Stimulatory Effect of the Aqueous Extract of *Ruta chalepensis* on the Sex Organs and Hormones of Male Rats

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ABSTRACT

The oral administration of an aqueous extract of the leaves of *Ruta chalepensis* to mature male rats in daily oral doses of 0.5 g (Group I), 1.0 g (Group II) and 2.0 g (Group III) per animal for 30 days resulted in an increase of the testicular weights, volume, and index, and epididymis weight with no change in the body weight. The plant had a spermotrophic action demonstrated by the increase in sperm count, motility, living percent, and decrease in encountered sperm abnormalities. The hormonal profile was also influenced by the *R. chalepensis* extract. The testosterone and FSH levels were significantly increased with no change in the LH and prolactin levels.

INTRODUCTION

Ruta chalepensis has been used medicinally in many ancient cultures. In ancient Turkish[1,2] and Chinese[3] literature, its use as an abortifacient and uterine stimulant were reported. Recently, it has been studied for its antirheumatic, anti-inflammatory, antipyretic, analgesic, and CNS depressant properties.[4,5,6] In preliminary studies, toxicity studies in mice on the total ethanolic extract of *Ruta chalepensis* failed to reveal any spermatotoxic effects.[7] Yet, Zeichen de Sa et al[8] found that dried leaf infusions of *Ruta chalepensis* caused perinatal changes in mice, confirming the embryotoxic effect of the plant.

In an effort to develop new orally active gonadotrophic agents from indigenous plants to improve human fertility and libido, a comprehensive screening program is being pursued at this institute, Department of Veterinary Medicine, Faculty of Agriculture and Veterinary Medicine, AlQassim University.[9,10] This study was undertaken to unravel the possible influence of *R. chalepensis* on male sex organs administered in a form (ie, as an aqueous extract) commonly used by people in the Middle East.

MATERIALS AND METHODS

Animal Model

Colony bred adult Sprague Dawley rats weighing 150 g to 200 g were derived from a colony at the King Saud University, Faculty of Medicine, Riyadh, Saudi Arabia. They were maintained in

air-conditioned quarters (22 ± 1˚C) under uniform husbandry conditions in the laboratory house of the Department of Veterinary Medicine and with a light cycle from 06:00 to 20:00 hours. Animals were kept in groups of 10 each in a wire-bottomed cage with free access to pelleted diet (AlQassim Animal Feed Factory) and tap water ad libitum. General procedures for animal care and housing were in accordance with the US Department of Agriculture through the *Animal Welfare Act (7USC 2131) 1985* and *Animal Welfare Standards* incorporated in *9 CFR Part 3, 1991*.

Plant Material

Leaves of *Ruta chalepensis* were collected from AlTaif region on the west coast of Saudi Arabia during the period of September 2003 and authenticated by Professor Saleh Bazaid, Department of Biology, Faculty of Science, Umm Al-Quora University, Mecca, Saudi Arabia. The fresh leaves were macerated and extracted with distilled water (1:2 wt/vol) for 48 hours at 4˚C. The crude filtrate was freeze dried in a labconco model 75018 lyophylizer and the required doses were made up in double distilled water.

Dose and Duration of Treatment

The crude aqueous extract was administered orally through gastric gavage at daily dose regimens of 0.5 g (Group I), 1.0 g (Group II), and 2.0 g (Group III) per animal for 30 days. Control animals received saline.

Autopsy

The animals were weighed and autopsied under pentobarbital anesthesia (50 mg/kg given intraperitoneally) 24 hours after the last dosing of the respective treatment. Blood samples were collected at 10 AM from the inner canthus in heparinized tubes and the plasma was immediately separated and stored at –20˚C until used for hormonal assays.

Body and Testes Measurements

Initial and final body weights of the animals were recorded. At autopsy, the reproductive and accessory sex organs (testes, epididymis) were dissected out, freed from adherent tissues, and weighed up to the nearest 0.01 g. The maximum length and width of each testis was measured to the nearest 0.1 mm. A testicular index (TI) was calculated for each rat (testis length testis width/body weight); TI and testis width are correlated with testis weight, which reflects spermatogenesis and testosterone production.[9,11] After measurement of the three dimensions of each testis, the testicular volume was calculated using the "Ellipsoid formula".[12]

Sperm Motility, Count and Morphology

Cauda sperm forward motility. After anesthetizing the rat, the epididymis was exposed by scrotal incision, and spermatozoa were expressed out by cutting the distal end of the cauda epididymidal tubule. Spermatozoa with epididymal fluid was diluted with physiological saline and placed on a thin glass slide, and forward motility (rate and percentage) of 100 spermatozoa per rat was observed under a microscope using precalibrated ocular micrometer.[13]

Sperm count. Spermatozoa were counted as per the method of Zaneveld and Polakoski.[14] Sperm suspension was placed on both sides of Neubauer's hemocytometer and allowed to settle in a humid chamber (wet) for 1 hour. The number of spermatozoa in the appropriate squares of the hemocytometer was counted under the microscope at 100 magnification.

Sperm morphology. Morphology was evaluated on sperm from both the cauda

and caput epididymis. Cauda sperm were taken from the original dilution for motility and diluted in a 1 to 3 ratio with 10% neutral buffered formalin. Caput sperm were diluted in 500 µL of motility medium and allowed to disperse, and 1 mL of 10% formalin was added. Five hundred sperm from each cauda and caput sample were scored for total morphological abnormalities as described previously.[15] Briefly, in wet preparations using phase-contrast optics, sperm were categorized as one of the following: (1) normal head and tail, (2) isolated heads (whether the head was misshapen or not), (3) head-only defects (ie, misshapen head with normal tail), (4) tail defects (ie, normal head with abnormal tail or misshapen head with abnormal tail), and (5) fused sperm.

Hormonal Assays

The collected plasma was assayed for testosterone, follicle stimulating hormone (FSH), leutinizing hormone (LH), and prolactin. The hormones were measured by means of a radioimmunoassay Coat-A-Count kit (Diagnostic Products Corporation, Los Angeles, Calif) using a Packard Cobra gamma-counter.

Statistical Analysis

All of the recorded values of body / organ weight, testicular dynamics and hormonal estimations were expressed in terms of mean ± SEM. The treated groups were compared to control using the Student *t* test.

RESULTS

The results recorded in Table 1 reveal that although the body weights in the three groups did not change yet, the testicular and epididymal weights significantly increased and showed a consequent significant increase in the testicular volume and index ($P < 0.01$). In the three dose regimen groups, the sperm count and motility were significantly increased ($P < 0.01$). The living percent was also increased and showed a significant decrease in the total number of abnormalities.

The plasma sex hormones exhibited a significant increase in the levels of testosterone and FSH, while the levels of LH and prolactin did not reveal any significant change (Table 2). The stimulatory effects were dose-dependent.

DISCUSSION

Interestingly, although a number of alkaloids and coumarins have been isolated from *Ruta chalepensis var. latifolia*,[16] no attempt appears to have been made so far to determine the spermtrophic and/or hormonal effects of *Ruta chalepensis* in mammals.

Testicular size is the best primary assessment of spermatogenesis, since the tubules and germinal elements account for approximately 98% of the testicular mass.[17] The oral administration of *R. chalepensis* aqueous leaf extract resulted in a significant increase in testicular weight, which is known to be mostly related to the number of spermatids and spermatozoa present in the tissue.

Although Gijon et al[18] indicated that the ethanolic extract of *Ruta chalepensis* immobilized frog sperms yet, on the contrary the results of the present investigation indicate an identical stimulatory mode of action of the aqueous extract of *Ruta chalepensis* leaves on the gonads and the spermogram of the male rat. This discrepancy could be attributed to the difference in the *R. chalepensis* extraction procedures. This differential effect has been previously recorded with species and extraction methodology.[19-21] The increase in sperm density and motility in cauda epididymis is of importance with regard to fertilization. Therefore, the crude aqueous extract caused an androgen stimulatory effect on the target organs, beneficial alterations in the

Table 1. The Effects of the Crude Aqueous Extract of *Ruta chalepensis* on Body Weight, Male Sex Organs and Spermogram of Rats (n = 10)

Parameter	Groups			
	Control (saline)	I (0.5 g)	II (1.0 g)	III (2.0 g)
Body weight (g)	261.8 ± 4.71	266.0 ± 1.05	261.8 ± 4.71	242.2 ± 9.24
Testicular weight (g/100 g body weight)	1.09 ± 0.03	1.02* ± 0.01	1.24† ± 0.02	1.24† ± 0.04
Testicular volume	1.35 ± 0.03	1.57† ± 0.06	1.68† ± 0.03	1.49† ± 0.02
Testicular index	0.41 ± 0.01	0.59† ± 0.05	0.59† ± 0.05	0.59* ± 0.04
Epididymis weight (g)	0.41 ± 0.02	0.39† ± 0.01	0.40† ± 0.01	0.45† ± 0.02
Sperm count 10^6/mL	7.27 ± 0.44	9.00* ± 0.47	11.25† ± 1.02	15.8† ± 2.88
Sperm motility %	36.0 ± 1.63	65.0† ± 1.49	68.0† ± 1.33	73.0† ± 1.33
Total sperm abnormalities %	58.0 ± 1.74	30.6† ± 1.78	31.2† ± 1.51	32.0† ± 2.06
Live sperm %	44.0 ± 1.12	89.0† ± 1.07	94.0† ± 1.33	92.0† ± 2.23

*$P < 0.05$
†$P < 0.01$

Table 2. Levels of Plasma Testosterone, Gonadotrophic and Prolactin Hormones Following Oral Administration of a Crude Aqueous Extract of *Ruta chalepensis* Leaves*

Groups	Hormones			
	Testosterone (ng/mL)	FSH (MIU/M)	LH (MIU/M)	Prolactin (ng/mL)
Control (saline)	1.21 ± 0.05	0.22 ± 0.01	0.61 ± 0.02	1.03 ± 0.01
I (0.5 g)	3.65 ± 0.13†	0.88 ± 0.02†	0.60 ± 0.00	1.13 ± 0.05
II (1.0 g)	4.26 ± 0.12†	0.92 ± 0.01†	0.61 ± 0.02	1.05 ± 0.03
III (2.0 g)	4.76 ± 0.48†	0.91 ± 0.01†	0.66 ± 0.02	1.04 ± 0.02

*At daily dose regimens of 0.5, 1.0, and 2.0 g per animal in adult male rats.
†$P < 0.01$

motility, morphology and metabolism of the spermatozoa, and thereby, increased fertility in male rats.

In the present investigation the observed increase in the cauda epididymal sperm motility might be due to an alteration in the microenvironment in the cauda epididymis, which also had a synergistic action on the metabolism of the spermatozoa of the treated rats as a result of the androgen-stimulatory effect of the aqueous extract of *R. chalepensis*. The increase in the cauda epididymis sperm count in the treated animals substantiates the spermatogenic nature of the extract. The extract had a direct effect on the testes resulting in an increase in the number of spermatozoa and the increased level of testosterone production. Also, the extract had no spermatotoxic effect as previously indicated by Shah et al[7] and as evidenced by the recorded spermogram in Table 1.

The extract did not show an antigonadotrophic nature, demonstrated by the increased level of FSH and normal LH levels in the treated rats.

The increased level of FSH reveals a possible role of *R. chalepensis* extract in influencing the release of gonadotrophic hormones from the pituitary. The rise of FSH by itself is of critical importance in the initiation and expansion of spermatogenesis in mammals, as is generally agreed.[22] In addition, *R. chalepensis* had no influence on prolactin secretion, a

factor which if present, could cause testicular dysfunction in mice and humans.[9]

An extensive literature search revealed that *M. paniculata* and all other species of the subtribe Clauseninae demonstrated no effect on human reproduction. This subtribe shared a common phytochemical profile, particularly in carbazole alkaloids and coumarins.[23]

Among the other naturally occurring coumarins, only the 3-phenylcoumarins have been reported to possess potent estrogenic activity, due to their trans-stilbene character.[24] This might enhance the secretion of the accessory organs by affecting the neural control of the secretory process and/or by increasing the amount of secretory epithelium in the glands

In conclusion, the stimulatory effects of *Ruta chalepensis* seem to be mediated through a pituitary-testicle axis participating in the physiological events of spermatogenesis.

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