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Research Article
Sperm Profile and Testicular Weight Assessment of Albino Rats Administered African Nutmeg (*Monodora myristica*) and African Basil (*Ocimum gratissimum*)

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Abstract

Background and Objectives: *Monodora myristica* and *Ocimum gratissimum* are herbs widely consumed for nutritional and medicinal purposes in developing countries including Nigeria. This study was therefore designed to assess the effects of *M. myristica* and *O. gratissimum* on sperm parameters, testicular and epididymal weight of albino rats. **Materials and Methods:** Forty-nine male Albino rats (170-200 g) were divided randomly into 7 groups (A-G) of 7 animals each. Rats in group A served as the control; groups B and C were given 300 and 500 mg kg^{-1} b.wt., of *M. myristica*, respectively; groups D and E received 300 and 500 mg kg^{-1} b.wt., of *O. gratissimum*, respectively; while groups F and G received 300 and 500 mg kg^{-1} b.wt., of *M. myristica*+*O. gratissimum*, respectively. After 8 weeks of treatment, the animals were sacrificed and sperm samples collected for analysis. **Results:** Sperm analysis revealed that extract of *M. myristica* increased epididymal sperm motility, viability and count significantly ($p<0.05$) and dose dependently; whereas extract of *O. gratissimum* and the combined plant extract reduced sperm motility, viability and count significantly. Extract of *O. gratissimum* alone increased sperm head abnormalities significantly. **Conclusion:** Findings of this study therefore, suggest that *M. myristica* promotes male fertility, whereas *O. gratissimum* and the combination of both herbs could have anti-fertility effects.

Key words: *Monodora myristica*, *Ocimum gratissimum*, sperm analysis, testicular and epididymal weight, Albino rats, herbal medicine, ailments

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The use of herbs to treat various ailments and diseases in Africa has been in practice for so many years now. Man's continuous dependence on herbs for therapeutic and nutritional reasons cannot be overemphasized. This application predates human history and constitutes the origin of modern medicine[1]. Plant materials have remained pivotal in traditional medicine and a vital resource for the production of new drugs. Although, orthodox medicine is widely acceptable, alternative healthcare is still sought after all over the world[2].

It is believed that herbal medicines are harmless, affordable and readily available, hence, inhabitants of developing countries predominantly, subscribe to the use of herbs for therapeutic purpose. These herbs can be used singly or in combination to prepare medicines for the treatment of different ailments[3]. The combination of herbs to achieve a desired result has been practiced by people of different cultures. This crude form of combinatorial chemistry is not new and practitioners have several motives for combining herbs. Amongst the reasons for such combination is the possibility of a synergistic activity, moderation of effects or using the activity of one herb to reduce toxicity and undesired effects of another[3].

Several plants have been associated with nutritional and therapeutic benefits. *Monodora myristica* and *Ocimum gratissimum* are examples of such plants reported to have both nutritional and medicinal values. These plants are widely consumed as food in Nigeria and used on several occasions to treat various diseases. On one hand, *O. gratissimum*, commonly known as African basil is an annual herbaceous plant found in the tropics and sub-tropics, especially India and Nigeria[4]. It belongs to the family of plants called Labiaceae[5]. Indians call this herb tulsi, while in Nigeria, it is known as nchuanwu by the Igbos, the Yorubas refer to it as efinrin and the Efiks call it nton. The leaves of this plant are used to treat fungal infections, fever, cold and catarrh and management of babies' umbilical cord[6].

On the other hand, *M. myristica* is a shrub or tree of evergreen and deciduous forests distributed across Liberia, Cameroon and Nigeria. This plant belongs to the Annonaceae family and is commonly called African nutmeg. In Nigeria, it is known as ehuru or ehiri by the Igbo tribe, the Yorubas and Efiks call it ariwo and enwin, respectively. Uwakwe and Nwaguikpe[7] reported that the bark of this plant could be used to treat arthritis, rheumatism, diarrhea and hypertension.

In view of this backdrop, this study was designed to assess sperm profile and testicular weight of albino rats administered *M. myristica* and *O. gratissimum* extracts singly and in combination.

MATERIALS AND METHODS

Experimental site: This study was carried out between July, 2016 and November, 2016 at the Animal House of Genetics and Biotechnology Department, University of Calabar, Calabar, Nigeria.

Collection and preparation of plant materials: Fresh leaves of *O. gratissimum* and seeds of *M. myristica* were purchased from Watt market, Calabar and taken to the Department of Botany, University of Calabar, Nigeria for identification and authentication. Plant materials were washed, air-dried and then pulverized with the help of an electric blender (Model 4250 Braun, Germany). Each pulverized plant material was extracted using Soxhlet method. The filtrate was obtained using rotary evaporator at 45°C, while the extract was reduced into pastes using hot-air oven at 60°C. The pastes obtained were stored in plastic screw capped bottles, labeled and kept in refrigerator for use.

Experimental animals and procedure: Forty-nine sexually mature male Albino rats with body weight ranging from 170-200 g were purchased from the Department of Zoology and Environmental Biology, University of Calabar, Calabar, Nigeria. They were kept in aluminum cages covered with wire mesh, fed with growers mash and allowed access to clean water *ad libitum*. Animals were handled in accordance with the guidelines for care and use of laboratory animals as provided by the Animal Research Ethical Committee of the Department (GBT/16/006).

After a week of acclimatization, they were divided into 7 groups (A-G) of 7 rats each, in a completely randomized design. Animals in group A served as the control and received feed and water only. Animals in groups B and C received 300 and 500 mg kg^{-1} b.wt., of *M. myristica* extract, respectively. Animals in groups D and E received 300 and 500 mg kg^{-1} b.wt., of *O. gratissimum* extract, respectively. While those in groups F and G received 300 and 500 mg kg^{-1} b.wt., of *M. myristica*+*O. gratissimum* extract, respectively. Plant extracts were administered via oral gavage for 8 weeks and animals were sacrificed under chloroform anesthesia 24 h after the last dose.

Estimation of sperm profile: Testes and epididymes were surgically removed and weighed using an electronic weighing balance (Scout-pro; 3000 g). The epididymes were placed in physiological saline in the ratio of 1:10 (weight/volume)[8,9] and macerated to release sperm cells. After pipetting, the suspension was filtered with an 80 mm stainless wire mesh and then used for sperm parameters estimation.

Estimation of sperm motility: Sperm motility was estimated by placing two drops of sperm suspension on a sterile slide and then placing a cover slip over it. Five slides were prepared for each sample in quick succession and examined. The number of motile cells divided by the total number of spermatozoa counted under x40 objective lens was determined and expressed in percentage[10].

Determination of sperm viability: Sperm viability was determined using the eosin-nigrosin staining technique[8,9]. Two drops of sperm suspension was mixed with an equal volume of stain. The mixture was smeared on sterile slides and allowed to air-dry. Five air-dried smears were prepared for each sample and examined using x40 objective lens of light microscope. Live sperm cells appeared whitish, while dead ones took up the stain and appeared pinkish. The number of live cells divided by the total number of cells examined was expressed in percentage.

Estimation of sperm head abnormality: To estimate sperm head abnormality, two drops of sperm suspension was mixed with an equal volume of 1% eosin Y solution. The mixture was smeared on sterile slides and allowed to air-dry. Five air-dried smears were prepared for each sample and examined with x100 objective lens of light microscope. The number of abnormal sperm heads in every 200 spermatozoa was determined and expressed in percentage[9].

Estimation of sperm count: A cover slip was placed on the improved Neubaeuer (2.5×10^4 mm^3; Hawksley, England) hemocytometer and a fine pore capillary tube was used to charge it with sperm suspension[8,9]. Sperm cells were examined and counted under x40 objective lens of light microscope. Sperm count was expressed in million-cells mL^{-1}.

Statistical analysis: Data obtained from this study were subjected to one way analysis of variance (ANOVA) using predictive analysis software (PASW) version 18.0. Least Significant Difference (LSD) was used to separate means that were significant at $p<0.05$.

RESULTS

Weight of testes and epididymes: The result obtained revealed that there was no significant ($p>0.05$) difference between the mean testicular weight of rats in the control group and those that received plant extract (Table 1). The mean testicular weight of rats in the control group was 1.10 g. However, the highest mean testicular weight (1.12 g) was recorded in rats administered 500 mg kg^{-1} b.wt., of *M. myristica* extract, while the least testicular weight (1.08 g) was recorded in rats administered 500 mg kg^{-1} b.wt., of *O. gratissimum* extract. Similarly, there was no significant ($p>0.05$) difference between the mean epididymal weight of rats in the control and rats administered the extract (Table 1). Least epididymal weight (0.36 g) was recorded in rats administered 500 mg kg^{-1} b.wt., of *O. gratissimum* extract; whereas the highest epididymal weight (0.40 g) was recorded in rats administered 500 mg kg^{-1} b.wt., of *M. myristica* extract.

Sperm profile: Sperm profile result revealed that sperm motility, viability and count of rats administered plant extract differed significantly ($p<0.05$) from those in the control group (Table 1). Sperm motility, viability and count increased significantly in rats administered *M. myristica* extract and reduced in those that received *O. gratissimum* extract and the combined plant extract. The highest mean sperm motility

Table 1: Effects of *M. myristica* and *O. gratissimum* on sperm profile, epididymal and testicular weight of male albino rats

Sperm parameters	A	B	C	D	E	F	G
Motility (%)	66.0 ± 0.58^{d}	70.3 ± 0.32^{e}	79.0 ± 0.57^{f}	38.3 ± 0.67^{b}	29.0 ± 0.50^{a}	55.0 ± 0.33^{c}	$57.1^{c}\pm0.20$
Viability (%)	77.0 ± 0.58^{d}	80.7 ± 0.33^{e}	88.0 ± 0.58^{f}	54.3 ± 0.33^{b}	48.7 ± 0.67^{a}	61.7 ± 0.88^{c}	69.3 ± 0.33^{c}
Abnormalities (%)	5.7 ± 0.45^{a}	5.4 ± 0.88^{a}	5.8 ± 0.56^{a}	7.0 ± 0.58^{b}	7.7 ± 0.30^{b}	5.7 ± 0.33^{a}	6.0 ± 0.62^{a}
Count (10^6 mL^{-1})	59.5 ± 0.33^{d}	70.9 ± 0.83^{e}	77.9 ± 0.20^{f}	34.2 ± 0.67^{b}	32.6 ± 0.58^{a}	41.2 ± 0.57^{c}	35.1 ± 0.58^{b}
Testes weight (g)	1.10 ± 0.01^{a}	1.11 ± 0.01^{a}	1.12 ± 0.03^{a}	1.09 ± 0.01^{a}	1.08 ± 0.02^{a}	1.09 ± 0.01^{a}	1.09 ± 0.01^{a}
Epididymes weight (g)	0.38 ± 0.03^{a}	0.38 ± 0.01^{a}	0.40 ± 0.01^{a}	0.38 ± 0.01^{a}	0.36 ± 0.01^{a}	0.38 ± 0.03^{a}	0.39 ± 0.01^{a}

Values are presented as Mean±SEM, Means followed by the same case letter along the horizontal array indicate no significant difference ($p>0.05$), A: Control (0 mg kg^{-1} b.wt.), B and C: 300 mg and 500 mg kg^{-1} b.wt., of *M. myristica* extract, respectively, D and E: 300 mg and 500 mg kg^{-1} b.wt., of *O. gratissimum* extract, respectively, F and G: 300 mg and 500 mg kg^{-1} b.wt., of *M. myristica*+*O. gratissimum* extract, respectively

(79.0%), viability (88.0%) and count (77.9 M mL^{-1}) were observed in rats administered 500 mg kg^{-1} b.wt., of *M. myristica* extract. While the least mean sperm motility (29.0%), viability (48.7%) and count (32.6 M mL^{-1}) were observed in rats administered 500 mg kg^{-1} b.wt., of *O. gratissimum* extract. Conversely, mean sperm head abnormality increased significantly ($p<0.05$) in rats administered *O. gratissimum* extract compared to those in the control. Rats in the group administered 500 mg kg^{-1} b.wt., of *O. gratissimum* extract recorded the highest mean sperm head abnormality (7.7%) compared to those in the control, which had the least (5.7%).

DISCUSSION

The result for epididymal and testicular weights of rats revealed that *M. myristica* and *O. gratissimum* extracts had no significant ($p>0.05$) effects on the weight of these organs. However, an insignificant reduction was observed in testicular and epididymal weights of rats administered *O. gratissimum* extract and the combined plant extract which could be due to decrease in tubule size and number of germ cells[11]. Conversely, *M. myristica* extract increased testicular and epididymal weights which is in agreement with Akinola *et al.*[12], Cajuday and Pocsido[13] and Ekaluo *et al.*[14], perhaps due to increase in the number of germ cells.

The result for sperm profile analysis revealed that *M. myristica* extract increased sperm motility, viability and count significantly ($p<0.05$) and dose dependently which is in accordance with Akinola *et al.*[12], Ekaluo *et al.*[14] and Uboh *et al.*[15]. Increase in sperm count of rats administered *M. myristica* extract may be due to increase in testosterone production, since testosterone promotes spermatogenesis. Furthermore, increase in sperm motility could be due to increase in mitochondrial activity and fructose synthesis, as well as high ATP content[16]. Interestingly, in this study, it was observed that increase in sperm viability corroborated increase in sperm motility. This increase could be due to the ability of *M. myristica* to trigger the production of hormones responsible for spermatogenesis. On the other hand, *O. gratissimum* extract and the combined plant extract reduced sperm motility, viability and count significantly ($p<0.05$) which is in agreement with Ekaluo *et al.*[17], whereas *O. gratissimum* extract increased sperm head abnormalities which is in tandem with Ekaluo *et al.*[18], Ikpeme *et al.*[19] and Obianime *et al.*[20]. It was also observed that the combined plant extract did not affect sperm quality and quantity as much as sole administration of *O. gratissimum* extract. This may be due to moderation effect of *M. myristica* on antifertility potential of *O. gratissimum*. It thus suggested that decrease in sperm quality and quantity could be due to reduction in circulating androgen level or anti-androgenic property of the plant extract; Ugonna[3] reported that any substance that affects reproductive activity will as well affect the quality and quantity of sperm. Impairment of male fertility has also been reported in *Azaridichta indica*, *Phyllanthus niruri* and *Gongronema latifolium*[21,22].

CONCLUSION

The findings of this study indicate that *M. myristica* has the ability to enhance male fertility, whereas *O. gratissimum* and the combined plant extract could have antifertility effects. Further research should be carried out to investigate the single and more importantly the combinational effects of these herbs on reproductive hormones.

SIGNIFICANCE STATEMENT

This study discovered the antagonistic effect that *O. gratissimum* has on *M. myristica* when administered or consumed together. Therefore, this study will help researchers to uncover the critical areas of combining herbs with antagonistic rather than synergistic effects that many researchers were not able to explore.

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