

Effects of ethanolic extract of *Raphanuse sativus* leaves on fertility hormone and sperm parameters in male wistar rats

¹ Manhal M Dafaalla, ² Omer Fadl Idris, ¹ Abdel Wahab Hassan, ¹ Ahmed S Kabbashi

¹ Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), P.O. Box 2404, National Center for Research, Khartoum, Sudan.

² Department of Biochemistry and Molecular Biology, Faculty of Science and Biotechnology, University of El-Neelain, Sudan.

Abstract

This study was conducted to assess the effect of *Raphanuse sativus* leaves extract on the reproductive performance of male wistar rats. A total of 24 male rats were divided into 4 groups (6 of each) namely group A, B, C and D. All groups were given orally 0.0, 100, 200 and 400 mg/kg body weight extract respectively for 30 days. Symptoms caused by acute toxicity were absorbed through the experimental period. At the end of the experiment body weights and sexual organ weights (testes and epididymis) and semen characteristic were determined. Serum was analyzed for testosterone, Follicle-stimulating hormone (FSH), and Luteinizing hormone (LH) levels. The results showed the body weights was significantly ($P \leq 0.01$) decreased, while testicular weight increased. In a dose dependant manner following administration of *R. sativus* extracts. The extract also improved semen characteristics and elevated significantly the level of fertility hormones. It study conclude that the administration of the *Raphanuse sativus* leaves extract improve normal male rats fertility.

Keywords: *Raphanuse sativus*, ethanolic extract, semen characteristic, fertility hormone.

1. Introduction

Sexual health is an important component of an individual's quality of life and well-being (WHO, 2002) [22]. Traditional herbs have been used to improve sexual performance or to treat infertility. A large number of plants have been tested throughout the world for the possible fertility regulatory properties (Bhatia *et al.*, 2010) [2]. Some medicinal plants are extensively used as aphrodisiac to relieve sexual dysfunction, or as fertility enhancing agents. They provide a boost of nutritional value thereby improving sexual performance (Yakubu *et al.*, 2007; Sumalatha *et al.*, 2010) [23, 19]. *R. sativus* (Radish) is an annual or biennial bristly herb with a white or brightly colored tuberous tap root and coarsely toothed leaves. It belongs to the (family: *Brassicaceae*). A plant cultivated in West and Central Africa and exported worldwide, possesses aphrodisiac activity. Radish leaves were found to contain alkaloid like coumarins, saponins, flavonoids and anthocyanins (Sanaa, 2001) [15]. They decrease uric acid level in the serum which related to circulating markers of inflammation and free radical reactions (Zaman, 2004) [25]. The anthocyanins are important group of dietary antioxidants that have many physiological functions. They protect living cells from oxidative damage resulting in the prevention of diseases (Matsufuji *et al.*, 2003) [7]. Besides, radish leaves contain isothiocyanate that has antimicrobial activity, antimutagenic, anticarcinogenic and anti-atherosclerosis activity (Suh *et al.*, 2006) [18]. Radishes are rich in ascorbic acid, folic acid, vitamin B6, riboflavin, potassium, magnesium, copper and calcium (Jan and Badar, 2012) [5]. The aim of this study was to investigate the aphrodisiac effect of the leaves of *R. sativus* in mature male rats.

2. Materials and methods

Plant materials and extraction procedure:

Raphanuse sativus leaves were obtained from the local market of Khartoum North well cleaned and kept in poly ethylene bags at room temperature. The plants were authenticated by taxonomical identification was confirmed by a senior plant taxonomist. Leaves of *R. sativus* were first dried in the shade, left in ethanol (85%) for more than two days in Soxhlet apparatus. Then the 85% ethanol extract was dried in Rotary Evaporator apparatus, weighed and dissolved in distilled water to give the final concentration of 100 mg extract/kg, 200 mg extract /kg and 400 mg extract /kg and were administrated orally by Gavage for the three groups of rats; A, B, and C, for 30 days.

Experimental animals

The study was conducted on healthy male Wistar albino rats. Out breed Albino rats *Rattus norvegicus* weighing 160-200g were obtained from the Medicinal and Aromatic Plants Research Institute, National Center for Research. From this stock, rat colonies were raised in the laboratory. They were kept in accommodation with a mean temperature 30 °C. They were kept in wide metal cages. Individuals were identified by marks on their tails using the system of Ramesh, (2004) [13].

Extract administration

Twenty-four male Wistar rats weighing between (160-200g) for all experiments, were maintained under standard environment conditions and fed with standard pellet diet and water *adlibitum*. After a week of adaptation, the rats were randomly divided into 4 groups A, B, C and D of 6 rats each. Group a, b and c received orally *R. sativus* leaves ethanol extract in a dose

of 100, 200 and 400 mg/kg respectively while group D served as a control (Sabu, and Subburajub, 2002) [14].

Body weight determination

Body weights of experimental animals before and after experiments were measured using small balance (0-5 kg capacity), following an overnight fasting. The body weights were used to calculate the daily weight gain.

Sexual organs weights determination

All the control (standard) and experimental groups of male rats were evaluated for their body weight. The animals were completely anaesthetized with anesthetic ether (Narsons Pharma), sacrificed by cervical decapitation and then testis and epididymis were carefully removed and weighed using digital electronic balance. The organ weight of each organ were determined (Thakur and Dixit, 2006; 2007 [20, 21]; Amini and Kamkar, 2005) [11].

Semen collection

The testicles were then removed through a lowered abdominal incision and testes were then separated from the epididymis. The right and left epididymis were trimmed off the body of the testes and semen sample were collected from the tail of the epididymis through an incision made with a scalpel blade. Sperm cells were sucked into apasteur pipette from the caudal epididymis. The incisions were also flushed with 2-3 drops of 2.9% buffered sodium citrate kept at body temperature.

Sperm analyses

Sperm motility and count

This experiment was conducted following the method adopted by (Prasad *et al.*, 1972). by To determine sperm motility and sperm counts, 100 mg of caudal epididymis was minced in 5 ml of physiological saline. One drop of an evenly mixed sample was applied to a Neubauer's counting chamber under a cover slip. Quantitative motility expressed as index was determined by counting both motile and immotile spermatozoa per unit area. Epididymal counts was made by routine procedure and expressed as million/ml of suspension.

Percentage of abnormal and normal spermatozoa

The smears were prepared by placing a drop from semen sample and one or two drops of previously warmed (37 °C) eosin-nigrosin stain at one of clean slide and another side (spreader) was brought towards the mixture until it touched it. The smears were allowed to dry in the air and then examined using high power (100X) microscope oil immersion objective. 200 sperm cells from different fields were examined and the number of abnormal ones was calculated as percentage.

Estimation of sperm viability

This was estimated using the improved one step eosin-Nigrosin staining technique. A fraction of each suspension of the sperm samples was mixed with equal volume of eosin-Nigrosin stain and air dried smears were prepared on glass slides for each

samples. The slides were coded randomly and examined under the microscope for percentage viability. Normal live sperm cells exuded the eosin-nigrosin while dead sperm cells took up the stain. Percentage viability was calculated based on the number of viable (live) sperm cells divided by the number of sperm cells within 30 minutes multiply by 100.

Testosterone assay

Serum collected at termination was used for assaying for total testosterone. Testosterone was measured using a commercial ELISA kit (IBL) which is based on competitive binding of testosterone on immobilised antibody. Radish peroxidase was used for colour development and absorbance at 420 nm measured on a plate reader (Multiskan EX). Values are reported as ng/ml of serum.

Statistical analysis

Data generated were subjected to Statistical Analysis System (SAS). One-way Randomized Complete Design (RCD) was assessed and then Duncan's Multiple Range Test (DMRT) was performed for mean separation.

3. Results

Body and sexual organ weights

Rats treated with higher doses (200,400 mg/kg body weight) of *R. sativus* leaves showed significant ($P \leq 0.01$) reduction in their body weights (Table 1).

While rats treated with either *R. sativus* leaves showed significant ($P \leq 0.05$) dose dependant increase in testes weight (Table 1) However, epididymis change slightly but not significant ($P \geq 0.05$).

Table 1: Changes in body and sexual organ weights (gm) of experimental rats fed different dose of Radish (*Rhaphanus sativus*) leaves extract:

Parameters	Control	Treatments (Mg/kg body wt.)			P-value
		100	200	400	
Initial body weight	181.50 ^c ±0.31	178.67 ^d ±0.23	185.33 ^a ±0.37	183.17 ^b ±0.26	0.009**
Final body weight	185.68 ^a ±0.37	173.78 ^b ±0.26	170.60 ^c ±0.23	161.56 ^d ±0.18	0.010**
Testes weight	2.13 ^c ±0.11	2.14 ^c ±0.12	2.30 ^b ±0.14	2.38 ^a ±0.15	0.047*
Epididymis weight	0.80 ^a ±0.04	0.80 ^a ±0.04	0.84 ^a ±0.05	0.85 ^a ±0.05	0.361 ^{n.s}

Key: Values are mean ± SD. Means bearing different superscript letters in a row are significantly different ($P \leq 0.05$) according to DMRT. $P \leq 0.02-0.05$ * significant; $P \leq 0.01$ ** highly significant.

Sperms analysis

Mean value of rats treated with *R. sativus* leaves showed significant ($P \leq 0.05$) dose dependant improvement in semen characteristics [motility (%), sperm count (million/ml), normal morphology (%), viability (%)] (Table 2).

Table 2: Semen characteristics of experimental rats fed different dose of Radish (*Rhaphanus sativus*) leaves extract

Parameters	Control	Treatments (Mg/kg body wt.)			P-value
		100	200	400	
Motility (%)	70.89 ^c ±0.18	71.60 ^b ±0.19	71.60 ^b ±0.19	72.02 ^a ±0.20	0.044*
Sperm count (million/ml)	50.72 ^b ±0.10	50.86 ^b ±0.10	51.33 ^a ±0.11	51.84 ^a ±0.11	0.038*
Normal morphology (%)	89.40 ^b ±0.29	89.80 ^b ±0.29	91.30 ^a ±0.32	91.60 ^a ±0.32	0.007**
Abnormal morphology (%)	10.20 ^a ±0.09	9.20 ^b ±0.08	9.00 ^b ±0.08	7.40 ^c ±0.07	0.032*
Viability (%)	87.20 ^d ±0.27	88.20 ^c ±0.30	90.00 ^b ±0.31	92.00 ^a ±0.31	0.024*

Key: Values are mean ± SD. Means bearing different superscript letters in a row are significantly different ($P \leq 0.05$) according to DMRT. $P \leq 0.02-0.05$ * significant; $P \leq 0.01$ ** highly significant.

Fertility hormone

Mean values of rats treated with *R. sativus* leaves showed significant ($P \leq 0.01$) dose dependant increase in fertility hormone (testosterone, FSH and LH, Table 3).

Table 3: Hormone fertility of experimental rats fed different dose of Radish (*Rhaphanus sativus*) leaves extract

Parameters	Control	Treatments (mg/kg.B.W)			P-value
		100	200	400	
Testosterone (ng/mL)	1.90 ^b ±0.08	1.95 ^b ±0.09	2.40 ^a ±0.11	2.47 ^a ±0.12	0.046*
FSH (mIU/ml)	9.87 ^b ±0.25	9.92 ^b ±0.12	11.21 ^a ±0.14	11.38 ^a ±0.17	0.039*
LH (mIU/ml)	11.44 ^c ±0.18	11.61 ^c ±0.17	12.37 ^b ±0.15	13.41 ^a ±0.19	0.035*

Key: Values are mean ± SD. Means bearing different superscript letters in a row are significantly different ($P \leq 0.05$) according to DMRT. $P \leq 0.02-0.05$ * significant; $P \leq 0.01$ ** highly significant.

4. Discussion

The present study showed that oral administration of ethanol extract of *R. sativus* doses 100, 200 and 400 mg/kg body weight in male albino Wistar rats for 30 days caused a significant increase in fertility parameters especially in higher dose. The model employed in this work has been used previously by several investigators to assess the effects of different compounds on fertility and reproduction in laboratorial animals (Lilibeth and Glorina, 2010) [6].

Administration of ethanol leaves extract of *R. sativus* at daily dose 100, 200 and 400 mg/kg for 30 day significantly ($P \leq 0.01$) decrease in body weight, when difference between initial weight and final body weight were compared (Table 1), support earlier reports that (Mozammel *et al.*, 2014) [9].

The mean testicular weights of the rats in the treatment groups were significantly different ($P \leq 0.05$) from the control. However, the results showed positive correlation between levels of incorporation of *R. sativus* leaves extract and testis weight. Morris *et al.*, (1979) reported that there is a correlation between testicular weight and sperm production. Although significant ($P \leq 0.05$) difference in the weight of testis was observed, there was however, a slight increase in the range of values obtained. According to Perry and Petterson (2001) [11], testis length reflects the present and future sperm production as well as breeding quality of the male. The testes, epididymis and other reproductive organs are structurally and physiologically

dependent upon the testosterone and other androgens. Testosterone stimulates growth and secretory activity of the reproductive organs (Singh *et al.*, 1995; O' Donnell *et al.*, 1994) [16, 10] so a significant increase of these hormones in our study could increase the number and function of somatic and germinal cells of testis and in results increase the testis and epididymis weight.

The present study showed that sperm count, motility and viability at dose 100, 200 and 400 mg/kg significantly improvement. It has been observed that rats treated for 8 weeks with ascorbic acid, a potent antioxidant, showed a significantly increased epididymal sperm concentration (Sonmez *et al.*, 2005). Treatment with isoflavones resulted in an increase in sperm count and antioxidant activity in male rabbit (Yousef *et al.*, 2004). In previous study, sperms quality improved a significant increase emphasizes the fact that (El-Tahomi *et al.*, 2010). It is a well confirmed that, these parameters in mammals are regulated by the two Gonadotropins, LH and FSH. FSH binds with receptors in the sertoli cells and directly stimulates spermatogenesis.

The plant extracts also significantly increased male fertility hormone particularly testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), the saponins boost the level of testosterone in the body (Gauthaman and Adaikan, 2008). LH stimulates the production of testosterone in Leydig cells, which in turn may act on the Sertoli and peritubular cells of the seminiferous tubules and indirectly stimulates spermatogenesis via testosterone (Singh *et al.*, 1995; O'Donnell *et al.*, 1994) [16, 10]. Therefore, a significant increase in LH hormone concentration in our study treated rats could lead to increased testosterone secretion from leydig cells (O'Donnell *et al.*, 1994).

5. Conclusion

The present results confirm that the leaves *R. sativus* ingestion produce increased effects on male fertility hormone and sperm analyses in adult male rat. It also lends support to the claims for traditional usage of *R. sativus* as a sexual function enhancing medicine. Work is in progress on the isolation and characterization of the spermatogenic principle in the plant extract.

6. References

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