**Effect of *Phyllanthus amarus* on Some Reproductive Indices of Male Albino Rats**

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**Authors’ contributions**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**ABSTRACT**

**Background:** Medicinal plants have been a good source of drugs for humans, but chronic and prolong use of medicinal plants like *Phyllanthus amarus* for the treatment of malaria and other disorders are issues of concerns. This study evaluated the effect of *Phyllanthus amarus* on reproductive organs and sperm parameters in albino rats.

**Materials and Methods:** Twenty-four healthy male albino rats of 12 weeks old were assigned into four groups with six rats in each group using a Completely Randomized Design (CRD). The experimental animals were orally treated with *Phyllanthus amarus*. Group A served as the control and was given only water and feed; Group B, C and D received 100 mg/kgBW, 200 mg/kgBW and 300 mg/kgBW of *Phyllanthus amarus* respectively. Data obtained were analyzed using analysis of variance (ANOVA). The treatments lasted for a period of 65 days after two weeks of acclimatization.

**Results:** The results showed statistically significant (p<0.05) reduction in weight of testes and epididymes, sperm motility, sperm viability, sperm count and sperm head abnormalities in male rats treated with *Phyllanthus amarus* when compared to the control. The sperm pH was not significantly (p>0.05) affected by *Phyllanthus amarus* among the different treatment groups in the experimental animals.
Conclusion: Findings from the present study indicate that *Phyllanthus amarus* possesses a dose-dependent anti-fertility activity in male albino rats under a sub-chronic course of administration.

Keywords: *Phyllanthus amarus*; reproductive organ; sperm count; sperm viability; sperm head abnormality.

1. INTRODUCTION

Plants are natural products and their derivatives were the fundamental basis for early medicines and continue to provide a rich source of drugs and plant metabolites today [1-3]. Approximately 60% of approved new chemical entities emanates from natural sources [1-4], making man’s continuous quest on herbs for therapeutic and nutritional values to be a day to day activity. Phytochemical and pharmacological research on *Phyllanthus amarus* showed that it is a rich source of active constituents and chemicals [5]. These active constituents are biological active lignans, phyllanthin, hypophyllanthin, flavonoids, alkaloids and phenylpropanoids found in the leaf, stem and roots of *Phyllanthus amarus* [5].

*Phyllanthus amarus* is a small erect herbal plant that grows up to 10-50 cm high belonging to the family *Euphorbiaceae*. This tropical annual herbal shrub have small leaves, its stem have green capsules with flowers and very small fruits that burst open when dry. This plant is commonly called “stonebreaker”, locally called “Iyin-olobe” in Yoruba; South-west Nigeria [6], while the Ibibo tribe and Asaba people called it “Oyomo ke Iso Amankedem” and “Buchi oro” respectively [7] in South-south Nigeria. In Eastern Nigeria, the Igbo tribe called it “Ngwu” [7]. Traditionally, this plant has been utilized for its antidiabetic, antihypertensive, hepatoprotective, analgesic, antimicrobial and anti-inflammatory properties as documented by researchers [6,8-10]. Anticarcinogenic and antimutagenic properties of *Phyllanthus amarus* has been documented [11]. Also *Phyllanthus amarus* has been reported to be utilized locally to treat skin ulcer, swelling skin, gastrointestinal disorder, jaundice, diarrhea and sores [10,12-13]. It is also known to have an inhibitory effect on endogenous hepadnavirus, DNA polymerase [5,14-15]. Due to the abundance of *Phyllanthus* species, they are widely used for the treatment of malaria in endemic regions [16-18] and also to improve libido in males by local medicine practitioners.

There have been reports of decrease in male fertility potentials after treatment of animals with anti-malaria herbs like *Phyllanthus niruri* [17] resulting from impairment in sperm parameters. Therefore, this research aimed at investigating the effect of *Phyllanthus amarus* on the reproductive organs of male albino rats and their weights in a dose-dependent manner in mammalian experimental model.

2. MATERIALS AND METHODS

2.1 Location and Duration of the Study

The study was conducted at the animal house, Department of Genetics and Biotechnology, University of Calabar, Calabar. The duration of the study was two months and three weeks.

2.2 Collection and Preparation of Plant Samples

*Phyllanthus amarus* were collected with in the University of Calabar campus and authenticated in the herbarium unit of the Department of Plant and Ecological Studies, Faculty of Biological Sciences, University of Calabar, Calabar. The entire plant was washed with clean water, air dried for two weeks before powdering using electric blender (Qlink-Q15L40). The powdery form of the plant was subjected to extraction using Soxhlet method with 70% ethanol as solvent. The filtrate was obtained using rotary evaporator at 45°C and reduced into pastes with hot-air oven at 40°C. The pastes obtained were stored in plastic screw capped bottles, labeled and stored in refrigerator for usage. Appropriate weights of the pastes were prepared in normal saline to obtain the various concentrations used for the different experimental groups of animals, except the control group.

2.3 Experimental Animals

Twenty-four healthy male albino rats of 12 weeks old, with an average body weight of 185.5 grams were obtained from the animal house, Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Calabar, Calabar for this study. The rats were housed in well ventilated wire mesh cages under standard laboratory conditions.
They were allowed free access to water and pelleted commercial feed throughout the period of the experiment. Generally, the study was conducted in accordance with the recommendations from the declarations of Helsinki on guiding principles in care and use of animals.

2.4 Experimental Design and Procedure

The twenty-four male albino rats were assigned into four groups of six rats each using a completely randomized design. The animals were acclimatized for two weeks before the commencement of the treatment. The daily treatments were given orally via oral gavage which lasted for 65 days and the protocol is shown in Table 1. The albino rats were sacrificed under chloroform anaesthesia 24 hours after the administration of last treatment. The epididymes and testes were dissected out and weighed using Scout Pro SPU 601 electronic weighing balance. The epididymes were processed for epididymal sperm count, motility, viability and sperm head abnormality.

2.5 Semen pH and Motility

Immediately after dissection, a puncture was made in the epididymis with a sterile pin. The semen smeared on the pin was rubbed on a pH paper of range 4.0-10.0. The colour change corresponding to the pH of the semen, which was read from the paper. Two drops (0.05 ml each; that is 0.1 ml) of sperm suspension were put on a microscope slide and cover slip was placed on it. The number of progressively motile cell (cells that swim in a mostly straight line or very large circles) was recorded and divided by the total number of sperm cells counted under x40 lenses and expressed in percentage.

2.6 Sperm Viability

The sperm viability test was determined using Eosin-Nigrosin staining technique [19]. A portion (0.1 ml) of the sperm suspension was mixed with equal volume of Eosin-Nigrosin stain (0.05 ml each) and air-dried smears were prepared on glass slide for each sample. The slides were examined for percentage of viability. Viable sperm cells appeared whitish, while dead sperm cells took up stain and appeared pinkish. The percentage viability was calculated based on the number of viable sperm cells out of the total number of cells observed.

2.7 Sperm Count

The epididymal sperm samples were obtained by macerating known weights of caudal epididymis in physiological saline in the ratio of 1:10 weight by volume [20]. The epididymis was pipetted to release the sperm cells and filtered using 80 μm stainless mesh. Epididymal sperm count was obtained by using the improved Neubauer cytometer (Model: BRT723014) and was expressed in x10⁶/mL of the sperm suspension [20].

Table 1. Protocol for daily treatment of experimental animals for 65 days

<table>
<thead>
<tr>
<th>S/N</th>
<th>Groups</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A (Control)</td>
<td>No administration of Phyllanthus amarus</td>
</tr>
<tr>
<td>2.</td>
<td>B</td>
<td>100mg/kgBW of Phyllanthus amarus</td>
</tr>
<tr>
<td>3.</td>
<td>C</td>
<td>200mg/kgBW of Phyllanthus amarus</td>
</tr>
<tr>
<td>4.</td>
<td>D</td>
<td>300mg/kgBW of Phyllanthus amarus</td>
</tr>
</tbody>
</table>

2.8 Sperm Head Abnormality Test

A portion of the sperm suspension was mixed with 1% eosin Y solution (10:1) for 30 minutes and air-dried smears prepared on glass slides for the sperm head abnormality test. The slides were examined for percentage sperm head abnormalities in every 200 sperm cells observed on each slide for each sample. The percentage of sperm head abnormality was calculated according to the protocol of Ekaluo et al. [21].

2.9 Statistical Analysis

All data obtained on weight of testes, weight of epididymis, semen pH, sperm motility, sperm viability, sperm count and sperm head abnormalities were subjected to analysis of variance (ANOVA) test for significant difference by using SPSS statistical software. Statistical significance were considered if P<0.05 while Least Significant Difference (LSD) test was used to separate the means.

3. RESULTS

3.1 Reproductive Organs Weight changes

The results presented on Table 2 showed the effect of Phyllanthus amarus on the weight of testes and epididymes. There was significant
decrease in the mean weight of testes and epididymes across the treated groups (B, C and D) when compared with 1.52 g and 0.41 g for testes and epididymes respectively in the control group A.

3.2 The Effect of Phyllanthus amarus on Reproductive Parameters of Male Albino Rats

3.2.1 Sperm pH and sperm motility

The sperm pH was not significantly (P>0.05) affected by the Phyllanthus amarus among the different treatment groups. The sperm pH ranges from 6.92 to 6.96 (Table 3). There was significant reduction in sperm motility of male albino rats treated with Phyllanthus amarus from 68.89% in group B to 72.4% in group D as displayed on Table 3.

3.2.2 Sperm viability

The sperm viability was significantly (P<0.05) affected by Phyllanthus amarus when compared to the control group in a dose-dependent manner, especially on group D (Table 3).

3.2.3 Sperm count

Sperm count was reduced significantly (p<0.05) in male albino rats treated with Phyllanthus amarus when compared to the control group (group A) and other groups (B, C, and D) as shown in Table 3.

3.2.4 Sperm head abnormalities

The results obtained from the male animals treated with Phyllanthus amarus had the highest percentage of sperm head abnormalities in the treatment groups and was statistically significant (P<0.05) when compared to the male animals not treated with the plant in the control group (Table 3).

4. DISCUSSION

Medicinal plants have been a good source of drugs for humans since ancient times. Despite advances in pharmacology and medicine, the cost of some renounced anti-malaria drugs available are not readily affordable by the poor people in developing countries. Against this backdrop, the chronic use of medicinal plants like Phyllanthus amarus for the treatment of malaria and other disorders are documented [5,9-10,16-18,22]. The association between male infertility and antimalarial plants commonly used locally by poor people in developing countries are issues of great concerns. In this study, Phyllanthus amarus significantly reduced the mean weight of reproductive organs like testes and epididymes. The reduction in weight of testes and epididymes significantly influenced some reproductive parameters like sperm motility, sperm viability, sperm count and sperm head abnormalities when compared with the male rats in control group. This is agreeably with the reports of Obianime and Uche [23], Ogbomade et al. [24] who observed dose-dependent decreases in the reproductive parameters due to the effect of Phyllanthus amarus extract administered to male guinea pigs and male wistar albino rats respectively.

Asare et al. [22] reported that aqueous leaf extract of Phyllanthus niruri (another phyllanthus species) administered over prolonged periods generated hormonal imbalances and possible testicular degradation with potential male infertility. This could therefore suggests that prolonged treatment of rats with Phyllanthus amarus have altered hormonal imbalances, testicular degradation and suppresses spermatogenesis with a possible potential side effects; which may lead to male infertility. In the same line of thought, Ikpeme et al. [25] noted that disruptions in fertility male mammals was directly correlated to disruptions in spermatogenesis. So this may suggests that Phyllanthus amarus treatment altered spermatogenic pathways and processes; with a concomitant reduction in reproductive sperm parameters as observed from the results of this study. The reduction in sperm count supports the decrease in weight of epididymes observed in group of male rats treated with Phyllanthus amarus which imply disruptions in sperm maturation in the epididymes. The sperm motility was drastically decreased from the result of this present finding, which may be due to the morphological disruption of the sperm cells by Phyllanthus amarus. This is similar to the documented researches by Ikpeme et al. [26] and Ekaluo et al. [21], where test substances (medicinal plants) decreased sperm motility due to morphological disruption of sperm cells.

Reproductive organs of the male albino rats were significantly reduced in their mean weight when treated with Phyllanthus amarus. This is in harmony with the findings of Ezeonwu [27] which
reported that *Phyllanthus niruri* decreased the reproductive organs weight after the administration, indicating toxic effect of the plant due to its chemical constituent(s). The researcher [27] further stated that any chemical agent or constituent that can affect reproductive organs negatively, will also affect the quality and quantity of sperm.

The sperm counts, motility and viability of the treated samples were significantly decreased in group B, C, and D (P<0.05) when compared to the control group A. These decreases can be attributed to anti-androgenic property of the plant extract, which is in harmony with other findings [27,22]. Bandekar et al. [28] reported that *Phyllanthus niruri* could cause oxidative stress in wistar rats leading to testicular toxicity, which will have negative impacts on reproductive potential of animals; reflecting in reduction of sperm parameters. Although the oxidative stress of *Phyllanthus amarus* was not investigated in this present study, but the reduction in reproductive parameters among the treated animals could be due to oxidative stress resulting in testicular toxicity. Also several other studies recorded significant reduction in sperm parameters of animals treated with medicinal plants when compared to the control [29-31], which are similar to the results of this study.

Sperm count or volume and sperm density were significantly reduced in animals treated with *Carica papaya* as reported by Ikpeme et al. [29] due to presence of phytochemicals like alkaloids, saponins, flavonoids, etc which might have altered the spermatogenic processes and pathways. These active phytochemicals are found in *Phyllanthus amarus* [5], therefore contributing to the alterations of spermatogenic processes and pathways in the male rats treated with the plant under investigation. This assertion is in conformity with other reports [30,32-34] who documented on the antifertility properties of medicinal plants. The length of time and intensity of spermatozoa motility, sperm density and the percentage of motile sperm are reproductive parameters that have received numerous research attentions in man, mammals and fish [18-19,22-26,29-34] in order to assess sperm quality and quantity. Testicular weight reduction in animals treated with plants extracts revealed distortions in the testicular integrity [31]. The decrease in the mean weight of testes and epididymes treated with *Phyllanthus amarus* support the concomitant decrease observed in the sperm count or volume and other sperm parameters observed in the experimental male animals model treated. These findings are similar to other published researches [27-31].

Results obtained from this study also indicated a significant (P<0.05) increase in sperm head abnormalities among animals treated with *Phyllanthus amarus*, which is denotive of induced mutation(s) on the sperm cells during spermatogenesis. Increase in the incidence of abnormal sperm has been reported after treatment of male albino rats with formaldehyde [35] and analgesics [36]. *Phyllanthus amarus* extracts are locally used as anti-inflammatory analgesics [9,10], pointing that it may induce mutations on sperm cells during spermatogenic processes; though it may be administered as an

<table>
<thead>
<tr>
<th>Reproductive organ</th>
<th>Group A (control)</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testes (g)</td>
<td>1.52±0.02a</td>
<td>0.89±0.04b</td>
<td>0.87±0.20c</td>
<td>0.84±0.16c</td>
</tr>
<tr>
<td>Epididymes (g)</td>
<td>0.41±0.01a</td>
<td>0.38±0.02b</td>
<td>0.35±0.10b</td>
<td>0.32±0.38b</td>
</tr>
</tbody>
</table>

*Means with different superscripts are significantly different at 5% level based on ANOVA along each horizontal array.*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm pH</td>
<td>7.08±0.01a</td>
<td>6.92±0.03a</td>
<td>6.96±0.02b</td>
<td>6.96±0.02a</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>69.52±1.13a</td>
<td>68.89±1.10a</td>
<td>71.20±1.51b</td>
<td>72.47±1.39b</td>
</tr>
<tr>
<td>Sperm viability (%)</td>
<td>91.95±3.14a</td>
<td>88.29±1.46b</td>
<td>86.51±1.22c</td>
<td>82.9±1.31d</td>
</tr>
<tr>
<td>Sperm count (x10⁹/mL⁻¹)</td>
<td>9.14±0.33a</td>
<td>8.01±0.91b</td>
<td>7.27±1.01b</td>
<td>6.91±0.89c</td>
</tr>
<tr>
<td>Sperm abnormalities (%)</td>
<td>7.28±1.10a</td>
<td>7.98±1.24b</td>
<td>8.20±0.99c</td>
<td>8.41±1.26c</td>
</tr>
</tbody>
</table>

*Means with different superscript are significantly different at 5% level based on ANOVA along horizontal array.*
ana
gesics, anti-malaria drug or for other purposes. Sperm head abnormalities in animals treated with caffeine was documented by Ekaluo et al. [37] and Uno et al. [38-39] also disclosing induced mutation on sperm cells during spermatogenic processes, resulting in sperm head abnormalities. These reports are similar to our present findings on sperm head abnormalities due to disruption during spermatogenesis.

5. CONCLUSION

In conclusion, Phyllanthus amarus orally administered to male albino rats reduced the mean weight of testes, epididymes and significantly altered some reproductive parameters like sperm motility, sperm viability, sperm count and sperm head abnormalities negatively. This plant have anti-fertility effects on male albino rats in a dose-dependent manner; as a mammalian model.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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