity of *Ruta graveolens L* in male Albino rats with emphasis on sexual and aggressive behavior

Nabil Azar Khouri[1] & Zeyad EL-Akawi[2]

[1] Department of Anatomy, Faculty of Medicine, Jordan University of Science & Technology;
[2] Department of Biochemistry, Faculty of Medicine, Jordan University of Science & Technology, Irbid, Jordan.

Correspondence to: Dr. Nabil, A. Khouri. MD, Ph.D.,
Assist. Professor, Department of Anatomy
or
Dr. Zeyad EL-Akawi. MD, Ph.D.,
Assist. Professor, Department of Biochemistry
Faculty of Medicine, Jordan University of Science & Technology.
P. O. Box 3030, Irbid, 22110 JORDAN
PHONE: 962 02-7201000 Ext: 23842 FAX: 962 02-720-5010
EMAIL: nabeelkhouri@yahoo.com

Submitted: September 20, 2005 Accepted: October 15, 2005

Key words: ***Ruta graveolens L;*** **Fertility*;* spermatogenesis; male and female albino rats; Behavior.**

Neuroendocrinol Lett 2005; **26**(6):823–829 **PMID:** 16380694 NEL260605A18 ©Neuroendocrinology Letters **www.nel.edu**

Abstract

BACKGROUND: The *Ruta graveolens L.* is currently used by the Jordanian populations systemically for its antispasmodic, diuretic, sedative, and analgesic effects and externally for its antirheumatic effect.

OBJECTIVE: To study plant effects on the reproductive system and fertility using adult male albino rats with special emphasis on the aggressive behavior and sex behavior. The aqueous extracts of *Ruta graveolens L.* solution was fed orally to male albino rats at a dose of 500 mg/kg body weight for 60 days.

RESULTS : This dose induces a significant decrease in the weight of reproductive organs (P<0.01) when compared to controls. The sperm motility and density in cauda epidydimides and testicular ducts were significantly decreased (P<0.01). A significant decreased (P<0.001) in spermatogenesis activity is observed in somniferous tubule. Treated rats testicular cell population showed a decrease in number of spermatocytes and spermatids (P<0.001) when compared to controls. Serum hormonal assay indicated a decrease in Testosterone and Follicular Stimulating Hormone levels in treated rats. A decreased in number female rats impregnated by males receiving treatment was observed and demonstrated by a decrease in the implantation sites and viable fetuses number (P<0.01). The ingested extract also suppresses the sexual behavior in adult male rats expressed by a prolongation of first mount time, increase in intromission latency, decrease in intromissions number, and prolongation of the post-ejaculatory interval. This led to reduce the ejaculation time and increase the post ejaculatory intervals. Ingestion of *R. graveolens* markedly abolished aggressive behavior parameters in adult male treated rats namely, a suppression in lateralization, boxing bouts and ventral presenting postures.

CONCLUSION : that the aqueous extracts of *Ruta graveolens L* might have adverse effects on territorial aggression and sexual behavior in male albino rats.

1. 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 (Sinha,1990; Purohit and Daradka,1999).

Ethanobotanical knowledge provide a very useful basic clues not only in the problem relating to nomenclature identification of crude drugs extract but also in the discovery and the use of medicinal plants. The *Ruta graveolens L.* is currently used by the Jordanian populations systemically for its antispasmodic, diuretic, sedative, and analgesic effects and externally for its anti-rheumatic effect.

The role of this indigenous plant products in the induction of male and female fertility in experimental animals has drawn the attention of researchers over the turn of the century (Fransworth, et al. 1975; Bhargave, 1988a; Bhargave, 1988b). Several authors have reported the anti-fertility outcome activity of this plant (Pakarashi & Pakarashi (year); Sethi et al.1990; Desta, 1994). Many others researchers also focused on the study of the fertility regulations by this plant in different other countries such as Japan (Uuki & Ichikawa, 1989; Ushiroyama et al.1991; Noda et al.1993), China (Li, 1988), India (Bhargave,1988a; Purohit & Daradka, 1999), Brazil (Elisabetsky & Posey, 1989), as well as in Ethiopia (Desta, 1994)

In light of this facts, this work was conducted to monitor the plant effects on the reproductive system and fertility using adult male albino rats with special emphasis on the aggressive behavior and sex behavior.

2. Material and method

2-1. Animals:

Adult male and female albino rats of Sprague Dawley strain, weighing about 300 g were raised in the Animal House Unit in Faculty of Medicine, Jordan University of Science and Technology under controlled temperature of 21 ±1°C and 12 hours light: 12 hours darkness schedule (lights on 06.00 AM–18.00PM hr). Food and Water were available *ad labitum*.

2-2. Plant and treatment:

Ruta graveolens L plant were obtained from a local market in Irbid (Jordan). The plant was dissolved by boiling in distilled water and administered orally to rats after cooling using animal feeding intubations needles (Popper and Sons, New York)in concentration of 500 mg/kg.

2-3. Experimental Design

Male rats were divided into following groups.

Group 1- Intact (Control) rats of this group received vehicle (distilled water) treatment for 60 days.

Group 2- Rats of this group received an aqueous extracts of *Ruta graveolens L* in a dose of 500 mg/kg body weight for 60 days representing the full reproductive cycle.

After 24 hours of the last dose, animals were weighed and autopsied under light ether anesthesia. The blood was collected through cardiac puncture using a sterile syringe for serum analysis.

2-4. Fertility Test

Fertility was estimated in adult male rats treated with Aqueous extracts of *Ruta graveolens* and in the control males counterparts. Each male was placed in an individual cage with two virgin untreated females of the same strain they were left together for 10 days during which two estrous cycles had elapsed (Rugh, 1968). One week after the removal of the exposed males, pregnant females rats were killed by cervical dislocation under light ether anesthesia and the number of implantation sites, the number of viable fetuses and the number of resorption sites were recorded.

2-5. Body and Organ Weights

The initial and final body weight of the animal were recorded. The reproductive tract was taken out trimmed free of fat and each organ was weighed separately on electronic balance. The reproductive organ taken into account for study in male include testes, epididymides, ventral prostrate, seminal vesicle, and vas deferens. Some vital organs such as the liver and heart were also obtained, weighed and kept in 90% formaldehyde for further analysis. Reproductive organs along with a small piece of the obtained liver, heart and kidney were fixed in Bouin's fixative for histological studies.

2-6. Sperm Motility and Count

To determine the sperm count and motility, a 100mg of cauda epididymides was minced in 2 ml of physiological saline and one drop of the evenly mixed sample was applied to a Neubauer's counting chamber under cover slip. Quantitative motility expressed as percentage was determined by counting both motile and immotile spermatozoa per unit area. Cauda epididymal and testicular sperm counts were made by routine procedure and expressed as million/ml of suspension (Prasad, 1972).

2-7. Histological analysis

The Bouin's fixed reproductive organs (testes, epididymides, seminal vesicle, ventral prostate, vas deferens) along with liver, kidney and heart were cut into small pieces and processed for histological slides. After dehydration using different concentration of alcohol, specimens were embedded in paraffin blocks and sectioned at 5 μm, placed on a clean histological glass slide and stained using Haematoxyline and Eosin.

2-8. Histometry

With the help of Camera Lucida, one hundred of circular appearing somniferous tubules were traced at x80 magnification and the diameter of each tubule was measured separately. The measurement was expressed

in terms of mean of all the traced tubules. Similarly, Leydig cell and their nuclei were traced at × 800. In addition, epithelial cell height of cauda epididymides, caput epididymides and seminal vesicle were also traced at X-360 and recorded.

2-9. Testicular Cell Population Counting

Spermatogenic elements namely spermatogonia, spermatocytes and spermatids were counted in 5 um thick cross sections of 10 somniferous tubules obtained from 10 animals of each group. All raw counts were transformed to 'true' counts by an adaptation of Abercrombie formula (Aberrcrombie, 1946) from germ cell diameter measurement. Interstitial cell types (such as fibroblast, immature and mature Leydig cells and degenerating cells) were estimated, applying a differential count over 200 cells population and statistically verified by the binomial distribution (Dixon and Massey, 1957).

2-10. Serum Biochemistry

Total protein, cholesterol, triglycerides, Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) were determined in blood serum using commercial kits (Cis BIO International Gif sur Yvette, France)

2-11. Hormonal Assays

Blood Plasma FSH and Testosterone concentrations was measured by Radioimmunoassay using two commercial kits (Cis BIO International Gif sur Yvette, France).

2-12. Statistical analysis

All values of body/organ weight biochemical estimation and histometric analysis were expressed in terms of mean value ± S.D. The different treatment groups were compared with control group using chi-square test and Student's "t" test (Ipstein and Poly 1970). Data were expressed as mean ± S. D. Differences between control and sodium fluoride exposed groups were analyzed using either the Chi-square test or Student "t" test [31].

2-13. Aggressive Behavior Testing

A rectangular observation cage with a Plexiglas front (45 cm length × 27cm breadth X 40cm height was used for rats aggression testing behavior. A stud male rat was placed in the testing arena for 10 days. A second male (control) was placed in the test arena with the stud male for 5 minutes, and the following parameters were recorded: (1) Lateralization by the stud male (2) Boxing bouts with stud male (3) fights of stud with the control male (4) Ventral presenting postures of the stud male (Bataineh et al 1997; Bataineh et al 1998).

2-14. Sex Behavior Testing Procedures

Animals were placed in the same observation rectangular cage with a Plexiglas front and a lightened bulb of 15 W was used above this cage. This observations were performed between 9:00 am and 3:00 pm. All behavioral measures were monitored by a single observer unfamiliar with the exposed groups.

Female rats of the same strain were used in this experiment receiving sequential subcutaneous treatment with 12.5 mg/ animal estradiol benzoate (Sigma Chemical Co. St Louis, MO, USA) 54 h before testing and 0.5 mg /animal progesterone (Gift from Roussel Uclaf, Paris, France) 6 h before testing in order to be brought into estrus cycle. The hormones were dissolved in corn oil (ALFCO: Arab International Food and Oil Processing Co.) in a total volume of 0.1 ml. Male rats were left in testing arena placed in for 5 minutes before the receptive females were introduced. Mating performance of male rats and the number of ejaculations were classified as follows:

1. Number of mounts: The time to the first mount (Body Contact) and the number of mounts without penile intromission.
2. Intromission latency: time in minutes from the presentation of the female to the first intromission.
3. Intromissions: number of mounts with penile intromissions.
4. Ejaculation latency: time from the first intromission until ejaculation.
5. Post-ejaculatory interval (latency period): time from the end of ejaculation until the next intromission.

These observations were terminated when: (1) No intromission had occurred within 15 min. after presentation of the female. (2) The male had not ejaculated within 30 minutes after the first intromission. (3) The first intromission following an ejaculation had occurred. (4) No intromission had occurred within 15 minute after ejaculation (Klint and Larsson 1995; Bataineh et al 1997; Bataineh et al 1998).

2-15. Statistical analysis

All the values of body/organ weight biochemical estimation and histometry were expressed in terms of mean value ± S.D. The different treatment groups were compared with control group using chi-square test and Student's "t" test (Ipstein and Poly1970). Data were expressed as mean ± S. D. Differences between control and Boric acid exposed groups were analyzed using either the Chi-square test or Student "t" test (Siegel, 1956 and Ipstein and Poly 1970)

3. Results

3-1. Effect of *R. graveolens* on Body and Body and Organ Weights

Table.1 shows the effect of intra-gastric administration of *R. graveolens* caused an increased in body weight in treated animals, when initial and final body weight were compared with controls. The weight of the testes, epididymides, seminal vesicle, ventral prostate and vas deferens however, were found to be significantly decreased in treated male rats when compared with the weights of the same organs obtained from control rats.

Table 1: Body and organ weights of male albino rats treated with *Ruta graveolens* for 60 days and comared to controls.

Condition	Body weight (gm)		Testes	Epididymides	Seminal vesicle	Ventral Prostate	Vas deferens
	Initial	Final	-------- (mg/100 gm body weight) --------				
Control group	304± 2.80	319± 2.65	895± 25.21	367± 21.61	376± 14.38	226± 4.1	113± 4.36
Treatment group	322± 4.55	348±7.6	797**± 32.1	365.2** ± 19.3	434**± 28.1	213**± 11.35	83.75*± 4.20

Results are expressed as mean ±S.D.
n rats were included per group.
*p < 0.05, **p < 0.01 significantly different from control group (Student's "t" test).

Table 2: Histometerical parameters and sperm dynamics findings in male albino rats treated with *Ruta graveolens* for 60 days compared to controls.

Condition	Sperm densitymillion/ml		Sperm motility %	Seminiferous tubule	Leydig cell nuclear	Epithelial cell height		
	Testes	Cauda	Cauda	diameter	diameter	Caput	Cauda	Seminal vesicle
				---------- µm ----------				
Control Group	4.75±0.47	56.0±1.94	74.1±1.94	290.6±3.2	6.45±0.96	38.8±0.4	26.08±0.32	17.32±0.17
Treatment group	3.55**±0.14	31.185**±1.08	47.26***±1.z08	218.27**±2.35	4.18**±0.762	32.68**±2.66	20.4**±2.68	14.45**±0.27

Results are expressed as mean ±S.D.
n rats were included per group.
*p < 0.05, **p < 0.01, ***p < 0.001 significantly different from control group (Student's "t" test).

Table 3: Testicular cell population dynamics findings in male albino rats treated with *Ruta graveolens* for 60 days compared to controls.

Condition	Germinal cell types					Interstitial cell type		
	Spermato-gonia	Spermatocyte (primary)	Spermatocyte (secondary)	Spermatids	Fibroblast	Immature Leydig cell	MatureLeydig cell	Degenerating cell
Control group	23.99±0.93	18.85±0.80	64.126±3.51	147.71±4.87	63.83±1.64	65.195±3.47	70.64±1.03	18.34±1.67
Treatment group	17.05±4.44	12.96***±2.41	17.97***±3.73	9.32***±6.82	38.66±1.33	41.66***±1.65	46.66***±0.78	69.6 ***±0.76

Results are expressed as mean ±S.D.
n rats were included per group.
*p < 0.05, **p < 0.01 ***p < 0.001 significantly different from control group (Student's "t" test).

3-2. Effect of *R. graveolens* on sperm dynamics and histometrical parameters.

Table. 2 shows that the sperm motility in cauda epididymis was decreased to a significant levels (P<0.001) in treated animals with *R. graveolens L* when compared with the controls. A significant decrease in sperm density, somniferous tubule diameter and leydig cell nuclear diameter was also observed in treated rats (P< 0.01). Epithelial cell height in epididymides (cauda and caput) and seminal vesicle were found to be significantly decreased as well in treated group(P< 0.01).

3-3. Effect of *R. graveolens* on Testicular Cell Population Dynamics

Table.3 shows that the administration of *R. graveolens* extract causes a significant decreased (P< 0.001) in rats germinal cell population namely spermatocytes (primary and secondary) and spetmatids. Similarly the immature and mature Leydig cells number were also decreased to a significant levels, whereas the degenerating cell numbers were observed to be significantly increased (P<0.001). Fibroblast and spermatogonia numbers were not altered in treated rats.

3-4. Effect of *R. graveolens* on Biochemical Changes Analysis

The results presented in Table 4 shows that blood glucose level was increased to a significant values (P<0.01), while bilirubin, total cholesterol and triglycerides level remain within a normal range. Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT) were observed to increased (P<0.05) in treated groups when compared to controls. Levels of plasma FSH and Testosterone were found to be significantly decreased (P< 0. 01) in treated group when compared with controls.

3-5. Effect of *R. graveolens* on Male Rat Fertility

The results presented in table 5 shows that intragastric administration of *R. graveolens* diet at dose (500 mg/kg body weight) for 60 days to male rats causes a significant decreased (P< 0.01) in the number of females impregnated by male treated rats. The number of implantations and the number of viable fetuses calculated after cesarean suctions were significantly decreased (P< 0.01) in female rats impregnated by treated males when compared with females impregnated with untreated rats. On other hand the number

Table 4: Serum Biochemistry findings in male albino rats treated with *Ruta graveolens* for 60 days compared to controls.

Condition	Glucose	Cholesterol	Triglycerides	Bilirubin	SGOT	SGPT	Testesteron	FSH
	---------- Mmol ----------			µmol	---------- U/ L -------		nmol / l	IU /L
Control group	7.3± 0.212	1.4± 0.147	0.8±.0.07	3.175±.0.142	36.7±1.98	77.7±7.14	14.4± 2.53	21.87±0.44
Treatment group	8.9**± 1.03	1.56± 0.07	1± 0.05	3.3± 0.22	68.33*±2.22	78.33*± 8.87	8.66**± 1.88	16.33**±0.68

Results are expressed as mean ±S.D.
Ten rats were included per group.
*p < 0.05, **p < 0.01 ***p < 0.001 significantly different from control group (Student's "t" test).

Table 5: The effect of *Ruta graveolens L* fed to intact on male albino rats on fertility

Condition	No. of male	No. of female	No.of pregnant females	No.of implantationSites	No. of viablefetuses	Total No. of resorption sites	No.of resorption/ total no.of implantation
Control group	10	20	18/20(90%)	9.62± 2.66	9.37± 1.16	8	8/173(5%)
Treatment group	10	20	15/20(75%)	8.2**± 3.31	6.63**± 1.54	19	19/123(15.4%)

Results are expressed as mean ±S.D.
ו rats were included per group.
*p < 0.05, **p < 0.01 ***p < 0.001 significantly different from control group (Student's "t" test).

Table 6: The effect of *Ruta graveolens L* fed to intact male albino rats on sex behavior.

Treatment	No.of animals	Time to first mount (min)	Number of mounts	Intromissions Latency (min)	Number of intromissions	Ejaculatory Latency (min)	Post-ejaculatory interval (min)	% of males ejaculating
Control group	10	2.38± 1.30	7.15± 1.21	4.19± 1.88	12.46± 2.72	9.90± 4.43	5.1± 1.31	80%(8/10)
Treatment group	10	1.83± 1.09	5.24***± 2.11	3.44± 1.25	13.08± 1.85	13.24***± 2.33	8.33***± 1.66	50%(5/10)

Results are expressed as mean ±S.D.
ו rats were included per group.
*p < 0.05, **p < 0.01 ***p < 0.001 significantly different from control group (Student's "t" test).

Table 7: The effect of *Ruta graveolens L* fed to intact male albino rats on aggressive behavior.

Treatment	No.of animals	Lateralization by stud male	Boxing bouts with Stud male	Fights with Stud male	Ventral presenting
Control group	10	13.20 ± 3.76	3.70 ± 0.82	2.40 ± 0.69	1.60 ± 0.69
Treatment group	10	6.85***± 2.09	1.31***±0.44	1.25***±0.25	1.04*± 2.89

Results are expressed as mean ±S.D.
ו rats were included per group.
*p < 0.05, **p < 0.01 ***p < 0.001 significantly different from control group (Student's "t" test).

of resorptions sites were found to be increased to a significant values (P< 0.05) in females impregnated by treated male rats when compared to controls.

3-6. Effect of *R. graveolens* on Sex Behavior

The results presented in Table 6 shows the effect of *R. graveolens* on the parameters related to male rats sex-behavior. Male treated rats presented with a significant decrease (P< 0.001) in the number of mounts where a significant increased (P< 0.001) in ejaculation time was observed when compared to controls. In addition, a significant increase (P< 0.001) in the post ejaculatory interval was found evident when treated rats where exposed to female rats of the same strain and when compared with control rats.

3-7. Aggressive behavior parameters

Table 7 shows the effects of long-term ingestion of *R. graveolens* on the parameters of territorial aggression in adult male rats. Male treated rats had significantly reduced effect on lateralization and boxing bouts (p < 0.001). A significant reduction (P<0.05) in the number of ventral presenting postures was observed in treated rats when compared with controls. Although there was a higher tendency rate to reduce the fighting when adult treated male have been exposed to untreated male rats, this effect did not reach a recognized significant level. Therefore, so was unlikely possible to draw any conclusion from these results.

4. Discussion

The *R. graveolens* is currently used by many people in Jordan as a phrodisiac and fertility promoting agent. In this experiment, the animal model has been used previously by several workers to assess the adverse effects of this extract obtained from medicinal plants on reproductive functions in male (Pakrashi, A. and Pakrashi, P.L.,1977; Lohiya et al.,1994)

In rats the whole spermatogenic cycle process requires 53 days out which spermatozoa spends the last 6 to 7 days in its final transition through epididymides (Ke and Tso,1982). Treatment presented as an extract obtained from *R. graveolens* was administrated for one complete spermatogenic cycle. The present investigation clearly shows that oral administration of *R. graveolens* promoted a decreased male albino rats fertility. This is further illustrated from the data obtained regarding the weight of reproductive organs were a markedly decreased in the organs weight was observed as indicated by table number 1. It is well known that the weight, size and the secretory function of testes, epididymes, seminal vesicles, ventral prostate, and vasa differentia are closely regulated by androgens hormones (Choudhary and Steinberger,1975, Agrawal et al.,1986). Therefore, it could be that the treatment may act directly or indirectly on the pituitary gland secretory function leading to an increase in the main hormones controlling spermatogenesis process. It has been demonstrated that the process of spermatogenesis and the accessory reproductive organs function are androgen dependent. Therefore, an changes in the androgen production would reflect and explain the decrease in number of mature Leydig cells and their functional status. In this present study our findings indicated that the number of degenerating Leydig cells were significantly decreased that lead to a decrease in the serum androgen level observed in our results. Our findings that a decrease in number of spermatocytes (primary and secondary) and spermatids observed together with this perversely mentioned observations go hand in hand with and further confirm our hypothesis leading to conclude that these stages are completely androgen dependent (Dym et al.,1979).

Histometric analysis of reproductive organs, on the other hand, further confirmed the androgenic effect. This is illustrated by the finding that a significant decrease in the sperm motility was observed in the cauda epididymis in treatment group. The results presented in this paper also indicated that ingestion of *R. graveolens* by adult male rats reduces the number of females impregnation as shown in table number 5. In addition, the number of implantations and the number of viable fetuses were decreased, this decreased could be a reflect and may be due to the decrease in sperm motility and sperm density observed in this study. This may be due to the activity effects of *R. graveolens* on the enzymes involved in the oxidative phosphorylation process.

In conclusion *R. graveolens* ingestion diet possesses strong compound or principles that decreased fertility mainly by affecting pituitary gland cells. Further studies are in progress to isolate and identify the active principle(s) with precise mode of its action.

REFERENCES

1 Aberrcrombie M. Estimation of nuclear population from mictome section. Anat Rec 1946; **94**:238-248.
2 Agrawal S. Chauhan S. and Mathur R. Andrologia 1986; **18**:125.
3 Bennetts HW, Underwood EJ, Shier FL. A specific breeding problem of sheep on subterranean clover pastures in westen Australia. Aust Vet J 1964; **22**: 2-12.
4 Bharagava SK. Antifertility agent from plants. Fitoterapia 1988a; **LIX** 163-177.
5 Bataineh H, Al–Hamood MH, Elbetieha A. and Bani Hani I. Effect of long-term ingestion of chromium compounds on aggression, sex behavior and fertility in adult male rat. Drug and Chemical Toxicology 1997; **20**:133-149.
6 Bataineh H, Al-Hamood MH and Elbetieha A. Assessment of aggression, sexual behavior and fertility in adult male rat following long-term ingestion of four industrial metals salts. Human and Experimental Toxicology 1998; **17**:570-576.
7 Klint T and Larsson K. Clozapine acts as a 5-HT2 antagonist by attenuating DOI- induced inhibition of male rat sexual behavior. Psychopharmacology 1995; **119**:291- 294.
8 Bharagava SK. Antifertility effects of Sapindus trifoliatus l. Fruit extract in rats. Internal J Crude Drug Res 1988b; **26**:229-233.
9 Choudhary A and Steinberger E. Effect of 5a-reduced androgen on sex accessory organs, initiation and maintenance of spermatogenesis in the rat. Biol Reprod 1975; **12**:609-617.
10 Desta B. Ethiopian traditional herbal drugs: Part III. Antifertility activity of 70 medicinal plants. J Ethnopharmacol 1994; **44**:199-209.
11 Dixon W and Massey FJ. Introduction of statistical analysis. McGRaw Hill Book Co. ubs. NewYork: 1957. pp. 228.
12 Dym MR, Raj HGM, Lin YC, Chemes HE, Kotitie NJ, Nayfeh SN. and French FS. Is FSH required for maintenance of spermatogenesis in adult rats? J Reprod Fertil 1979; **26**(Supp l):175-181.
13 Elisabetsky E, Posey DA. Use of contraceptive and related plants by the Kayapo indians (Brazil). J. Ethnopharmacol 1989; **26**:299-316.
14 Fransworth NR, Bingle AS, Cordell GA, Grane FA. and Frong HHS. Potential value of plants as a source of new fertility agents. J Pharm sci 1975; **64**: 535-598.
15 Ipstein J and Poly F. In: Banchroft's introduction to biostatics II Ed. (Harper international) 1970. pp. 44-64.
16 Ke YB and Tso WW. Variations of Gossypol susceptibility in rat spermatozoa during spermatogenesis. Int J Fert 1982; **27**(1):42-46
17 Lohiya NK, Goyal RB, Jayaprakash D, Ansari AS, Sharma S. Antifertility effects of aqueous extract of Carica papaya seeds in male rats. Planta Medica 1994; **60**:400-404.
18 Li P. Preliminary study on anti-implantation and anti-early fertility effect of Chinese herbal medicine. Herbal Leonuri up on mice. J of XI AN. 1988. pp. 317- 320.
19 Lynch JM, Raphel SS, Mellor LD, Spare PD. and Inwood MJH. Medical laboratory technology and clinical pathology. WB Saunders Company. Ig. Ku Shoin Ltd. Toky: 1969. pp. 626, 647, 662.
20 Man T, Davies DV and Humphrey GF. Fructose and citric acid assay in the secretion of the accessory of glands of reproduction as indicator testes of male sex hormone activity. J Endocr 1949; **6** :75-85.
21 Mann T, Ed. The biochemistry of semen and of the male reproductive tract. Metauen, London: 1964. pp. 239.
22 Noda Y, Natsuyama S, Moria T. Effect of traditional herbal medicines on cultured hamster epididymal cells. Jap J Fertil Steril 1993; **38**:262-268.
23 Pakrashi A and Pakrashi PL. Antispermatogenic effect of the extract of Aristolocuia indica Linn. on male mice. Ind J Expt Bio 1977; **15**:256-259.
24 Pakarashi A, Pakarashi P. Antifertility efficacy of the plant Aristolochi indica Linn on mouse. Contraception 1979; **20**:49-54.

25 Petry R, Mauss J, Rausch-Stromann JG and Vermenten A. Reversible inhibition of spermatogenesis in men. Horm Metabol Res 1972; **4**:386-388.
26 Prasad MRN, Singh SP and Rajalakshmi M. Fertility control in male rats by continuous release of microquantities of cyproterone acetate from subcutaneous silastic capsules. Contraception. 1970; **2** :165-178.
27 Prasad MRN, Chinoy NJ, Chinoy NJ and Kadam KM. Changes in succinate dehydrogenase levels in the rat epididymis under normal and altered physiological condition. Fert Ster 1972; **23**:186-190.
28 Prasad MRN, Rajalakshmi M, Gupta G. and Karkun T. Hormonal control of epididymal function. J Reprod Fertil (Suppl) 1973; **18**:215-222.
29 Purohit A and Daradka HMM. Antiandrogenic efficacy of Curcuma longa (50%EtOH exttract) with special emphasis on testicular cell population dynamics. Indian Drugs 1999; **36**:142 – 143.
30 Ramanadham S, Paulette D and Thomos E. Effect of insulin replacement on SZ. Induced effect in the rat heart. Life Sciences 1983; **33**:289-296.
31 Rajalakshmi M and Prasad MRN. Changes in sialic acid in testes and epididymis of rats during onset of puberty. J Endocr 1969; **44**: 379-385.
32 Rajalakshmi M, Reddy PRK and Prasad MRN. Distribution of fructose, citric acid, sialic acid and lipids in male accessory glands of the hamstar Mesocricebus auratus. J Endocr 1973; **58**: 349-350.
33 Rugh R. The mouse,its reproduction and development.Burgess, Minneapolis: 1968.
34 Sethi N, Nath D, Snigh RK, Srivastava RK. Antifertility and teratogenic activity of some indigenous medicinal plants in rats. Fitoterapia 1990; **LXI**:64-66.
35 Sinha R. Post-testicular antifertility effects of Abrus precatorius seed of extractin albino rats. J Ethnopharmacol 1990; **28**:173-181
36 Usuki S. Blended effects of herbal components of Tokishakuyakusan on rat corpus luteum function in vivo. Am J Hin Med 1988; **16**(3-4):107-16.
37 Ushiroyama T, Tsubokura S, Saeki M, Okuda K, Kaneko T, Veki M, Gimoto O. Endocrinologic studies of Unkei-to (TJ-106) in young females with weight loss amenorrhea with an effect on pulsatile secretion of gonadotropins. Jap J Fertil Steril 1991; **36**: pp. 61-65.
38 Warren Ch, Hiyari F, Wingo PhA, Abdel-aziz AM and Morris L. Fertility and family planning in Jordan: Results from the 1985 Jordan Husband`s Fertility survey studies in family planning. 1990; 21, 1:33-39.
39 WHO. Protocol MB50 A method for examining the effect of plant extracts adminsitration orally on the fertility of male rats. 1983.