Greener Journal of Medical Sciences
Vol. 14(1), pp. 31-36, 2024
ISSN: 2276-7797

https://gjournals.org/GJMS

Sexual Function-Enhancing Potentials of *Monodora myristica* (African Nutmeg) Seed Extract in Experimental Animal Model

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ARTICLE INFO

Article No.: *041124045*
Type**:** *Research*
Full Text: *PDF, PHP, HTML, EPUB, MP3*

Accepted: *12/04/2024*
Published: *15/04/2024*

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Keywords: *Sexual behaviour, aphrodisiac effect, testosterone, Monodora myristica.*

ABSTRACT

Background: There is growing global concern about reports suggesting decline in male sexual functions, and the need for affordable and safe treatment options, especially in developing countries. *Monodora myristica,* is a widely distributed plant in the tropical forests of Africa, Asia, Central and South America, and Australia. The seeds are commonly used culinary spice in Africa, especially southern Nigeria, with ethnomedicinal usage in treating diabetes and sexual dysfunction. This study therefore aimed at evaluating the effects of the seed extract *M. myristica* on sexual function in males, using animal model.

Methods: Twenty male *wistar* rats randomized into 4 groups (n=5): Control (group 1) and 3 test groups were treated with extract at doses 75mg/kg, 150mg/kg, and 300mg/kg body weight, respectively for 54 consecutive days, then euthenised on day 55, blood collected and serum used for testosterone assay. Mating behavior test was performed on each male rat in the control, tests and a fifth group treated with sildenafil citrate (5mg/kg), on study days 53 and 54 using hormonally induced sexually receptive stimulus female rats in estrus.

Results: The results showed that the seed extract of *Monodora myristica* produced significant dose-dependent decrease in Mounting Latency, Intromission Latency and Post Ejaculatory Interval; with significant increase in Mounting Frequency, Intromission Frequency and Ejaculation latency, as well as increase in serum Testosterone level.

Conclusion: The results suggest that the seed extract of *M. myristica* could improve male sexual function by its enhancement of precopulatory sexual excitement and libido, delays ejaculation and improves recovery after sexual exhaustion in male *wistar* rats.

INTRODUCTION

There is increasing global concern about reports suggesting decline in male sexual functions, and the need for affordable and safe treatment options, especially in developing countries. The use of plants as medicine is believed to be as old as mankind[1]. Plants have provided a source of inspiration for novel drug compounds and plant medicines have made large contributions to human health and well-being. The plant-derived and herbal remedies continue to be a popular alternative for men and women seeking to improve their sexual life despite the availability of effective conventional medical treatments[2]. A large part of the world population, especially in developing countries, confide on medicinal plants as their source of primary health care [3], since herbs are believed to be more affordable, have high efficacy with limited side effects[4]. Traditional herbs have contributed to revolutionary breakthrough in the management of sexual inadequacies and have become known worldwide as an "instant" treatment[5]. However, some of these herbs are also known to impair fertility and sexual libido[6].

M. myristica plant belongs to the family, *annonaceae*. It is a long branching tree with a gray-barked trunk and reaches 35m high in nature. They are widely distributed in the tropical forest of Africa, Asia, Central and South America, and Australia. It grows very well in West Africa, especially in the southern part of Nigeria[7]. It has many common names, including; Calabash nutmeg, False nutmeg, African nutmeg, Jamaican nutmeg[4]. Local Nigerian names include *ehuru* in Igbo, *Ariwo* (Yoruba), *Gijuya dan miya* (Hausa), *Erhe* (Urhobo), *Uyengben* (Edo), *Arigogo* (Ijaw)[7,8] and *Obatorr* (Abureni/Kugbo) languages.

Monodora myristica seeds contain several bioactive substances with widespread traditional usage ethnomedicinal potentials. It is used as culinary spice in preparing several delicacies in Africa and other countries[4]. Ethnomedicinally, this plant is used in treatment of arthritis, diarrhea, diabetes, stomach ache, sickle cell and sexual impotency. It is reported to possess antinociceptive, antimalarial, antioxidative, and antimicrobial potentials[7]. Till date, there is paucity of scientific information on its effects on male reproductive system, while its traditional use in treating sexual impotence needs scientific evaluation. The aim of this study was therefore to evaluate the effect of *M. myristica* seed on sexual behavior in males, using *wistar* rats as experimental models.

MATERIALS AND METHOD

Collection and Extraction of Plant materials: Dry seeds of *Monodora myristica* (Calabash Nutmeg) purchased from Choba general market, Port Harcourt, were identified and authenticated by specialists at the department of Crop and Soil Science, Faculty of Agricultural Sciences, Niger Delta University, Wilberforce Island, Bayelsa State Nigeria. The seeds were washed, dried in the sun for 48 hours and then dehulled. The dehulled seeds were properly grounded with a hand blender (Corene, A.5 Lander YCIASA) to fine powder.

Figure 1. Fruit and seeds of *Monodora myristica* (authors).

The cold maceration extraction method was used[9]. The seed powder (100g) was soaked in 2 of a mixture of hydro-ethanol (30:70) solvent for 72 hours, with periodic agitations. The solution was then filtered with a Whatman No. 1 filter paper, and filtrate evaporated to dryness with Rotary Evaporator at 80^0c. A dry oily brown coloured extract was obtained, weighed and kept refrigerated in closed container until needed.

Animal Models: Twenty-eight adult male *wistar* rats (160-180g) were bred at the animal house, Faculty of Basic Medical Sciences, University of Port Harcourt. The

animals were kept in wooden cages with adequate ventilation. Feeds (TopFeeds (Grower Mash), SuperDelux Animal Feeds. Nigeria) and water were provided at *libitum*. They were acclimatized for two weeks with natural light/dark cycles and ambient temperature of 25^0c. Ethical approval was obtained from the University of Port Harcourt research ethics committee. Experimental procedures involving the animals and their care were conducted in conformity with International guidelines for the care and use of laboratory animals in Biomedical Research and as described by the American Physiological Society[10].

Study Design: Thirty male *wistar* rats (160g-200g) were randomly selected into five groups of 6 rats each. Group 1 received 1ml distilled water which served as normal control. Group 2 received 75mg extract/kg body weight of rat. Group 3 received 150mg of extract/kg body weight of rat, Group 4 received 300mg of extract/kg body weight of rat, while group 5 received sildenafil citrate (5 mg/kg b. wt) 4 hours before the sexual behavior test. The extract was administered by oral gavage between 9am-10am daily for 54 consecutive days. Five rats from each group were used for the sexual behavior test on days 53 and 54 of the study; while 5 rats from groups 1 to 4 were each sacrificed on day 55, after a 12hour overnight fast, blood collected and serum used for analysis of testosterone level.

Sexual behavior test: Sexual behavior tests were carried out by method previously described[11, 12.] The tests were carried out between 19:00 and 21:00 h under a dim red light. Female adult *wistar* rats of proven fertility were used as stimulus for the male sexual behaviour test. Briefly, Estrus was induced in each of the stimulus female rats by sequential subcutaneous administration of 0.5mg estradiol valerate (S &M Medical Ltd., Batch WEP43/004, England) 48h before the test, and 5mg progesterone (Embassy Pharm. & Chem. Batch No. 151203) 4hrs before the test. Female rats were tested for receptivity before mating. The male sexual behavior test began with the introduction of a male rat in an observation cage (56×35×31 cm), and allowed to adapt for 10 minutes. Thereafter, a stimulus female was quietly introduced. The male rat was then observed for 30 minutes for precopulatory and copulatory sexual behaviours by measuring or calculating the following parameters according to standard protocols[13-15]: mounting latency (ML), mounting frequency (MF), intromission latency (IL), intromission frequency (IF), ejaculation latency (EL) and post ejaculatory interval (PEI).

Determination of serum Testosterone concentration: The animals were anaesthetized with chloroform, laparotomy done and 3mls of blood collected through cardiac puncture were transferred into a plane sample bottles at room temperature. On clotting, samples were centrifuged at 1000rpm for 10 minutes, serum separated with Pasteur pipette used within 6 hours for assay of Testosterone concentration by the enzyme linked immunoassay (ELISA) technique following the procedure outlined in the manufacturer's instruction manual.

RESULTS

Effects of *M. myristica* seed extract on sexual behaviour parameters:

The results from this study show that *Monodora myristica* seed extract causes a significant ($p< 0.05$) dose dependent reduction in Mounting latency (ML) and Intromission latency (IL) (figure 2) in all the extract treated groups, when compared with the normal control group. However, the observed significant ($p< 0.05$) reductions in ML at higher doses of the extract (150mg/kg and 300mg/kg); as well as in the IL (75mg/kg, 150mgg/kg and 300mg/kg doses) were observed to be similar ($p>0.05$) when compared to that produced by the sildenafil citrate treated animals (figure 1).

Also, as shown in figure 3, a dose dependent significant ($p< 0.05$) increase was observed in the Mounting frequency (MF) in all the extract treated doses; and an increase in Intromission frequency (IF) at 75mg/kg($p>0.05$), 150mg/kg ($p< 0.05$), and 300mg/kg($p< 0.05$) extract treated doses respectively, when compared to the normal control group. The increase in MF (at 150mg/kg and 300mg/kg) and IF (at75mg/kg and 150mg/kg) were statistically similar ($p> 0.05$) when compared to that produced by sildenafil citrate; while at 300mg/kg dose, the extract produced a significantly ($p< 0.05$) greater increase in IL compared to the sildenafil citrate treated rats.

In addition, there was a non-significant difference ($P>0.05$) in Ejaculation latency (EL) (figure 4) in the low doses (75mg/kg and 150mg/kg) extract treated groups, while at higher dose (300mg/kg), the extract caused a significant ($P<0.05$) increase in EL similar to the sildenafil citrate treated groups, when compared with the normal control group (figure 4). The results (figure 4) further show a dose dependent reduction in Post ejaculatory interval (PEI) in the extract treated groups:75mg/kg ($P>0.05$), 150mg/kg ($P<0.05$) and 300mg/kg ($P<0.05$); and Sildenafil citrate ($P<0.05$) treated groups; when compared to the normal control group.

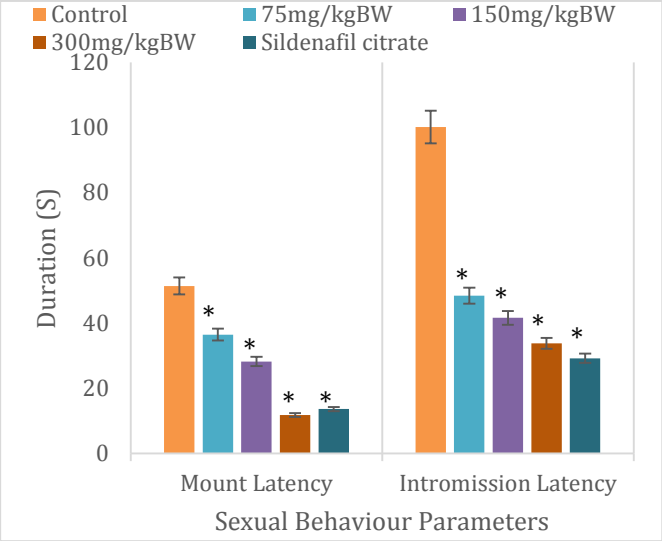

Figure 2: Effects of *Monodora myristica* fruit extract on mounting Latency (ML) and intromission latency (IL) in male *wistar* rats. N=5, values are presented as Mean ± SEM

*Shows significant at P < 0.05.

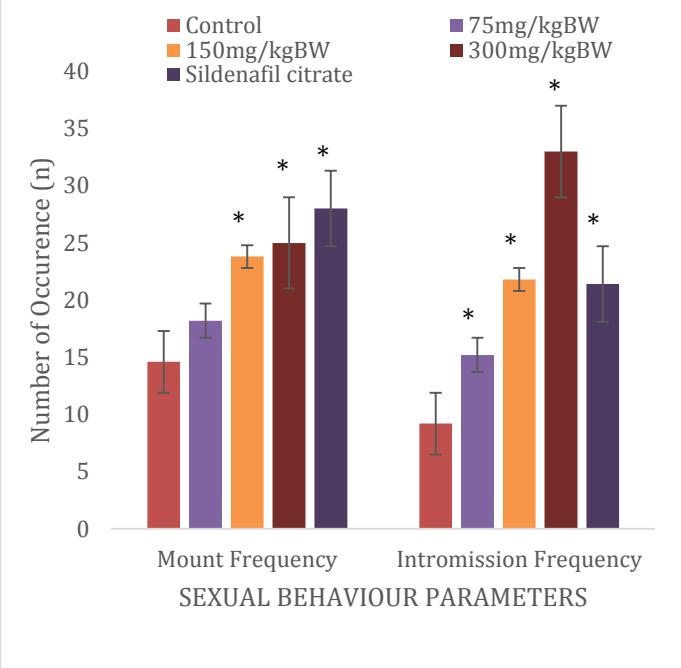

Figure 3: Effects of *Monodora myristica* fruit extract on Mounting Frequency (MF) and Intromission Frequency (IF) in male *wistar* rats.

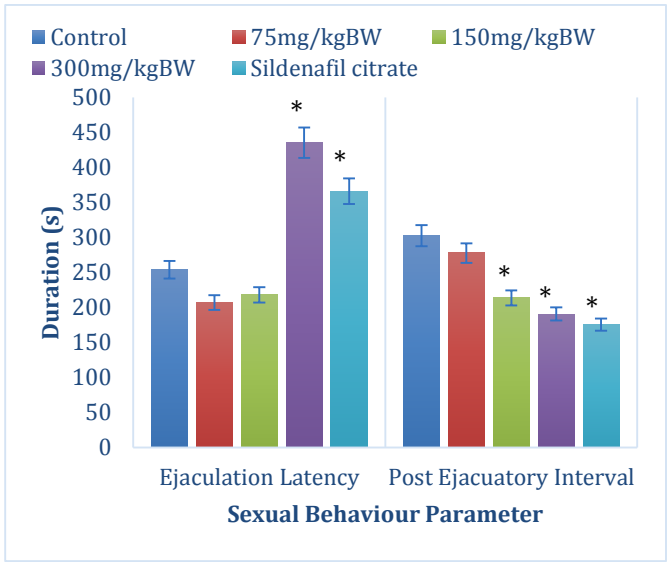

Figure 4: Effects of *Monodora myristica* fruit extract on Ejaculation Latency (EL) and Post Ejaculatory Interval (PEI) in male *wistar* rats.

Effects of *M. myristica* seed extract on serum Testosterone: The result further reveals a significant ($p<0.05$) dose dependent increase in the serum concentration of testosterone (figure 5), in all the extract treated groups (75mg/kg, 150mg/kg and 300mg/kg), when compared with the normal control group.

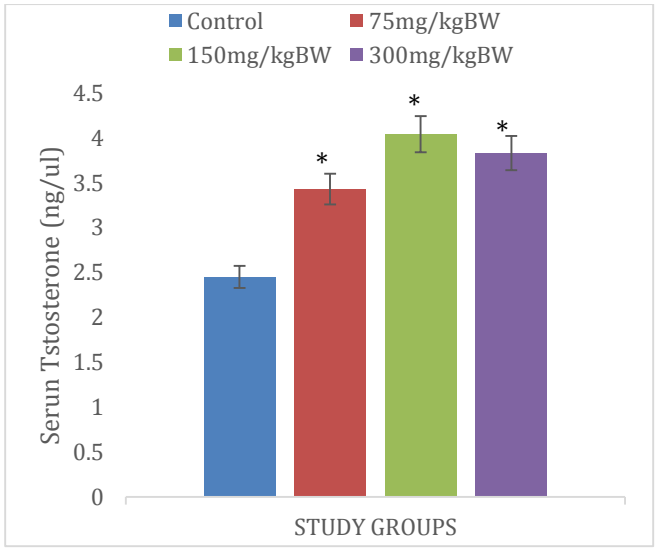

Figure 5: Effects of *Monodora myristica* fruit extract on Serum Testosterone level in male *wistar* rats.

DISCUSSION

This study evaluates the effects of *Monodora myristica* seed extract on sexual behavior in male *wistar* rats. The results show that the extract significantly ($P<0.05$) reduced the mounting latency (ML), intromission latency (IL), and post-ejaculatory interval (PEI); while increasing the mounting frequency (MF), intromission frequency (IF)

and ejaculation latency (EL), similar to sildenafil citrate. It also increases the serum concentration of testosterone.

Mounting frequency (MF), Intromission frequency (IF) are considered to be indices of libido (sexual desire) and potency, while mounting latency (ML) and intromission latency (IL) are also indicators of sexual arousal[16-18]. Therefore, the observed reductions in the mounting and intromission latencies with associated increase in mounting and intromission frequencies, indicate that *Monodora myristica* seed extract increases sexual arousal, libido and potency in male rats. This may be due to the increase in serum testosterone level by the extract, as testosterone has been reported to stimulate sexual desire and arousal resulting to erection in males[19-21]. For a successful mating to occur, adequate and sustained penile erection must be achieved. The increase in intromission frequency and ejaculation latency, suggest that *M. myristica* seed stimulate adequate and sustained penile erection, thereby prolonging the duration of intercourse; while the reduction in post ejaculatory interval indicates quick recovery from exhaustion, after a mating series. Reductions in IL and PEI have been correlated with invigoration of endocrine system thereby, resulting in enhanced sexual performance and motivation, as observed in this study. The implications of findings from this study, which agrees with similar report[22], is that *Monodora myristica* seed extract improves both precopulatory (sexual arousal, excitement and libido), and copulatory (sustained penile erection, increased intromission and delaying ejaculation) sexual behaviours,

Plants with aphrodisiac effects have been reported[14] to contain bioactive substances such as steroidal saponins and flavonoids which act centrally, or alkaloids which act peripherally. *Monodora myristica* contains several of such bioactive substances including tannins, saponins, flavonoids, alkaloids and phytoestrogens[23-25]. Also, some phyto-alkaloids can sustain penile erection either: by dilating the blood vessels of the *corpus carvenosum* directly or through centrally inducing nitric oxide (NO) release; by directly inhibiting the enzyme phosphodiesterase type-5 (PDE-5); or by increasing testosterone synthesis as observed in this study. Therefore, it is possible that these bioactive compounds in *Monodora myristica* could have contributed to the enhancement in sexual function observed in this study, by increasing testosterone synthesis. In addition, the similarities in the results of the sexual behavioural parameters obtained in the extract treated groups with those of the sildenafil citrate treated group strongly suggest a possible similarity in their mechanism of action.

In conclusion, this study shows that the seed extract of *Monodora myristica* improves male sexual function by enhancing pre-copulatory as well as copulatory sexual behaviors, with associated improvement in recovery after sexual exhaustion. These improved behavioural parameters, together, prolong the duration of sexual intercourse in the male rats, and therefore could have potential values in managing male sexual dysfunction.

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Cite this Article: Adienbo, OM; Azosibe, P (2024). Sexual Function-Enhancing Potentials of *Monodora myristica* (African Nutmeg) Seed Extract in Experimental Animal Model. *Greener Journal of Medical Sciences*, 14(1): 31-36.