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EFFECT OF HYDROALCOHOLIC EXTRACT OF MORINGA OLEIFERA LEAVES ON FERTILITY HORMONE AND SPERM QUALITY OF MALE ALBINO RATS

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Abstract

Objective: The aim of the study is to evaluate the effect of the Hydroalcoholic extract of *Moringa oleifera* leaves on fertility hormone and semen quality of male albino rats.

Materials and method: The Hydroalcoholic extract of Moringa oleiferaseed at doses of 100, 200 and 400 mg/kg were administrated for 30 days. The effect of the extract on body weight and sexual organs weights (testes and epididymis) were determined. The fertility hormone and semen characteristic was studied.

Resultsand and discussion: Oral administration of Hydro alcoholic extract at doses of 100, 200 and 400 mg/kg were significantly increased body weight and sexual organ weight. Also significantly increased serum Testosterone, Follicle-stimulating hormone (FSH) and Luteinizing hormone (LH) compared to the control group, in addition, significantly increased semen characteristic in experimental animals study.

Conclusion :The results of the present study demonstrate the effectiveness of *Moringa oleifera*seed extract on fertility hormones stimulator and improvement of semen characteristic which justify the traditional use of the plant as aphrodisiac and for management of male certain sexual disorders**.**

***Key Words*:** Albino rats, fertility hormone, Moringa oleifera, body weight

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INTRODUCTION

A large number of plants have been tested throughout the world for the possible fertility regulatory properties[1]. 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[2, 3]. Moringa oleiferais a medicinally important plant, belonging to (family: Moringaceae). The plant is also well recognized in India, Pakistan, Bangladesh and Afghanistan as a folkloric medicine. [4] Different parts of the tree have been used in the traditional system of medicine. In India the Moringa oleiferaseeds is being used traditionally as an aphrodisiac[5]. The leaves of Moringa oleiferahas many different chemical components, including crude fiber, Reducing sugars, resins, alkaloids, flavnoids, organic acids, sterols, Tannins, Saponins, and proteins. Moringa has been found to be a good source of polyphenols and antioxidants[6]. Phytochemicals such as vanillin, carotenoids, ascorbates, tocopherols, beta-sitosterol, moringine, kaempferol, and quercetin have been reported in its leaves, roots, flowers fruits and seeds. In addition, it has content of unsaturated fatty acids, especially linoleic, oleic and palmitic acids. Moringa oleiferais rich in amino acids vitamins and minerals particularly iron[7, 8]. The leaves have been used in indigenous medicine for over many decades as traditional medicine. Moreover, *Moringa oleifera*was found to be of a nutritional value as it contains a number of important vitamins, including: vitamins A, B complex (B1, B3, B6 and B7), C, D, E and K[9]. The *Moringa oleifera*are used to exert its protective effect by decreasing liver lipid peroxides, as an antimicrobial agent[10]. Moringa tree has become an outstanding indigenous source of highly digestible protein, calcium (Ca), iron (Fe) and antioxidants, these nutritional characteristics of the plant may be, potentially beneficial to the developing regions of the world where undernourishment is a major concern[11, 12, 13]. Therefore, the present work was undertaken to effect of Hydroalcoholic extract of *Moringa oleifera*leaves on fertility hormone and semen quality of male albino Rats.

MATERIALS AND METHODS

Plant materials and extraction procedure

The seeds of Moringa oleiferawere collected from local area of Narasaraopet and the taxonomical identification of the leaves was confirmed by Dr. MadhavaSetty, a botanist from the Department of Botany, S. V. University, Tirupati Specimen Voucher no:v3148, Preserved for further reference at our laboratory first dried in the shade, left in Hydroalcoholic (85%) for more than two days in Soxhlet apparatus. Then the 85% Hydroalcoholic 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 Gavages' for the three groups of rats; A, B, and C, for 30 days.

Experimental Design Extract administration

Twenty-four male Wistar rats weighing between (160-200 g) for all experimental, will be maintained under standard environment conditions and fed with standard pellet diet and water ad libitum, will be used for the present study. After a week of adaptation, the rats will be randomly divided into four groups A, B, C and D (n=6) for seeds Hydroalcoholic extract treated with different doses (100, 200 and 400 mg/kg for extract of M. oleifera) by orally for 30 days, group (D) as control group[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 decapacitation and then testis and epididymis were carefully removed through allowed abdominal incision and testes were then separated from the epididymis and weighed using digital electronic balance. The organ weight of each sexual organ was determined[15, 16].

Semen collection

The testicles were then removed through allowed 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 ascalpel 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[17]. 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 a 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 spermatozoa

The smears were prepared by placing a drop from semen sample and one or two drops of previously warmed (37^{o}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.

Sperm viability

To determine sperm vitality, 40µl of freshly liquefied semen was thoroughly mixed with 10µl of eosin-nigrosin (Merck, Germany), and 1 drop of this mixture was transferred to a clean slide. At least 200 sperms were counted at a magnification of ×100 (Olympus Japan) under oil immersion. Sperms that were stained pink or red were considered dead and those unstained were considered viable[18, 19].

Hormone assay

At the end of experiments, blood was collected by cardiac puncture. Serum was separated by centrifugation at 3000 rpm for 15 min and stored frozen at -20oC until use. Plasma testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and were measured by radioimmunoassay (RIA) using special kits (Radim, Italy) as described in the instructions provided with the kits.

Statistical analysis

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

RESULTS

BODY AND SEXUAL ORGAN WEIGHTS

Rats treated with ether *Moringa oleifera* leaves showed significant (P≤0.01) dose dependant increase in body weight and sexual organs (testes and epididymis) (Table 1).

Table.1 Changes in body and sexual organ weights (gm) of experimental rats fed different dose of Moringa (*Moringa oleifera*) leaves extract.

Parameters	Control	Treatments (mg/kg body wt.)			P-value
		100	200	400	
Initial body weight	181.50d±0.31	187.93c±0.34	193.56b±0.45	195.67a±0.48	0.005**
Final body weight	185.68d±0.37	199.83c±0.58	215.33b±0.67	227.50a±0.72	0.001**
Testes weight	2.13d±0.11	2.23c±0.15	3.00b±0.10	3.25a±0.11	0.047*
Epididymes weight	0.80d±0.04	0.84c±0.05	0.89b±0.06	0.91a±0.07	0.049*

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

SPERMS ANALYSIS

Mean values of rats treated with Moringa oleiferaleaves showed significant (P ≤0.05) 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 Moringa (Moringa oleifera) leaves extract.

Parameters	Control	Treatments (mg/kg body wt.)			P-value
		100	200	400	
Motility (%)	70.89d±0.18	72.76c±0.20	75.20b±0.22	80.40a±0.24	0.001**
Sperm count (million/ml)	50.72d±0.10	52.15c±0.11	54.56b±0.13	59.17a±0.15	0.047*
Normal Morphology (%)	89.40d±0.29	90.40c±0.31	93.00b±0.33	95.00a±0.34	0.002**
Abnormal Morphology (%)	10.20a±0.09	8.25b±0.08	6.60c±0.06	4.00d±0.04	0.036*
Viability (%)	87.20d±0.27	90.40c±0.31	92.00b±0.31	93.00a±0.33	0.029*

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

FERTILITY HORMONE

Mean values of rats treated with Moringa oleiferaleaves showed significant (P≤0.01) increased in fertility hormone (testosterone, FSH and LH), (Table 3).

Table .3 Hormone fertility of experimental rats fed different dose of Moringa (Moringa oleifera) leaves extract.

Parameters	Control	Treatments (mg/kg body wt.)			P-value
		100	200	400	
Testosterone (mg/mL)	1.90c±0.17	3.47b±1.93	6.38a±2.08	6.80a±1.88	0.005**
FSH (mIU/ml)	9.87d±0.07	10.50c±0.17	13.86b±0.43	15.05a±0.61	0.004**
LH (mIU/ml)	11.44c±0.08	11.30c±0.11	12.35b±0.09	15.67a±0.48	0.039*

Values are mean ± SD. Means bearing different superscript letters in a row are significantly different (P≤0.05) According to DMRT. P ≤ 0.02-0.05 * significant; P ≤ 0.01 highly significant**

DISCUSSION

This research demonstrates that oral administration of alcoholic extract of Moringa oleiferadoses 100, 200 and 400 mg/kg body weight in male 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 laboratory animals[20].

Administration of Hydroalcoholic leaves extract of Moringa oleiferaat the dose of 100, 200 and 400 mg/kg for 30 day, significantly (P≤0.01) increased body weight of rats, when difference between initial weight and final body weight were compared (Table 1), support earlier reports that Moringa oleiferais of a high nutritional value[21, 22]. The weight of the reproductive organs like testes and epididymis, increased significantly (P≤0.05) when compared with that of control animal group (Table 1). Steroids are one of the causes of increased body and sexual organ weight and an increase in these parameters could be regarded as a biological indicator for effectiveness of the plant extract in improving the genesis of steroidal hormones. Since androgenic effect is attributable to testosterone levels in blood, it is likely that the plant extracts have a role in testosterone secretion allowing better availability of hormone to gonads. The testes, epididymis and other reproductive organs are structurally and physiologically dependent upon the testosterone and other androgens. Testosterone stimulates growth and secretary activity of the reproductive organs[23] 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. Administration of Hydroalcoholic leaves extract of Moringa

oleiferaat the dose of 100, 200 and 400 mg/kg, significantly (P<0.001) increased the sperm (motility, sperm count, normal morphology, viability) in epididymis as compared to control group (Table 2). emphasis the fact that, Moringa oleifera(50 mg/kg/orally for 100 days) an improved sperm concentration and motility, these were also evident in previously reported work [24]It has been observed that rats treated for 8 weeks with ascorbic acid, a potent antioxidant, showed a significantly increased epididymal sperm concentration[25]. Treatment with isoflavones resulted in an increase in sperm count and antioxidant activity in male rabbit[26]These results may be due to presence of flavonoids. Flavonoids are well known antioxidants that can ameliorate oxidative stress- related testicular impairments in animal tissue[27,28,29].It also stimulates testicular androgenesis and is essential for testicular differentiation, integrity and steroidogenic functions[30,31,32]. Our results correlates with other authors who studied the effect of Nigella sativa on spermatogenesis and fertility of male albino rats[33]. Testosterone supplementation has previously been shown to improve sexual function and semen quality[34], in addition to the intensity of ejaculations which might also be expected to improve[35,36]. In previous study, sperm count, motility and viability had a significant increase. [37] 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), *Moringa oleifera* (50 mg/kg/orally for 100 days) an improved plasma testosterone these were also evidence[24]. The saponins boost the level of testosterone in the body[38]. 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[39]. Therefore, a significant increase in LH hormone concentration in our study treated rats could lead to increased testosterone secretion from leydig cells[40].

CONCLUSION

The present results confirm that the seeds *Moringa oleifera* 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 *Moringa oleifera* as a sexual function enhancing medicine. Work is in progress on the isolation and character-ization of the spermatogenic principle in the plant extract.

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