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Effect of Aqueous Extract of *Moringa oleifera* Seed on Sexual Activity of Male Albino Rats

Varsha S. Zade*, Dinesh K. Dabhadkar, Vaibhao G. Thakare and Shital R. Pare
Department of Zoology,
Government Vidarbha Institute of Science and Humanities, Amravati, Maharashtra, India

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ABSTRACT: The aim of the study is to evaluate the effect of the aqueous extract of *Moringa oleifera* on reproductive abilities of male albino rats. The aqueous extract of *M. oleifera* seed at doses of 100, 200 and 500 mg/kg were administrated for 21 days. The general mating behaviour and libido activity was studied. The effect of the extract on body weight, reproductive and vital organ weight were determined. The most effective aqueous extract dose was further studied for its effect on sperm count and histoarchitecture of testes of experimental animal. Similarly adverse effects and acute toxicity of the extract were evaluated. Oral administration of aqueous extract at doses of 100, 200 and 500 mg/kg significantly increased the Mounting Frequency, Intromission Frequency and Ejaculation latency with reduction in Mounting Latency, Intromission Latency and Post Ejaculatory Interval. It also significantly increased the libido and sperm count in experimental animal. The extract was also observed to be devoid of any adverse effects and acute toxicity. The results of the present study demonstrate that aqueous extract of *M. oleifera* seed enhance sexual behaviour in male rats. It also thus provides a rationale for the traditional use of *M. oleifera* as acclaimed aphrodisiac and for the management of male sexual disorders.

Keywords: Albino rats, Spermatogenic, Libido, Mating Behaviour, *Moringa oleifera*

INTRODUCTION

In the last few years, a marked decrease in the quality of semen has been reported (Carlsen *et al*., 1992). Infertility is one of the major health problems in couples' lives; approximately 30% of couple's infertilities are due to male factors (Isidori *et al*., 2006). Several conditions can interfere with spermatogenesis and reduce sperm quality and production. Many factors such as drug treatment, chemotherapy, toxins, air pollution, and insufficient vitamin intake may have harmful effects on spermatogenesis and the normal production of sperm (Mosher and Pratt, 1991). The classical approach to therapy of male infertility is via spermatogenic drugs such as Clomiphene (Clomid). However, prescription drugs like these are usually expensive, coupled with the possibility of adulteration as such. The gradual shift to herbal therapy with its attendant increasing acceptance, even among the elites, make the herbal practitioners lay claims to having the cure to a myriad of ailments, including male infertility, irrespective of the etiology of such diseases (Anthony *et al*., 2006). A large number of plants have been tested throughout the world for the possible fertility regulatory properties (Bhatia *et. al.*, 2010). 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 and libido (Yakubu *et al*, 2007; Sumalatha *et al*., 2010).

Moringa oleifera (Linn) is a medicinally important plant, belonging to family *Moringaceae*. The plant is also well recognized in India, Pakistan, Bangladesh and Afghanistan as a folkloric medicine (Mughal *et al*., 1992). *M. oleifera* is a small or medium sized tree up to 10 m tall, with thick, soft, corky, deeply fissured bark, growing mainly in semiarid, tropical and subtropical areas. Different parts of the tree have been used in the traditional system of medicine. Survey in the tribal belt of Melghat region (20° 51′ to 21° 46′ N and to 76° 38′ to 77° 33′ E) of Amravati district of Maharashtra state of India revealed that the *M. oleifera* seeds is being used traditionally as an aphrodisiac (Lalas and Tsaknis, 2002). The seeds have been used in indigenous medicine for over many decades as traditional medicine. The seeds are used to exert its protective effect by decreasing liver lipid peroxides, as an antimicrobial agent (Faizi *et al*, 1998). The leaves of *M. oleifera* are used as purgative, applied as poultice to sores, rubbed on the temples for headaches, used for piles, fevers, sore throat, bronchitis, eye and ear infections, scurvy and catarrh; leaf juice is believed to control glucose levels, applied to reduce glandular swelling (Morton, 1991; Dahot, 1988; Makonnen *et al*., 1997). The stem bark is used as abortifacint and antioxidant activity (Ghasi *et al*., 2000, Nath and Sethi, 1992).

The root of *M. oleifera* were shown to possess antilithic, rubefacient, vesicant, carminative, antifertility, anti-inflammatory, stimulant in paralytic afflictions; act as a cardiac/circulatory tonic, used as a laxative, abortifacient, treating rheumatism, inflammations, articular pains, lower back or kidney pain and constipation (Padmarao *et al.,* 1996; Dahot 1988). Therefore, the present work was undertaken to validate scientifically the spermatogenic role of *M. oleifera* seeds as acclaimed by the traditional tribal user of Melghat region of Amravati district, Maharashtra. But to the best of our knowledge, there is no information in the open scientific literature that has substantiated or refuted the spermatogenic claims of *M. oleifera* seeds in the folklore medicine.

MATERIALS AND METHODS

Collection of plant material: The seeds *M. oleifera* plant were collected from Melghat region of Amravati district during the flowering period of September to March, identified and authenticated by experts from Botanical Survey of India, Pune (Accession No. VZ-1).

Procurement and rearing of experimental animal: Healthy wistar strain male albino rats of about two month old and weighing 200- 300 gm were procured from Sudhakarrao Naik Institute of Pharmacy, Pusad (Maharashtra). The rats were housed in polypropylene cages and maintained under environmentally controlled room provided with a 12:12 hours light and dark cycle approximately at 25 °C. They were fed on pellets (Trimurti Lab Feeds, Nagpur) and tap water *ad libitum*. The rats were allowed to acclimatize to laboratory environment for 15 days before experimentation.
All experimental protocols were subjected to the scrutinization and approval of Institutional Animal Ethics Committee [registration number 1060/ac/07/CPCSEA (IAEC/1/2012)].

Preparation of extract: The seeds of *M. oleifera* were collected, shade dried, powdered and subjected to soxhlet extraction with distilled water. The extract was evaporated to near dryness on a water bath, weighed and kept at 4 °C in refrigerator until further use.

Phytochemical screening: The presence of various plant constituents in the plant extract was determined by preliminary phytochemical screening as per Thimmaiah (2004).

Acute toxicity study: Healthy male albino rats were starved for 3-4 hr and subjected to acute toxicity studies as per (OECD) Organization of Economic Co-operation and Development guidelines No: 423 (OECD, 2004). They were divided into 4 groups of 6 animals each and kept singly in separate cages during the experiment. Group 1 represented the control group, which received 10 ml/kg of distilled water orally. Groups 2- 4 received suspension of aqueous seed extract of *M. oleifera* orally at the doses of 1000, 2000 and 5000 mg/kg daily for 7 days respectively. The rats were observed continuously for 2 hours for behavioural, neurological and autonomic profile, and for next 24 and 72 hours for any lethality or death

Mating behaviour test: The test was carried out by the methods of Dewsbury and Davis Jr (1970) and Szechtman *et al* (1981), modified by Amin *et al* (1996). Healthy and sexually experienced male albino rats (200– 300 gm) that were showing brisk sexual activity were selected for the study. They were divided into 5 groups of 6 animals each and kept singly in separate cages during the experiment. Group 1 represented the control group, which received 10 ml/kg of distilled water orally. Groups 2–4 received suspension of the aqueous extract of *Moringa oleifera* seed orally at the doses of 100, 200 and 500 mg/kg, respectively, daily for 21 days at 18:00 hr. Group 5 served as standard and was given suspension of sildenafil citrate (Vigora tablets, German Remedies) orally at the dose of 5 mg/kg, 1 hr prior to the commencement of the experiment. Since the male animals should not be tested in unfamiliar circumstances the animals were brought to the laboratory and exposed to dim light at the stipulated time of testing daily for 6 days before the experiment. The female animals were artificially brought into oestrus (heat) (Sooriya and Dharmasiri, 2000) by the Szechtman *et al* (1981) method (as the female rats allow mating only during the estrus phase). They were administered suspension of ethinyl oestradiol (Lynoral tablets, Organon Pharma) orally at the dose of 100 µg/animal 48 hours prior to the pairing plus progesterone (Dubaget tablets, Glenmark Pharma) injected subcutaneously, at the dose of 1 mg/animal 6 hour before the experiment. The receptivity of the female animals was confirmed before the test by exposing them to male animals, other than the control, experimental and standard animals. The most receptive females were selected for the study. The experiment was carried out on the 21st day after commencement of the treatment of the male animals. The experiment was conducted at 20:00 hour in the same laboratory and under the light of same intensity. The receptive female animals were introduced into the cages of male animals with 1 female to 1 male ratio.

The observation for mating behaviour was immediately commenced and continued for first 2 mating series. The test was terminated if the male failed to evince sexual interest. If the female did not show receptivity she was replaced by another artificially warmed female. The occurrence of events and phases of mating were recorded on audio video-cassette (Sony Handycam) as soon as they appeared. Their disappearance was also recorded. Later, the frequencies and phases were determined from cassette transcriptions: number of mounts before ejaculation or Mounting Frequency (MF), number of intromission before ejaculation or Intromission Frequency (IF), time from the introduction of female into the cage of the male up to the first mount or Mounting Latency (ML), time from the introduction of the female up to the first intromission by the male or Intromission Latency (IL), time from the first intromission of a series up to the ejaculation or Ejaculatory Latency (EL) and time from ejaculation and the first intromission of the following series or Post-ejaculatory interval.

Test for libido: The test was carried out by the method of Davidson (1982), modified by Amin *et al* (1996). Healthy and sexually experienced male albino rats (200– 300 gm) that were showing brisk sexual activity were selected for the study. They were divided into 5 groups of 6 animals each and kept singly in separate cages during the experiment. Group 1 represented the control group, which received 10 ml/kg of distilled water orally. Groups 2– 4 received suspension of the aqueous extract of *M. oleifera* seed orally at the doses of 100, 200 and 500 mg/kg, respectively, daily for 21 days at 18:00 hr. Group 5 served as standard and was given suspension of sildenafil citrate orally at the dose of 5 mg/kg, 1 hour prior to the commencement of the experiment. Since the male animals should not be tested in unfamiliar circumstances the animals were brought to the laboratory and exposed to dim light at the stipulated time of testing daily for 6 days before the experiment. The female rats were made receptive by hormonal treatment and all the animals were accustomed to the testing condition as previously mentioned in mating behaviour test. The animals were observed for Mounting Frequency (MF) on the evening of 21th day at 20:00 hour. The penis was exposed by retracting the sheath and 5% xylocaine ointment (Lidocaine ointment, AstraZeneca Pharma) was applied 30, 15 and 5 min before starting observations. Each animal was placed individually in a cage and the receptive female rat was placed in the same cage. The number of mountings was noted. The animals were also observed for intromission and ejaculation.

Effect on sexual and vital organ weight: Healthy and sexually experienced male albino rats (200– 300 gm) that were showing brisk sexual activity were selected for the study. Sexually experienced male albino rats were divided into 5 groups of 6 animals each; Group 1 represented the control group, which received 10 ml/kg of distilled water orally. Group 2- 4 received suspension of the aqueous extract of *M. oleifera* orally at the doses of 100, 200 and 500 mg/kg, daily for 21 days at 18:00 hour. Group 3 served as standard and was given suspension of sildenafil citrate orally at the dose of 5 mg/kg, daily 1 hour prior to the commencement of the experiment. After 21 days of treatment, 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), then sacrificed by cervical decapacitation and testis, seminal vesicles, epididymis, vas-deference, penis and prostate glands along with vital organ like liver, kidney, adrenal gland, and spleen were carefully removed and weighed using digital electronic balance (Adair Dutt). The organ weight of each organ were determined (Thakur and Dixit, 2006; 2007; Amini and Kamkar, 2005).

Effect on sperm count: Healthy and sexually experienced male albino rats (200– 300 gm) that were showing brisk sexual activity were selected for the study. Sexually experienced male albino rats were divided into 5 groups of 6 animals each; Group 1 represented the control group, which received 10 ml/kg of distilled water orally. Group 2-4 received suspension of the aqueous extract of *M. oleifera* orally at the dose 100, 200 and 500 mg/kg, daily for 21 days at 18:00 hour. Group 5 served as standard and given suspension of sildenafil citrate orally at the dose of 5 mg/kg, 1 hour prior to the commencement of the experiment. After 21 days of treatment, the sperm count was carried out by using Haemocytometer (Mukherjee and Kanai, 1988). Haemocytometer is generally used for RBC as well as WBC count. It is provided with the pipettes for the dilution of the blood samples and Neaubaur's slide with special type of ruling. The counting is done in the ruled squares on the slide. The epididymis was removed and placed in a pre-chilled petri-plate. 2 ml. of 0.9% saline was added to it and the cauda epididymis was gently minced with the help of sharp razor. This sample was used for the sperm count. The sample was pipetted out with the help of pipette provided in the Haemocytometer. A clean and dry cover slip was kept on the Neaubaur's ruling. The ruling was loaded with the sample by touching the tip of the pipette to the slide.

The slide was kept on a bench for 2 min. to allow the sperms to settle down. The sperms were counted in four squares at the corner of the ruling covering an area of 4 sq. mm. under high power objective. The spermatozoa with head and tail were counted (Taylor *et al*, 1985; WHO, 1999).

Total sperm count (*C. epididymis*)= (Sperm count/4 x 0.1) x 1000

Histopathological studies: The 500 mg/kg body weight aqueous extract of *M. oleifera* seed was found to be the most active amongst the three doses in sexual activity testing. Hence it was subjected to a detailed investigation for the study of histopathology. Healthy and Sexually experienced male albino rats were divided into 3 groups of 6 animals each; Group 1 represented the control group, which received 10 ml/kg of distilled water orally. Group 2 received suspension of the aqueous extract of *M. oleifera* orally at the dose 500 mg/kg, daily for 21 days at 18:00 hour. Group 3 served as standard and given suspension of sildenafil citrate orally at the dose of 5 mg/kg, 1 hour prior to the commencement of the experiment. After 21 days of treatment, testis of animals belonging to control, experimental and standard groups were dissected out and immediately fixed in 10 % buffered neutral formalin solution. After fixation, tissues were embedded in paraffin, serial sections were cut at 5 µm, stained with hematoxylin and eosin, examined for histoarchitectural changes and photographed under Olympus BX51 light microscope (Kosif *et al*, 2008).

Statistical analysis: All the data are expressed as Mean ± S.E. Statistical analysis was done by Student's t-test and one way ANOVA (Mahajan, 1997).

RESULT AND DISCUSSION

The seed of *M. oleifera* has been in use by the tribals of Melghat region as a means of treating sexual inadequacy and stimulating sexual vigor even without recourse to the scientific validity of the claim. Hence this study was carried out to validate scientifically this tribal claim.

Preliminary phytochemical screening of the seed extract of *M. oleifera* revealed the presence of alkaloids, flavonoids, steroids, phenolics, tannins and saponines. The phytochemical screening helps to reveal the chemical constituent of the plant extract and the one that predominates over the other. It may also be used to search for bioactive agents as starting product used in the partial synthesis of some useful drugs (Harbone, 1998). It has been reported that steroid and saponin constituents found in many plants possess fertility potentiating properties, and they are useful in the treatment of impotence (Shukla and Khanuja, 2004). Saponins found primarily in the leaf *Tribulis terrestris* L. have been used as an aphrodisiac agent in both; Indian and Chinese traditional system of medicine (Singh and Gupta, 2011). The saponins may boost the level of testosterone in the body as well as trigger libido enhancing effect observed in this study (Gauthaman and Adaikan, 2008). The presence of flavonoids in the *M. oleifera* extract which has been implicated to have a role in altering androgen levels (Padashetty and Mishra, 2007) may also be responsible for the enhanced male sexual behaviour in this study The alkaloids can also cause facilitation of sexual behaviour and has effect on sexual behaviour (Adimoelja, 2003). The improvement in sexual function demonstrated in the current study might thus be due to the presence of such compounds in *M. oleifera* seed extracts. Further study are required to identify the active constitutes responsible for the sexual function improvement activities and the mechanism whereby these activities implanted are in progress.

Clinical toxicity symptoms such as respiratory distress, salivation, weight loss and change in appearance of hair as well as maternal mortality were not observed at any period of the experiment. Similarly no mortality and changes in the behavioural, neurological and autonomic profile were observed in treated groups of the rats up to highest dose of 5000 mg/kg body weight. Hence one tenth of treated dose was selected for the present investigation. This revealed that short term use for this purpose is apparently safe. Similar finding was also observed by Tajuddin *et al* (2005), while working on ethanolic extract of *Myristica fragrans.*

The administration of *M. oleifera* aqueous, chloroform and alcohol seed extract for 21 days to male rats resulted in remarkable increase in the sexual vigor of the male rats, as evidenced by the different parameters studied. The results of mating behaviour test show the aqueous seed extract of *M. oleifera* at the dose of 100, 200 and 500 mg/kg body weight significantly increased the Mounting Frequency (MF) ($P<0.001$), Intromission Frequency (IF) ($P<0.001$) and Ejaculatory Latency (EL) ($P<0.001$). Similarly it also causes significant reduction in the Mounting Latency (ML) ($P<0.001$) and Intromission Latency (IL) ($P<0.001$) in experimental animals as compared to control group. Similarly, the standard drug also increased the MF, IF and EL as well as decreased the ML ($P<0.001$) and IL ($P<0.001$) in a highly significant manner as compared to control animals. The most appreciable effect was observed in aqueous extract at the dose of 100, 200 and 500 mg/kg body weight of *Moringa oleifera* (Table 1).

The significant increase in Ejaculation latency (EL) suggests that the all experimental extracts and standard drug prolonged the duration of coitus, which is an indicator of increase in sexual motivation (Wattanathorn *et al*., 2012).

Table 1: Effect of aqueous extract of *Moringa oleifera* seed on mating behaviour in male rats.

Treatment groups	***Doses (mg/kg Body wt)***	***Parameters***					
		Mount Frequency(MF)	***Mount Latency (in Sec)***	***Intromission Frequency (IF)***	***Intromission Latency (in Sec)***	***Ejaculation Frequency (EF)***	***Ejaculation Latency (in Sec)***
Group- I Control	Vehicle	4.5±0.66	248.6±11.7	4.33±0.68	341.4±1.76	1±0.25	262.8±5.73
Group- II to IV Aqueous extract	100	8.5±1.77**	246.8±6.5 ^{ns}	9.83±1.89*	228.4±10.5*	1.83±0.30 ^{ns}	407.4±16.8**
	200	16±1.75***	180± 11.1***	19.16±3.14***	186.4±17.2*	2±0.36**	484.2±109.2*
	500	25.66± 4.98***	129.2±16.1***	21.5±3.33***	156±10.9**	2.5±0.30***	846.6±58.8***
Group- V Sildenafil citrate	5	6.5±0.36***	138.6±7.2*	5.66±0.33***	142.19±6.6***	2.5±0.42***	454.3±15.6***

Values in Mean± S.E. (Standard error), n=6, *P<0.05, **P<0.01, ***P<0.001, when compared with control, ns- non significant.

Table 2: Effect of aqueous extract of *Moringa oleifera* seed on mounting frequency (test for libido) in male rats.

Treatment Groups	***Doses (mg/kg body wt.)***	***Parameters***		
		Mounting Frequency (MF)	***Intromission Frequency (IF)***	***Ejaculation (EJ)***
Group-I Control	Vehicle	4.8±0.47	4±0.36	Absent
Group- II to IV Aqueous extract	100	8.5±0.88*	8.33±1.05 ^{ns}	Present
	200	8.83±1.70**	12±1.75**	Present
	500	28.33±5.4***	24.16±2.54***	Present
Group-V Sildenafil citrate	5	17.83±0.70***	9.5±0.56***	Present

Values in Mean± S.E. (Standard error), n=6, *P<0.05, **P<0.01, ***P<0.001, when compared with control, ns- nonsignificant

The present finding shows that the aqueous, alcohol seed extract of *Moringa oleifera* produces a striking enhancement of over- all sexual performance of normal animals. Our finding also corroborates with the aphrodisiac effect of *Allium tuberosum* seeds extract, investigated in male rats at 500 mg/kg for 21 days, which significantly reduced ML and IL increased MF, IF and EL (Gouhua *et al*., 2009).

The results obtained in the test for libido shows that the aqueous seed extract of *Moringa oleifera* at the dose of 100, 200 and 500 mg/kg, significantly increased the Mounting Frequency (MF) ($P < 0.001$, $P < 0.01$ and $P < 0.05$) as compared to control group. The standard drug also significantly increased the MF ($P < 0.001$) as compared to control animals. Intromission was also observed in control; aqueous extract treated and standard groups of animals. The intromission frequency increases in a significant manner in all the extract treated group in a dose dependent manner, however a strikingly increased libido activity was observed in the 500 mg/kg body weight aqueous extract treated animals (Table 2). Thus, it may be inferred that the test drug produced a striking increase in 'pure' libido. Similar finding was also recorded by Tajuddin *et al* (2003), while working on ethanolic extracts of *Myristica fragrans* and *Syzygium aromaticum* in male rats. The intragastric (i. g.) administration of aqueous seed extract of *M. oleifera* at the dose of 100, 200 and 500 mg/kg, significantly caused an increase in body weight, when difference between initial and final weight body weight were compared. The weight of the reproductive organ like testes, caput segment of the epididymis, ventral prostate, seminal vesicle, penis and vas-deferens ($P < 0.001$, $P < 0.01$ and $P < 0.05$) increased significantly. Similarly, there was significant increase in the relative weight of the vital organs like liver, adrenal gland and spleen ($P < 0.001$, $P < 0.01$ and $P < 0.05$), when compared with that of control animal group (Table 3).

The significant increase in the weight gain of reproductive and vital organs was also observed in standard drug treated of animal group as compared to control. Genesis of steroids is 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 (Thakur and Dixit, 2007). Since androgenic effect is attributable to testosterone levels in blood (Amini and Kamkar, 2005), it is likely that the plant extracts may have a role in testosterone secretion allowing better availability of hormone to gonads. Testosterone supplementation has previously been shown to improve sexual function and libido (Aversa and Fabbri, 2001), in addition to the intensity of orgasm and ejaculations which might also be expected to improve (Morels, 1996). Similar conclusion was recorded by Watcho *et al*, (2005), while working on hexane extract of *Mondia whitei* on the reproductive organ of male rats.

Administration of aqueous seed extract of *M. oleifera* at the dose of 100, 200 and 500 mg/kg, significantly increased the sperm concentration (sperm count) ($P< 0.001$) in testes and epididymis as compared to control group. Similarly the standard group animal also showed significant increase in the sperm concentration (sperm count) ($P< 0.001$) as compared with the control group (Table 4). The present results clearly indicate that administration of aqueous seed extract of *M. oleifera* has positive effect on spermatogenesis in rats. These results may be due to presence of flavonoids. Flavonoids are well known antioxidants that can ameliorate oxidative stress- related testicular impairments in animal tissues (El-Missiry, 1999; Ghosh *et al*., 2002; Kujo, 2004). It also stimulates testicular androgenesis and is essential for testicular differentiation, integrity, and steroidogenic functions (Dawson *et al*., 1990; Luck, 1995; Salem *et al*., 2001). Our finding was also corroborates with the finding of Mukhallad *et al* (2009), who studied the effect of *Nigella sativa* on spermatogenesis and fertility of male albino rats.

The testis of control group animals showed normal histological texture. All stages of spermatogenesis were clearly observed *viz*. spermatogonia, spermatocytes, spermatid and spermatozoa, beside connective tissue, blood vessels, lymph ducts and leydig's cells were observable and distinct. The cuboidal germinal epithelium exhibited normal shape and size. Sertoli cells had many cytoplasmic processes which were normal in size. Spermatozoa were embedded in the sertoli cells and showed normal cytoplasmic granulation. Leydigs cells had normal nuclear size. Luminal part of the tubule were normal in number with bundles of spermatozoa. Spermatozoa with long tail and small distinct head were more visible (Fig. 1a).

The animal in the extract treated group showed pronounced effects in terms of testis weight and histological alterations. Since the weight and size of the testis was greater in extract treated groups almost all semniferous tubules showed greater morphological difference in size (i.e. it showed increase in the size of seminiferous tubules). The solid packing of seminiferous tubule was quite evident as compared to control. Basement membrane was tightly bound with germinal epithelium. The lumen of seminiferous tubule was filled with bundles of spermatozoa.

Table 3: Effect of aqueous extract of *Moringa oleifera* seed on body weight, male reproductive organ and vital organ weights of male rats.

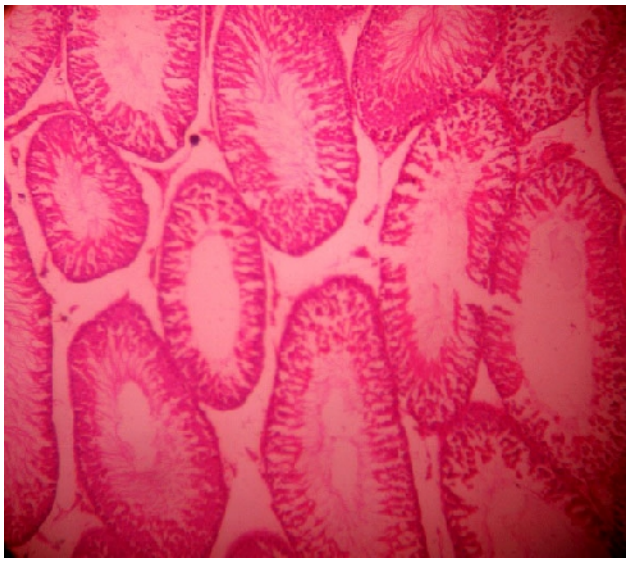
Treatment Groups	***Doses (mg/ kg body wt.)***	***Body weight (gm)***	***Testes (gm)***	***Epididymis (gm)***	***Seminal vesicle (gm)***	***Ventral prostate (gm)***	***Vas-Deferens (gm)***	***Penis (gm)***	***Liver (gm)***	***Kidney (gm)***	***Adrenal Gland (gm)***	***Spleen (gm)***
Group-I Control	Vehicle	200.16±3.28	212.83± 2.11	2.25± 0.08	0.408±0.09	0.809± 0.02	0.286± 0.01	0.338± 0.01	0.258± 0.11	7.198± 0.13	1.505± 0.01	0.035± 0.03	0.537± 0.03
Group-III to IV Aqueous extract	100	236.33± 6.81	248.33± 5.51*	2.371± 0.11***	0.509±0.01**	1.549± 0.09***	0.303± 0.01*	0.301± 0.008*	0.310± 0.008*	7.957± 0.18***	1.526± 0.07 ns	0.044± 0.06**	0.423± 0.04***
	200	210± 1.38	221.8± 2.56**	2.928± 0.20*	0.396±0.02 ns	1.039± 0.07***	0.314± 0.04*	0.371± 0.01 ns	0.314± 0.02**	8.325± 1.45***	1.645± 0.06*	0.040± 0.002*	0.482± 0.01 ns
	500	215.45± 3.14	234.33± 2.33***	3.154± 0.31ns	0.400±0.01 ns	1.086± 0.09***	0.581± 0.04***	0.273± 0.03**	0.301± 0.03**	7.642± 0.20**	1.837± 0.29***	0.046± 0.04**	0.477± 0.02**
Group-V Sildenafil citrate	5	230.16±1.18	251± 1.83**	3.874± 0.84***	0.468±0.21**	0.799± 0.07**	0.312± 0..07*	0.782± 0.04***	0.283± 0.03*	6.582± 0.68**	2.038± 0.32***	0.062± 0.02***	0.498± 0.01**

Values in Mean± S.E. (Standard error), n=6, *P<0.05, **P<0.01, ***P<0.001, when compared with control, ns- non significant.

Table 4: Effect aqueous extract of *M. oleifera* seed on sperm concentration of male albino rats.

Treatment group	***Doses (mg /kg body wt.)***	***sperm count (No. of sperm/ rat/ 10^6)***
Group-I Control	Vehicle	41.62±2.47
Group- II to IV Chloroform extract	100	41.83±2.36ns
	200	43.6±0.78**
	500	52±3.41***
Group-III Sildenafil citrate	5	49.64±1.58***

Values in Mean± S.E. (Standard error), n=6, *P<0.05, **P<0.01, ***P<0.001, when compared with control, ns- non significant.



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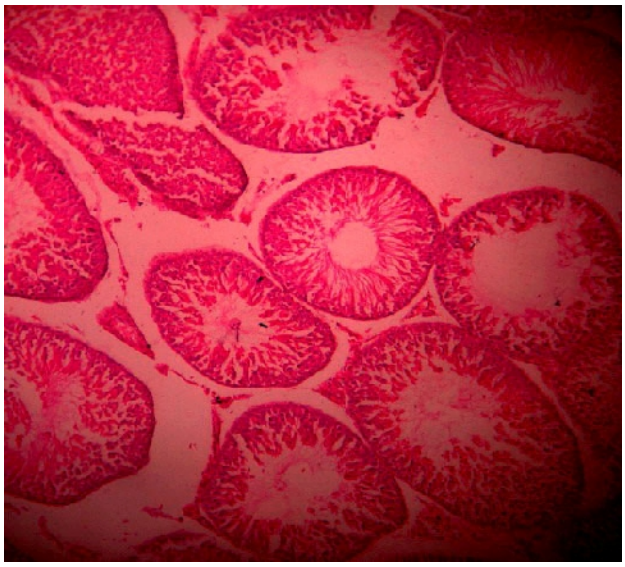

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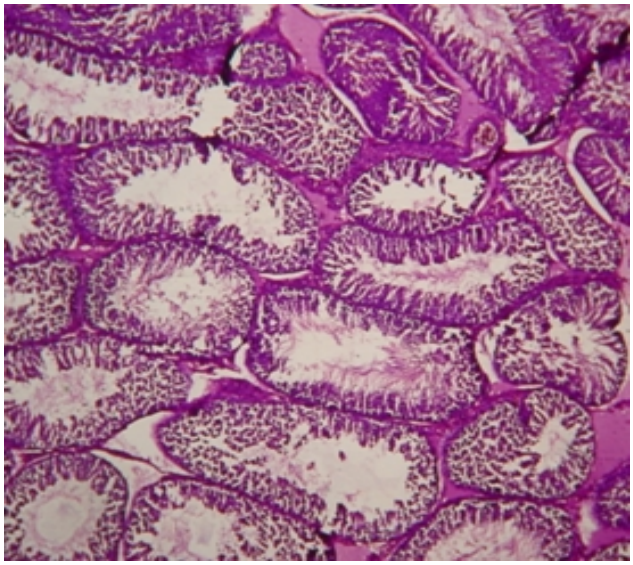

(c)

Fig. 1. Photomicrographs of testes tissue sections of rats [(a): control rats showing normal testes architecture, (b): treated with 500 mg/kg body wt. dose of aqueous extract of *M. oleifera* seed; (c): treated with 5 mg/kg body wt. dose of Sildenafil citrate. 100X].

In some tubules, spermatids were found scattered amidst spermatozoa. The germinal epithelium cells appeared to be hyperactive. Large numbers of different cells at different stages of spermatogenesis were evident. Sertoli cells were enlarged, highly processed and rich in nutrients as evidenced by highly granulated cytoplasm. This was the normal response of the sertoli cells when they were in readiness for providing nutritional supplementation to large number of spermatozoa (Mujumdar, 1995). Almost all leydig cells showed hypertrophy with enlarged nucleus and darkly stained cytoplasm. A very clear view of leydig cells was seen in photomicrograph of different doses of aqueous extract treated groups. Increment in the volume of cells and nucleus was strongly suggestive of steroid synthesis under the direct or indirect influence of the plant extract. The blood vessels of testis were slightly dilated (Fig. 1b). Histoarchitecture of sildenafil citrate treated groups also exhibited similar profile. The solid packing also suggests a supposed role of testosterone in increasing the vascularisation of testicular tissue. Increased spermatogenesis was evident by high number of spermatozoa in semniferous tubules and increase in spermatogenic elements as compared to control (Fig. 1c).

This histoarchitectural evidence was the clear indication of confirming the spermatogenic efficacy of extracts of *M. oleifera* seeds in male albino rats. The process of spermatogenesis and accessory reproductive organ function are androgen dependent. In present study the numbers of mature leydig cells as well as number of spermatocytes and spermatids were significantly increased, which reflect the increase of androgen level (Dym *et al*., 1979). Similar finding were also reported, in the study of spermatogenic effect of *Nigella sativa* (Mukhallad *et al*., 2009), and *Curculigo orchioides* in male rats (Chauhan and Dixit, 2008).

CONCLUSION

The present results confirm that the seeds *M. oleifera* ingestion produce increased effects on fertility and reproductive system in adult male rat. It also lends support to the claims for traditional usage of *M. oleifera* as a sexual function enhancing medicine. Thus, this study may prove to be an effective and safe alternative remedy in sexual disorders. Work is in progress on the isolation and characterization of the spermatogenic principle in the plant extract.

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