Libido Enhancement Potential of *Piper guineense* in Male Wistar Rats

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**Authors’ contributions**

This work was carried out in collaboration between all authors. Author JOO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SSE and HIE managed the analyses of the study. Authors PEO and OG managed the literature searches. All authors read and approved the final manuscript.

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**ABSTRACT**

**Background**: Some spices have been reported to play contributory role in enhancing male reproductive function.

**Aims**: To determine the effect of *Piper guineense* consumption on some libido indices and behavioral pattern of male Wistar rats at different concentrations.

**Study Design**: This is a Cross-sectional nonclinical study in animal model.

**Place and Duration of Study**: The study was carried out at the Department of Medical Laboratory Science, College of Medical and Health Sciences, Babcock University, Ilishan-Remo, Ogun State, Nigeria between the month of March and June, 2016.

**Methodology**: Thirty (30) male Wistar rats were divided into 6 groups of five rats each (n=5). Animals in Group 1 (Control) were fed with 100% Pelletized growers feed, while Group 2, 3, 4, 5 and 6 were fed with different concentrations of *P. guineense* supplemented diet: 0.5, 5, 10, 15 and 20%, respectively for 28 days. On the 28\(^{th}\) day of the experiment, female rats were introduced into the cages of the male rats (2 females to 1 male ratio) and from the cage side, the male rats were observed for mounting latency, mounting frequency and intromission frequency. The values of all

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the morphometric measurement, including change in sexual behaviours were analyzed statistically using SPSS 20 Software. P-value < 0.05 was considered statistically significant.

**Results:** The result of the libido index test show that the dry leaves of*Piper guineense*, in comparison with the control group, significantly (P<0.05) increased the Mounting Frequency and Intromission Frequency of the male rats, while it caused a significant (P<0.05) reduction in the Mounting Latency of the animals, especially at 10%, 15% and 20% concentration. The plant appears to induce aggression in the treated rats in a dose-dependent manner with the highest occurrence (100%) in Group 6 fed with 20% of* P. guineense*. No significant difference (P>0.05) was observed in the % body weight gain and testicular weight of the test rats when compared with the control group.

**Conclusion:** *P. guineense* showed potential value as a natural sexual enhancement (aphrodisiac) agent as shown by significant increase in some of the libido indicator parameters assessed.

**Keywords:***Piper guineense; rats; libido; mounting latency; mounting frequency; mounting latency.**

**1. INTRODUCTION**

Libido, colloquially known as sex drive, is a person’s overall sexual drive or desire for sexual activity. It is an important component of human reproduction which is influenced by biological, psychological and social factors [1-3]. According to Guyton and Hall [4], libido results from inherent reflex mechanisms integrated in the sacral and lumbar spinal cord, and these mechanisms can be initiated by either psychic stimulation from the brain or actual sexual stimulation from the sex organs, but usually it is a combination of both.

Sexual dysfunction is a serious medical and social symptom among 10-52% men worldwide and has been for years and in different ethnic groups [5]. It is a repeated inability to achieve normal sexual intercourse, which includes various forms like premature ejaculation, retrogradated, or retarded ejaculation, erectile dysfunction, arousal difficulties, etc [6,7]. Etiologies of sexual dysfunction include: hereditary, physical damage, bicycling, surgery, psycho-social disorders, neurogenic disorders, hormonal disorders, chronic diseases, aging, fatigue, smoking, alcohol abuse, substance abuse, medications, exposure to mumps virus, irradiation, pesticides and industrial chemicals [8-15].

The incidence of sexual inadequacy in human males has led to the search and development of a number of treatment options. Unfortunately, some of these available options are too expensive, not easily accessible and with some serious side effects. This problem has necessitated the need for more pharmacological research on cheaper and natural treatment options for this menace [16].

Nutrients and other constituents of medicinal plants have the potential to affect almost all aspect of the reproductive system. The relationship between the reproductive system and nutrients found in medicinal plants has been reviewed comprehensively, which shows that some medicinal plants play the contributory role in enhancing sexual drive [16-21].

Yongabi [22] listed *Piper guineense* (Family:*Piperaceae*) in the Nigerian phytomedical pharmacopoeia. According to Isawunmi [23], the plant, a popular spice in West African countries, especially Nigeria, is known to provide medicinal, insecticidal, culinary and dietary benefits to human beings due to the presence of alkaloids like piperidine and piperine. The common names of the plant are Black pepper, Benin pepper and Ashanti pepper. In Nigeria the plant is called Masoro (Hausa), Iyere (Yoruba), Uziza (Igbo), Adusa (Efik), Enie (Edo), Oziza (Ika) and Eshasha (Urhobo) [23,24].

It is used in West African cuisine where it imparts “heat” (piqaantness) and a spicy pungent aroma to “pepper” soups. The leaves are used phytomedically as contraceptive, antipyretic, antiemetic, carminative, antibiotic, febrifuge, aphrodisiac amongst others [24].

The leaves are aseptic in nature, with the ability to relieve flatulence. They are also useful for treating intestinal diseases, cough, bronchitis and rheumatism. The plant is known to contain phytoneutrients such as alkaloids, flavonoids, glycosides, essential oils, tannins, saponins, peptides and phenols among others [25,26]. Alkaloids which are natural products present in*P. guineense*, are made up of heterocyclic nitrogen, possesses antimalarial, anti-hypertensive, anti-arrhythmic and anticancer
Furthermore; *Piper guineense* has been reported to improve reproductive function including sexual strength and libido behavior in experimental animals when used singly or in combination with another herb. Its effects on reproductive function have been studied extensively [30-37] with diverse outcomes depending on the plant parts (Fruits, seeds or leaves) used. For instance, the work of Kamtchouing et al. [30] showed that aqueous extract of *Piper guineense* fruits at 122.5 mg/kg was able to stimulate sexual behaviour of mature male rats by decreasing intromission latencies and increasing mounting, ano-genital sniffing and penile erection index. A similar work by Mbongue et al. [31], show that there was a significant increase in the level of testosterone in the serum and testes, cholesterol in the testes, α-glucosidase in the epididymis and fructose in the seminal vesicles after 8 days of treatment, with 122.5 and 245 mg/kg doses of aqueous extract of dry fruit of *P. guineense*. Ekanem et al. [32] studied the effect of crude extracts of dry fruits of *P. guineense* on male fertility parameters using adult Sprague dawley rats. 200mg/kg of the extract was given to 2 groups of rats for four weeks and eight weeks respectively. The result showed that the extract improved male reproductive functions. It improved sperm motility, sperm function, testicular spermatogenesis and weight and this could be attributed to its androgenic and aphrodisiac properties.

Still, Kpomah et al. [33] found that albino male rats treated with di-herbal mixture of *Zanthoxylum leprieurii* and *Piper guineense* showed significant increase in libido parameters. Rahmawati and Bachri [34] also reported that male rats treated with a combined extracts of *Piper retrofractum*, *Centella asiatica* and *Curcuma domestica* showed a significant increase in sexual drive. The work of Memudu et al. [35], show that 200mg/kg crude extract of dry fruits of *Piper guineense* increased serum testosterone level in treated groups. In addition, semen analysis reveals normal parameters: no oligospermic or azoospermic condition was detected. Sperm morphology was normal and no teratozoospermic sperm found. The result of the work of Sutyarso et al. [36], through mating test, has also proved that extract of *P. guineense* can improve sexual drive in male mice. A recent work by Sutyarso et al. [37], also investigated the effect of fruit extracts of *P. guineense* on the fertility potential of male Albino Mice. When compared with control group, male mice treated with fruit extract of *P. guineense* showed significant increase in the serum testosterone level, epididymal sperm concentration, spermatoocyte counts, spermatid counts, and the weight of epididymis tubules. The work of Okoye et al. [38] on the other hand, show that there was no significant variation (p>0.05) in the mean serum testosterone concentration across the different groups after 30 days administration of 50, 100 and 200 mg/kg concentration of methanolic leaf extract of *P. guineense*. However, the mean gonadosomatic weights of the testes of the different treatment groups and the mean gonadosomatic weight of the epididymis of only group C (100 mg/kg body weight) were significantly higher (p<0.05) than that of the control.

Most previous studies on *P. guineense*, were carried out using the fruits and seeds. Very little is known about the libido enhancement potential of dry leaves of *Piper guineense*. Scarcity of information in this regard, necessitates this research. It is also hoped that the outcome of this study will help to clarify some of the discrepancy noted in previous similar studies.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out at the Department of Medical Laboratory Science, College of Medical and Health Sciences, Babcock University, Ilishan-Remo, Ogun State, located in Southwestern Nigeria, coordinates: 6.8862° N, 3.7055°E.

2.2 Duration of Study

The study was carried between March and June, 2016.

2.3 Plant Material

Fresh leaves of *Piper guineense* for this experiment were procured in bulk from Ilishan market, Ilishan-Remo, Ogun state. They were identified and authenticated by a Taxonomists in the Department of Agriculture, Babcock University, Ogun state, Nigeria, where voucher
specimen of the dry leaves are being kept in the Herbarium.

2.4 Preparation of Plant Material

The fresh leaves of *Piper guineense* were air-dried at room temperature (25±2°C) for 14 consecutive days and crushed into fine powdered form using a dry blender. The dry macerated leaves were weighed and stored in an air-tight container and kept in a cool dry area throughout the course of the research. This method was adopted in order to maintain the pungency of the spices. The dry macerated leaves were fed to the rats in a mixture of the pelletized growers rat feed which was procured. A calculated measure of the macerated leaves was given daily.

2.5 Experimental Animals

Ethical approval was obtained from Babcock University Health Research Ethics Committee (BUHREC), Babcock University, Ogun state, Nigeria, before commencement of the research. Thirty (30) adult male Wistar rats with average weight of 178±7.50 g were used. They were purchased from Covenant Farm (Nig.) Enterprises, Gbolasire Estate, Iwo road, Ibadan, Oyo state, Nigeria. The animals were authenticated in the Department of Agriculture, Babcock University, Ogun State, Nigeria. They were housed in well ventilated wire-mesh cages in the Animal house of the Department of Animal science, Babcock University, Ilishan-Remo, Ogun State. They were kept at room temperature of 25±2°C and exposed to 12:12 hr (natural light-dark) cycle. They were trained with sexually receptive females and fed with pelletized Growers rat feed, given water *ad libitum*, and allowed to acclimatize for two weeks before commencement of experiment.

2.6 Animal Ethics

All studies on animal experimentation were conducted in accordance with the Current Animal Care Regulations and Standards approved by the Institute for Laboratory Animal Research [39].

2.7 Treatment of Experimental Animals

The 30 sexually active male rats were randomly assigned into 6 groups (n=5/group) and treated for 28 days as shown in Table 1.

2.8 Assessment of Behavioral Pattern

The animals were observed daily for any change in normal behavior characterized by aggression causing the death of some of the animals. They were classified either as aggressive or non-aggressive. Signs of aggression include: puffing up, up-right posture, hissing, pushing sideways, chasing, attacking, fighting and biting other rats in the cage.

2.9 Assessment of Libido Index

On the 28th day of the experiment, female rats were introduced into the cages of the male rats (2 females to 1 male ratio) and from the cage side, the male rats were observed for mounting latency, mounting frequency and intromission frequency. Mounting latency was defined as the time from the introduction of female into the cage of the male up to the first mount. Mounting frequency was defined as the number of
Table 1. Experimental protocol for *Piper guineense* treated rats

<table>
<thead>
<tr>
<th>Groups (n=5)</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (Control Group)</td>
<td>100% Pelletized growers feed</td>
</tr>
<tr>
<td>G2</td>
<td>0.5% of <em>P. guineense</em> + 99.5% of pelletized growers feed</td>
</tr>
<tr>
<td>G3</td>
<td>5% of <em>P. guineense</em> + 95.0% of pelletized growers feed</td>
</tr>
<tr>
<td>G4</td>
<td>10% of <em>P. guineense</em> + 90.0% of pelletized growers feed</td>
</tr>
<tr>
<td>G5</td>
<td>15% of <em>P. guineense</em> + 85% of pelletized growers feed</td>
</tr>
<tr>
<td>G6</td>
<td>20% of <em>P. guineense</em> + 80% of pelletized growers feed</td>
</tr>
</tbody>
</table>

times the males rat mounts the female. Intromission frequency was defined as the number of vaginal penetration made by the male following penile erection. Percentage mounted was calculated using the formula:

\[
\text{Percentage Mounted} \% = \left( \frac{\text{number mounted}}{\text{number paired}} \right) \times 100.
\]

The test was terminated if the male failed to evince sexual interest. If the female did not show receptivity she was replaced by another female.

2.10 Specimen Collection

Twenty four hours after the last administration of the spice, the animals were anesthetized with chloroform vapour to put them to sleep. Following dissection of the scrotum, the testes were harvested and carefully dissected out, trimmed of all fat and connective tissue, and blotted dry to remove blood stain.

2.11 Gross Morphometrical Measurement

The initial and final weights of the rats, as well as the weights of the testes in each group were measured using an electronic sensitive analytical weighing balance. Percentage weight gain was calculated using the formula below:

\[
\text{Final Weight} - \text{Initial Weight} \times 100
\]

\[
\text{Initial Weight} = \% \text{ Weight Gain}
\]

2.13 Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS-20 (Statistical packages for social Scientists – version 20.0) statistical program. The statistical analysis was carried out by one way analysis of variance (ANOVA). Data were expressed as mean±Standard error of mean (M±SEM). Test for significance was done using student t-test. Difference between mean was considered statistically significant when P-value <0.05.

3. RESULTS AND DISCUSSION

3.1 RESULTS

The effect of *Piper guineense* on some libido indicator parameters in Wistar male rats is presented in Table 2. The result of the libido test show that the leaves of *Piper guineense* especially at 10%, 15% and 20% concentration, in comparison with the control group, significantly (P<0.05) increased the Mounting Frequency (MF) and Intromission Frequency (IF) of the male rats, while it caused a significant (P<0.05) reduction in the Mounting Latency (ML) of the animals at the said concentrations. The percentage mounted were 100% across all the test groups, including the control group.

Table 2 represents the effect of *P. guineense* consumption on the behavioral pattern of the Wistar rats. Result show that consumption of the leaves of *Piper guineense* especially at 5%, 10%, 15% and 20% concentrations, in comparison with the control group had a significant (P<0.05) negative impact on the behavior of the animals. Aggression appears to be concentration-dependent with the highest occurrence (100%) in group 6 fed with 20% of *P. guineense*.

Effect of *P. guineense* on body and testicular weight in Wistar rats is presented in Table 4. No significant difference (P>0.05) was observed in the % body weight gain of the test rats when compared with the control group. Statistically analysis, also shows that there was no significant difference (P>0.05) in the testicular weight of the test rats when compared with the control group.

3.2 Discussion

An aphrodisiac is a substance that when consumed, increases sexual behavior [30]. The search for aphrodisiacs dates back to millennia and various substances of plant origin have been used in folk medicine as aphrodisiacs [5,16-21]. The present study investigated the effect of *Piper guineense* supplemented diet on libido,
Table 2. Effect of *Piper guineense* on some libido indicator parameters in Wistar male rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Mounting latency (Sec)</th>
<th>Mounting frequency</th>
<th>Intromission frequency</th>
<th>Percentage mounted (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Control</td>
<td>429±7.50</td>
<td>6±1.21</td>
<td>5±0.93</td>
<td>100</td>
</tr>
<tr>
<td>G2</td>
<td>0.5% of <em>P. guineense</em></td>
<td>390±3.54(^{ns})</td>
<td>8±1.44(^{ns})</td>
<td>8±1.39(^{ns})</td>
<td>100(^{ns})</td>
</tr>
<tr>
<td>G3</td>
<td>5% of <em>P. guineense</em></td>
<td>371±2.36(^{ns})</td>
<td>10±1.98(^{ns})</td>
<td>8±1.72(^{ns})</td>
<td>100(^{ns})</td>
</tr>
<tr>
<td>G4</td>
<td>10% of <em>P. guineense</em></td>
<td>265±2.29*</td>
<td>13±2.75*</td>
<td>14±2.75*</td>
<td>100(^{ns})</td>
</tr>
<tr>
<td>G5</td>
<td>15% of <em>P. guineense</em></td>
<td>245±4.77*</td>
<td>14±1.59*</td>
<td>15±1.41*</td>
<td>100(^{ns})</td>
</tr>
<tr>
<td>G6</td>
<td>20% of <em>P. guineense</em></td>
<td>220±6.55*</td>
<td>20±3.34*</td>
<td>22±3.51*</td>
<td>100(^{ns})</td>
</tr>
</tbody>
</table>

Data expressed as Mean ±SEM, *Value considered statistically significant (P<0.05), ns = not-significant (P>0.05)

Table 3. Effect of *P. guineense* consumption on the behavioral pattern of Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Aggressive n (%)</th>
<th>Non-aggressive n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Control</td>
<td>0 (0)</td>
<td>5 (100)</td>
</tr>
<tr>
<td>G2</td>
<td>0.5% of <em>P. guineense</em></td>
<td>0 (0)(^{ns})</td>
<td>5 (100)</td>
</tr>
<tr>
<td>G3</td>
<td>5% of <em>P. guineense</em></td>
<td>2 (40)*</td>
<td>3 (60)</td>
</tr>
<tr>
<td>G4</td>
<td>10% of <em>P. guineense</em></td>
<td>2 (40)*</td>
<td>3 (60)</td>
</tr>
<tr>
<td>G5</td>
<td>15% of <em>P. guineense</em></td>
<td>3 (60)*</td>
<td>2 (40)</td>
</tr>
<tr>
<td>G6</td>
<td>20% of <em>P. guineense</em></td>
<td>5 (100)*</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*Value considered statistically significant (P<0.05), ns = not-significant (P>0.05)

Table 4. Effect of *P. guineense* on body and testicular weight in Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Initial Body weight (g)</th>
<th>Final Body weight (g)</th>
<th>% Weight gain</th>
<th>Testicular weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Control</td>
<td>191.20±6.66</td>
<td>201.73±9.64</td>
<td>5.51</td>
<td>2.66 ± 0.09</td>
</tr>
<tr>
<td>G2</td>
<td>0.5% of <em>P. guineense</em></td>
<td>173.16±8.81</td>
<td>182.28±10.97</td>
<td>5.27(^{ns})</td>
<td>2.66 ± 0.04(^{ns})</td>
</tr>
<tr>
<td>G3</td>
<td>5% of <em>P. guineense</em></td>
<td>173.62±4.05</td>
<td>187.41±6.14</td>
<td>7.94(^{ns})</td>
<td>2.68 ± 0.09(^{ns})</td>
</tr>
<tr>
<td>G4</td>
<td>10% of <em>P. guineense</em></td>
<td>175.90±11.49</td>
<td>192.84±14.76</td>
<td>9.63(^{ns})</td>
<td>2.77 ± 0.20(^{ns})</td>
</tr>
<tr>
<td>G5</td>
<td>15% of <em>P. guineense</em></td>
<td>171.16±9.88</td>
<td>184.36±13.84</td>
<td>7.71(^{ns})</td>
<td>2.36 ± 0.05(^{ns})</td>
</tr>
<tr>
<td>G6</td>
<td>20% of <em>P. guineense</em></td>
<td>184.56±7.47</td>
<td>200.17±9.08</td>
<td>7.80(^{ns})</td>
<td>2.54 ± 0.13(^{ns})</td>
</tr>
</tbody>
</table>

Data expressed as Mean ±SEM, ns = Value considered statistically not significant (P>0.05)

Behavior pattern and the testicular structure in rat model. The outcome of this present study shows that *P. guineense* possesses a significant libido enhancement activity. The result of the libido index test show that the dry leaves of *Piper guineense* especially at 10%, 15% and 20% concentration, in comparison with the control group, significantly (P<0.05) increased the Mounting Frequency and Intromission Frequency of the male rats. The study also revealed that the test plant impacted upon the behavioural Pattern of the animals. The treated rats were more aggressive especially at the highest concentration (20%) tested and were mounting continuously during the libido index test compared with the control.

Mount Frequency (MF) and Intromission Frequency (IF) are useful indices of libido. Increase in the number of intromission shows the efficiency of erection, penile orientation and the ease by which ejaculatory reflexes are activated [40,41]. Therefore, the increase in MF and IF following consumption of *P. guineense* supplemented diet especially at 10%, 15% and 20% concentration suggests enhanced libido. Such enhancement of libido might have arisen from increase in the number of concentrations of several anterior pituitary hormones and serum testosterone, which in turn stimulated dopamine receptor synthesis and sexual behaviour [42]. This sexual behaviour may also be due to inherent androgenic and gonadotropic properties of *P. guineense*. It may therefore be logical to attribute these behaviours to the presence of phytochemicals like alkaloids, flavonoids and saponin in the plant, since they have been reported to alter androgen levels [43].
by the test plant in this study suggests that the mechanism of penile erection was activated. Therefore, *P. guineense* may increase potency by allowing or sustaining erection. Various phytochemicals have been reported to affect penile erection by different mechanisms. For example, alkaloids have been shown to have ergogenic properties by inducing vasodilation of the blood vessels which consequently result in erection [40].

Also, the outcome of the study shows that *P. guineense* caused a significant (P<0.05) reduction in the Mounting Latency (ML) of the animals in a concentration-dependent manner. Mounting latency is an indication of reduction in the hesitation time of the male rats towards the receptive females. It also indicates enhanced sexual appetitive behavior because these parameters are considered to be inversely proportional to sexual motivation or desire [44]. Therefore, the decrease in the mount latency observed at 10%, 15% and 20% concentration in this study might imply stimulation of sexual motivation and arousability. It may also be an indication of enhanced sexual appetitive behaviour in the male rats. All these further support the libido enhancement effect of the plant.

The results of the present study agree with the reports of several previous studies [30-37]. Kamtchouing et al. [30] for instance showed that aqueous extract of *Piper guineense* fruits at 122.5 mg/kg was able to stimulate sexual behaviour of mature male rats by decreasing intromission latencies and increasing mounting, ano-genital sniffing and penile erection index.

Testes weight generally establishes the normalcy of testis, enabling experimentally induced changes in testicular size and potential spermatozoa production to be assessed [45]. In the present study, there was no significant difference (P>0.05) in the % body weight gain and testicular weight of the test rats when compared with the control group.

This present work contradicts the work of Malini et al. [46], who reported that *P. guineense* at doses of 5 mg/kg and 10 mg/kg for administered for 30 days resulted in a decrease in testicular weight. Meanwhile, it partly agrees with the work of Mbongue et al. [31], who reported a significant (P<0.05) increase in % body weight gain, but no significant different in the testicular weight of the test animals, compared with the control. Furthermore, it disagrees with the work of Ekanem et al. [32] who reported increased testicular weight with administration of 200 mg/kg crude extracts of dry fruits of *P. guineense*.

However, it is not in accordance with the work of Memudu et al. [35] who reported a significant difference between the percentage body weight gain and testicular weight of test rats, compared to the control following 28 days treatment with crude aqueous extract of dry fruit of *P. guineense*. Still, it fails to agree with the work of Okoye et al. [38], who reported a significant higher testicular weight in animals treated with methanolic leaf extract of *P. guineense* compared with the control.

### 4. CONCLUSION

*P. guineense* showed potential value as a natural sexual enhancement (aphrodisiac) agent as shown by significant increase in some of the libido indicator parameters assessed. If these findings are extrapolated to humans, they underscore its libido enhancement potential and may likely produce similar benefit in humans. Extraction of active principles, safety assay, as well as qualitative and quantitative phytochemical assay of the different plant parts (leaf, fruit and seeds) using standard protocols are suggested for further studies.

### ETHICAL APPROVAL

Ethical approval was obtained from the Babcock University Health Research Ethics Committee (BUHREC), Ilishan-Remo, Ogun State, Nigeria.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

### REFERENCES


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