Adverse Effects of Rosemary (*Rosmarinus officinalis* L.) on Reproductive Function in Adult Male Rats

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Ingestion of rosemary (*Rosmarinus officinalis* L.) by two groups of adult Sprague-Dawley rats at levels of 250 and 500 mg/kg body wt for 63 days was investigated for its effects on fertility. Body weight and absolute and relative testes weights were not affected, but the average weights of epididymides, ventral prostates, seminal vesicles, and preputial glands decreased significantly. A significant decline in spermatogenesis in testes due to a decrease in the number of primary and secondary spermatocytes and spermatids in treatment group 2 (500 mg/kg) is attributed to a significant decrease in testosterone. Sperm motility and density were also significantly decreased in the cauda epididymis and in the testes of rosemary-treated male rats in group 2. In addition, the treatment markedly increased the number of fetal resorptions in female rats impregnated by group 2 males, thereby reducing their fertility. Exp Biol Med 232:809–813, 2007

Key words: *Rosmarinus officinalis*; antifertility; contraception; spermatogenesis; male rat

Introduction

Rosemary (*Rosmarinus officinalis* L.) is a common household plant grown in many parts of the world. It is used for flavoring food, as a beverage, and in cosmetics (1–3). In folk medicine, it is used as an antispasmodic in renal colic and dysmenorrhea, in relieving respiratory disorders, and to stimulate growth of hair (4). Extract of rosemary relaxes smooth muscles of the trachea and intestine, and it has choleretic, hepatoprotective, and antitumorogenic activities (5).

The most important constituents of rosemary are caffeic acid and its derivatives such as rosmarinic acid (6). These compounds have antioxidant effects (7). The phenolic compound rosmarinic acid obtains one of its phenolic rings from phenylalanine *via* caffeic acid and the other from tyrosine *via* dihydroxyphenyl lactic acid. Relatively large-scale production of rosmarinic acid can be obtained from a cell culture of *Coleus blumei* Benth. when it is supplied exogenously with phenylalanine and tyrosine (8). Rosmarinic acid is well absorbed in the gastrointestinal tract and on the skin. It increases the production of prostaglandin E_2 (9), reduces the production of leukotriene B4 in human polymorphonuclear leucocytes, and inhibits the complement system (10). It has been proposed that rosemary and its constituents, especially caffeic acid derivatives such as rosmarinic acid, have a therapeutic potential in treatment or prevention of bronchial asthma, spasmogenic disorders, peptic ulcers, inflammatory diseases, hepatotoxicity, atherosclerosis, ischemic heart disease, cataracts, and cancer (11, 12).

The aim of this work was to investigate the effect of ingestion of an extract of *R. officinalis* leaves on fertility and sexual maturation in the male rat. The decoction of this plant is widely used by the Jordanian population.

Materials and Methods

Thirty adult male and 60 female Sprague-Dawley rats weighing approximately 290 g each were bred in the Animal House Unit at the Jordan University of Science and Technology (JUST) School of Medicine between May and October 2005. Rats were maintained at a controlled temperature of 21°C ± 1°C and on a 12:12-hr light:dark cycle. Food and water were supplied *ad libitum*.

Fresh rosemary leaves were collected from JUST gardens in May 2005. The plant was identified and

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authenticated by the staff of the JUST herbarium. Each 500 g of dried and ground *R. officinalis* was then refluxed in 2 liters of 70% ethanol at 60°–70°C for 36 hrs in a continuous extraction (soxhlet) apparatus. The ethanol extract was filtered and concentrated under reduced pressure at 60°C using a rotary evaporator. The net yield was 34 g/kg. This material was then dissolved in distilled water and administered orally to rats at a concentration of 250 and 500 mg/kg body wt (1-ml volume) as single daily doses using animal-feeding intubation needles (Popper and Sons, New Hyde Park, NY). Similarly, the controls received gastric infusions of 1 ml of distilled water in the same way as the experimental rats.

Male rats were randomly assigned to control or experimental groups. Experimental male rats were divided into two groups: groups 1 and 2 were fed 250 and 500 mg/kg body wt rosemary for 63 days, respectively. All male rats were healthy and continued to receive their respective drinking water and food throughout the experimental period.

To estimate the fertility in both experimental and control male rats, each male was placed in an individual cage with two virgin untreated females of the same strain for 10 days; the female rats were brought into estrus by sequential subcutaneous treatment with 5.0 mg of estradiol benzoate (Sigma-Aldrich, St. Louis, MO) 54 hrs before testing and 0.5 mg of progesterone (gift from Roussel Uclaf, Paris, France) 6 hrs before testing. The hormones were dissolved in corn oil (Arab International Food and Oil Processing Co., Amman, Jordan) in a total volume of 0.1 ml. The animals were left together for 10 days during which two estrus cycles should have elapsed (13). One week after the removal of the males, the females were killed by cervical dislocation under light ether anesthesia, and the number of pregnant females, implantation sites, viable fetuses, and fetal resorptions were recorded (14).

All males were sacrificed by cervical dislocation under light ether anesthesia after 63 days of exposure. Blood was collected by heart puncture using a sterile syringe, and serum was separated and stored at –20°C for biochemical analysis. Body weight and weights of paired testes, seminal vesicles (stripped of fluid), and preputial glands were recorded. The reproductive organs of male rats, including the testes, epididymides, ventral prostates, seminal vesicles, and vas deferens were fixed in Bouin fixative for histologic studies.

Sperm motility and sperm counts of cauda epididymis were determined by the method described earlier (14). Quantitative motility (%) was determined by counting both motile and immotile spermatozoa per unit area. Cauda epididymis and testicular sperm counts were performed by routine procedure and expressed as millions/ml of suspension (15). Histologic evaluation and sperm analyses were performed by a person unfamiliar with rat groups. Bouin-fixed reproductive organs were processed for paraffin embedding, sectioning (5 μm), and staining (Harris hematoxylin and eosin).

One hundred circular-appearing seminiferous tubules were traced at ×80 with a camera lucida, and the diameter of each tubule was measured separately. The measurements were expressed in terms of the mean of all the traced tubules. Similarly, Leydig cell nuclei were traced at ×800. The epithelial cell heights of caput and cauda epididymides and seminal vesicles were traced at ×360.

Spermatogonia, spermatocytes, and spermatids were counted in 5-μm thick cross sections of 10 seminiferous tubules of 10 animals in each group. All raw counts were transformed into "true" counts by an adaptation of the Abercrombie formula (16) from germ cell diameter measurement. Interstitial cell types (such as fibroblasts, immature and mature Leydig cells, and degenerating cells) were counted by applying a differential count over a 200-cell population and statistically verifying the counts by binomial distribution (17).

Glucose, total cholesterol, triglycerides, bilirubin, serum aspartate aminotransferase (AST), and serum alanine aminotransferase (ALT) were measured using commercial kits from Cis BIO International (Gif sur Yvette, France). Serum testosterone concentrations were measured using enzyme immunoassay kits (Cayman Chemical Co., Ann Arbor, MI). Serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations were measured using rat LH and FSH enzyme immunoassay systems (Amersham, Buckinghamshire, UK), respectively.

Data are expressed as means ± SD and medians (SPSS for Windows version 11.5; SPSS Inc., Chicago, IL). Differences between control and rosemary-exposed male groups were analyzed using the chi-square test, Student's *t* test, or nonparametric (Kruskal–Wallis) test, as applicable. $P < 0.05$ was considered statistically significant (18).

Results

Table 1 shows that intragastric administration of *R. officinalis* extract had no significant effect on the body weights of treated males when compared with those of the control group. However, the absolute and relative weights of testes, epididymides, seminal vesicles, ventral prostates, and vas deferens were significantly reduced in group 2. Table 2 shows that sperm motility in cauda epididymis, sperm density, seminiferous tubule diameter, Leydig cell nuclear diameter, and epithelial cell height in epididymides (cauda and caput) and seminal vesicles were significantly decreased in group 2 animals in comparison with controls.

Table 3 shows that administration of rosemary extract caused a significant decrease in the germinal cell population: spermatocytes (primary and secondary) and spermatids were decreased to a significantly lower level in group 2. Similarly, the numbers of fibroblasts and immature and mature Leydig cells were also significantly decreased in group 2. The number of degenerating cells, however, was significantly increased in group 2.

Serum glucose, total cholesterol, triglycerides, bilirubin, AST, and ALT levels were not altered. Whereas there

Table 1. Body and Organ Weights of Male Rats That Ingested *R. officinalis*[a]

Treatment[b]	Body weight (g)		Testes	Epididymides	Seminal vesicle	Ventral prostate	Vas deferens
	Initial	Final	(mg/100 g body wt)				
Control group	284 ± 2.80	351 ± 2.65	895 ± 15.21	367 ± 11.61	376 ± 4.38	226 ± 4.1	68.8 ± 1.36
Group 1 (*R. officinalis* 250 mg)	291 ± 2.80	337 ± 2.65	868.6 ± 11.73	371.3 ± 8.33	384 ± 3.17	198.7 ± 2.11	66.3 ± 1.67
Group 2 (*R. officinalis* 500 mg)	287 ± 4.55	342 ± 7.6	789** ± 12.1	351.1** ± 3.9	348.1** ± 8.1	167.8** ± 8.35	62.2* ± 2.20

[a] Results are expressed as mean ± SD.
[b] Ten rats were included per group.
*$P < 0.05$, **$P < 0.01$ significantly different than control group (Student's *t* test).

Table 2. Histometric Parameters and Sperm Dynamics of Male Rats That Ingested *R. officinalis*[a]

Treatment[b]	Sperm motility (%)	Sperm density (million/ml)		Seminiferous tubule diameter (μm)	Leydig cell nuclear diameter (μm)	Epithelial cell height		
	Cauda	Testes	Cauda			Caput (μm)	Cauda (μm)	Seminal vesicle (μm)
Control group	74.1 ± 1.94	4.75 ± 0.47	56.0 ± 1.94	290.6 ± 3.2	6.45 ± 0.96	38.8 ± 0.4	26.08 ± 0.32	17.32 ± 0.17
Group 1 *R. officinalis* (250 mg)	71.83 ± 1.31	4.68 ± 0.27	48.17 ± 1.34	284 ± 2.66	5.76 ± 0.33	33.37 ± 1.21	22.4 ± 1.03	16.3 ± 0.14
Group 2 *R. officinalis* (500 mg)	63.26** ± 1.08	4.55* ± 0.14	37.185** ± 1.08	266.27** ± 2.35	4.79** ± 0.76	14.68** ± 1.14	17.4** ± 1.08	12.45** ± 0.27

[a] Results are expressed as mean ± SD.
[b] Ten rats were included per group.
*$P < 0.05$, **$P < 0.01$ significantly different than control group (Student's *t* test).

Table 3. Testicular Cell Population Dynamics of Male Rats That Ingested *R. officinalis*[a]

Treatment[b]	Germinal cell type				Interstitial cell type			
	Spermatogonia	Spermatocyte (primary)	Spermatocyte (secondary)	Spermatid	Fibroblast	Immature Leydig cell	Mature Leydig 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
Group 1 (*R. officinalis* 250 mg)	21.3 ± 1.04	17.47 ± 1.11	46.15 ± 3.16	113.14 ± 4.17	54.7 ± 2.76	57.13 ± 2.14	66.6 ± 1.43	23.7 ± 1.25
Group 2 (*R. officinalis* 500 mg)	17.05* ± 1.44	12.96** ± 2.41	17.97*** ± 3.73	9.32*** ± 6.82	38.66** ± 1.33	41.66** ± 1.65	46.66** ± 1.78	31.9*** ± 0.76

[a] Results are expressed as mean ± SD.
[b] Ten rats were included per group.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ significantly different than control group (Student's *t* test).

Table 4. Serum Biochemistry of Male Rats That Ingested *R. officinalis*[a]

Treatment[b]	Glucose	Cholesterol	Triglycerides	Bilirubin	AST	ALT	Testosterone	FSH	LH
	mmol			µmol	U/l		pg/ml	ng/ml	
Control group	7.3 ± 0.212	1.4 ± 0.147	0.8 ± 0.07	3.47 ± 1.142	36.7 ± 1.98	77.7 ± 7.14	754 ± 16.7	79.7 ± 18.6	13.3 ± 6.1
Group 1 *R. officinalis* (250 mg)	7.6 ± 0.87	1.32 ± 0.12	0.78 ± 0.03	3.21 ± 1.75	43.11 ± 3.22	82.75 ± 8.87	746 ± 20	74.6 ± 14.3	10.8 ± 7.27
Group 2 *R. officinalis* (500 mg)	7.4 ± 1.03	1.24 ± 0.07	0.72 ± 0.05	3.12 ± 2.22	42.32 ± 2.65	80.33 ± 8.87	631* ± 24.8	61.8* ± 15.5	8.59** ± 5.66

[a] Results are expressed as mean ± SD.
[b] Ten rats were included per group.
*$P < 0.05$, **$P < 0.01$ significantly different than control group (Student's *t* test).

was a significant reduction in serum levels of testosterone, FSH and LH levels were not changed in plant-treated group 2 rats in comparison with the control group (Table 4).

Table 5 shows that rosemary exposure reduced fertility as indicated by the smaller number of females impregnated by rosemary-exposed males. The number of implantations and number of viable fetuses were significantly decreased in those females impregnated by group 2 males. The total number of resorptions was also significantly increased in females impregnated by group 2 males.

Discussion

The animal model used in this work has previously been used to assess the effect of various extracts obtained from medicinal plants on reproductive functions in males (19–21). The spermatogenic process in rats requires 53 days, out of which spermatozoa spend the last 6–7 days in the final transit through epididymides (22). Rosemary was administrated for one complete spermatogenic cycle.

The present investigation showed that oral administration of rosemary reduced fertility in male albino rats. The weights of reproductive organs were markedly decreased. The weight, size, and secretory function of testes, epididymides, seminal vesicles, ventral prostates, and vasa deferentia are closely regulated by androgens (23). The process of spermatogenesis and function of accessory reproductive organs are androgen dependent (24, 25). The drug may act on the pituitary gland and decrease the main hormone of spermatogenesis (24). Decreased androgen production is reflected by a decreased number of mature Leydig cells and their functional status. In the present study, the number of degenerating Leydig cells was significantly increased; this may reflect a decrease in androgen levels. This decrease was further confirmed by the decreased number of spermatocytes (primary and secondary) and spermatids as these stages are completely androgen dependent (24). The decreased weights and histometry of reproductive organs further confirmed the decrease in androgen levels. A significant increase in the sperm motility of cauda epididymis was observed in the treated group. This may be due to the effect of *R. officinalis* on the enzymes of oxidative phosphorylation (26).

Results presented in this article also showed that the ingestion of *R. officinalis* by adult male rats caused a slight decrease in the number of females impregnated by the exposed males. The number of implantation sites and number of viable fetuses were also reduced. These observations may be due to the decrease in sperm motility and function. The reduction in implantation and fetotoxic effects may be due to cytotoxic effects that can result in decreased fertility, failure of preimplantation, or post-implantation death. Cytotoxic agents may disrupt pregnancy by interfering with mitotic division of the fetus (27). The increased resorptions is further evidence, along with the reduced implantation rate, that rosemary extract at 500 g/kg/day has adverse effects on sperm.

Table 5. Effect of *R. officinalis* Ingestion on the Fertility of Adult Male Rats

Treatment[a]	No. of pregnant females/each male (cage)		No. of implantation sites (mean ± SD [median])	No. of viable fetuses (mean ± SD [median])	Rate of resorptions/ total implantations (mean ± SD [median])
	One pregnant female/cage (*n* [%])	Two pregnant females/cage (*n* [%])			
Control group	2 (20.0)	2 (20.0)	17.3 ± 4.2 (19)	16.9 ± 4.0 (19)	0.021 ± 0.027 (0.0)
Group 1 (*R. officinalis* 250 mg)	3 (30.0)	7 (70.0)	15.4 ± 4.9 (18)	14.6 ± 4.9 (17)	0.057 ± 0.049 (0.052)
Group 2 (*R. officinalis* 500 mg)	6 (60.0)	4 (40.0)	12.3 ± 5.2 (9.5)	10.4 ± 5.0 (8.5)	0.174 ± 0.097 (0.118)
P	0.155[b]		0.029[c]	0.003[c]	<0.0005[c]

[a] Ten rats were included per group
[b] Chi-square test
[c] Kruskal-Wallis (nonparametric) test

These results suggest that the ingestion of *R. officinalis* may impose toxic effects on fertility in male rats. Further studies are in progress to identify and isolate the active component(s) in rosemary that affect fertility in male rats and to determine its mechanism of action.

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