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Preliminary Evaluation of The Effects of *Phyllanthus amarus* On Conception, Pregnancy Outcome And Litter Weights, In Female Wistar Rats

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ABSTRACT

Previous studies have reported both antifertility and fertility-promoting effects of extracts from *Phyllanthus amarus*. In this study, we evaluated the effects of the ethanol extract of the aerial parts of *Phyllanthus amarus* on conception, pregnancy outcomes, and litter weights in female Wistar rats. Twenty-five (15) female Wistar rats were divided into five groups (A–C), with five animals per group. Each group was paired with two male Wistar rats for mating. Group (A) animals received normal saline (0.5 mL) and served as the control, while Groups B and C were administered 12.5, and 50 mg/kg (which were the low and highest dose following pilot studies that was guided by literatures reviews) of the extract orally for 30 days respectively. The female rats were paired with the male rats from day 1, to 7 days, before the male Wistar rats were removed. The female Wistar rats were observed for conception and pregnancy outcomes after a 28-day gestation period, and litters from all groups were weighed and compared with the control. Results showed that the treated Groups of B and C produced eight (8) litters each, with mean litter weights of 12.8 ± 1.76 g and 13.69 ± 2.03 g, respectively. These values compared positively with the twelve (12) litters and mean litter weight of 15.33 ± 2.14 g observed in the control group ($p > 0.05$). It is therefore concluded that the ethanol extract of *Phyllanthus amarus* has the potential to promote fertility in female Wistar rats and exerts a positive influence on conception and pregnancy outcomes.

Keywords: Fertility, antifertility, conception, pregnancy outcome, gestation-period, litter-weight

INTRODUCTION

The World Health Organization (2022) defines reproductive health as "a state of complete physical, mental, and social well-being, and not merely the absence of disease or infirmity, in all matters relating to the reproductive system and its functions and processes." Within this context, reproductive health is a vital component of overall well-being, encompassing the physical, mental, and social dimensions of the reproductive system. It ensures that individuals can experience a responsible, fulfilling, and satisfying sexual life, while also exercising autonomy in reproductive decisions (WHO, 2022). Good reproductive health is therefore critical to sustaining fertility, enabling safe pregnancies, ensuring positive outcomes, and enhancing the quality of life for individuals of reproductive age. In Sub-Saharan Africa, conception and pregnancy remain central expectations within marriage, serving both as a means of procreation and as an affirmation of fertility. When conception does not occur after years of marriage or sustained relationships, infertility is considered to be present. Medically, infertility is defined as the inability to achieve pregnancy after one year of regular, unprotected sexual intercourse. Studies have shown that about one in six couples worldwide suffers from infertility, of which about 50% is attributed female related disorders (Akbaribazm *et al.*, 2021). Infertility presents significant challenges and is often accompanied by social and cultural stigmas, particularly in regions such as South Asia, Sub-Saharan Africa, North Africa, the Middle East, Central and Eastern Europe, and Central Asia (Singh *et al.*, 2024). Standard approaches to infertility management typically involve prevention, diagnosis, and medical interventions, including orthodox medicine and surgical procedures. However, the high cost of treatments such as in vitro fertilization (IVF) combined with relatively limited success rates, makes these options inaccessible for many couples (Alifariki *et al.*, 2022). As a result, a substantial number of women experiencing infertility turn to alternative and complementary medicine, seeking more affordable, safer, and potentially more effective solutions (Taylor, 2022).

Medicinal plants have been utilised for centuries to address various health conditions, including infertility in Africa, and particularly in Nigeria. This has been the practice in many cultures from the beginning of civilization (Ugiomoh *et al.*, 2023). Some of the plants commonly used in the treatment of infertility in Southern Nigeria include *Psidium guajava, Pennisetum purpureum, Oenothera biennis, Aloe barbadensis, Asparagus racemosus, Gossypium hirsutum, Newbouldia laevis, Phyllanthus amarus*, and *Afrormosia laxiflora*. These plants are reported to exhibit diverse biological activities, ranging from alleviating menopausal symptoms and influencing hormonal balance to enhancing fertility, exerting anti-fertility effects, and functioning as abortifacients or contraceptives (Osawaru and Ogwu, 2024).

The *Phyllanthus* genus, comprising approximately 2,000 species including *Phyllanthus amarus* has been reported to contain bioactive compounds that enhance reproductive functions and improve fertility in both males and females (Dasiman and Bahari, 2021). However, findings on the effects of *Phyllanthus amarus* on female reproductive health remain inconsistent. Ataman and Sakpa (2017) documented reproductive toxicity associated with the ethanol extract of *Phyllanthus amarus* leaves in female Wistar rats. In light of these conflicting reports, the present study investigates the effects of ethanol extracts from the aerial parts of *Phyllanthus amarus* on conception and pregnancy outcomes in female Wistar rats.

MATERIALS AND METHODS

Collection and Extraction of Plant Material

The aerial parts of *Phyllanthus amarus* were obtained from the whole plant collected within the premises of University of Benin, Ugbowo Campus, Benin City, Edo State Nigeria. The plantd had earlier been identified at Forestry Research Institute of Nigeria (FRIN), Idi Ishin, Jericho Ibadan, Oyo State by Mr. Olawale Akinpelu, with a Herberium Voucher Number (SH107456) issued (Amaechina and Omogbai, 2007). The plant material was air dried under shade for 21 days, and later in an oven at 40ºC for 20 minutes, to remove residual moisture. The plant material was thereafter pulverized at the Department of Pharmacognosy, University of Benin, using an electric milling machine. Weighed quantity (500 g) of the pulverized material was extracted with ethanol using Soxhlet apparatus and a heating mantle with temperature regulated at 78.5 ºC (Amaechina *et al.,* 2022). After exhaustive extraction, the extract was concentrated to slurry paste using rotary evaporator, and later to solid extract in a vacuum oven set at 40ºC. During the animal experiments, 2.0 g of the extract was weighed and dissolved with 10 mL of distilled water that contained 0.1% of polysorbate 80, to obtain an extract solution of 200 mg/mL, from which appropriate serial dilutions were made to obtain the various doses that were used for the animal experiments.

Experimental Animals

Adult female non-gravid and male Sprague-Dawley rats (190-230 g) were purchased, and maintained in the Animal House of the Department of Pharmacology and Toxicology, University of Benin. The animals were fed with standard animal feed pellets, and allowed access to water *ad libitum*, under ambient temperature and natural day and night light cycle. Ethical approval was obtained from the Faculty of Pharmacy Ethical Committee for animal experiments, and all the animals were handled in compliance with the Public Health Service policy on humane Care and Use of Laboratory Animals (Uchendu *et al*., 2025).

Evaluation of Pregnancy Outcome and Litter Weights

In the protocol for the evaluation of pregnancy outcome, fifteen (15) female Wistar rats were randomly selected and divided into 3 groups (A - C) of five animals each. Group (A) serve as the control and received 0.5 mL normal saline, Groups B and C were administered 12.5, and 50 mg/kg (which were the low and highest dose following pilot studies that was guided by literatures reviews) of ethanol extract respectively for 21 days. Each group was paired with two male adult Wistar rats from day-1 to day-7, of treatment with the extract. Coitus was confirmed by vaginal smear observed under a microscope (Uchendu *et al*., 2025), after which the male rats were withdrawn, while the respective groups were observed throughout the gestation period for pregnancy outcome, confirmed by the liters from each treatment group. Fourteen (14) days after, the litters from each of the treated groups were weighed, and compared to the litters from the control.

Statistical Analysis

All data were analyzed using GraphPad prism version9 in one way analysis of variance (ANOVA) expressed as mean ± standard deviation, with 95% confidence interval, and $p < 0.05$.

RESULTS AN DISCUSSION

The results of the effect on litter weights, conception and pregnancy outcome are presented in table 1, and figure 2 below:

Table 1: The Effect of Ethanol Extract of Phyllanthus Amarus on Litter Weights

Treatment Group	Number of Rats	Number of Litters	Mean Litter Weights (g)
Group (A) Normal Saline	5	12	15.33 ± 2.14
Group (B) 12.5 mg/kg	5	8	12.8 ± 1.76
Group (C) 50 mg/kg	5	8	13.69 ± 2.03

The results show that the litter numbers and the weights of the treated groups compare positively with that of control.

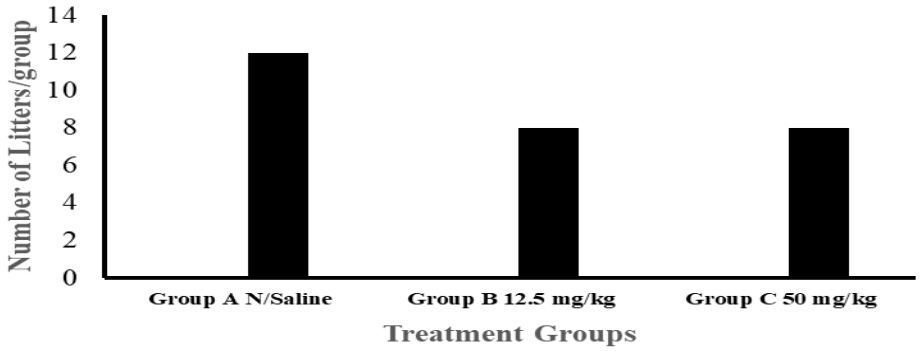

Figure 1: The Result of the Effect of the Ethanol Extract of The Aerial Part of *Phyllanthus Amarus* on Conception and Pregnancy Outcome

Discussion

Among the more than 2,000 species of the *Phyllanthus* genus, *Phyllanthus amarus* is the most abundant and extensively studied in Nigeria. Its application in ethnomedicine spans nearly all areas of health, a practice likely reinforced by findings from laboratory evaluations. Reported pharmacological activities include antidiabetic (Adedapo *et al.,* 2014), antihypertensive (Amaechina and Omogbai, 2007), anticancer (Rajeshkumar *et al*., 2002), and antiseizure/antiepileptic effects (Amaechina and Omogbai, 2013), among others. Despite these diverse therapeutic applications, previous studies have documented conflicting effects of *Phyllanthus amarus* extracts on female reproductive health. Nurudeen and Yakubu (2016) reported the restorative

effect of the aqueous leaf extract of *Phyllanthus amarus* on sexual competence in female rats with fluoxetine-induced sexual dysfunction. In contrast, Ataman and Sakpa (2017) documented the antifertility effects of the ethanol leaf extract of *Phyllanthus amarus* in female Wistar rats, attributing these outcomes to negative alterations in ovarian morphology and reproductive parameters.

The results of our preliminary evaluation of the ethanol extract of *Phyllanthus amarus* on conception and pregnancy outcomes in female Wistar rats showed that the treated groups compared positively with the control. This was demonstrated by the number of litters produced and the mean litter weights. The control group (Group A) produced twelve (12) litters with mean litter weights of 15.33 ± 2.14 g. In comparison, treatment with doses of 12.5 mg/kg and 50 mg/kg resulted in eight (8) litters in Group B and Group C, with mean litter weights of 12.8 ± 1.76 g and 13.69 ± 2.03 g, respectively. These values were not statistically different from the control ($p > 0.05$), indicating that the extract did not adversely affect conception or pregnancy outcomes. These preliminary results suggest a fertility-enhancing activity of the ethanol extract of *Phyllanthus amarus* in female Wistar rats, particularly at relatively lower dose ranges, with the maximal dose tested being 50 mg/kg. Importantly, our findings compare positively with the control, as conception and pregnancy outcomes were not adversely affected. This observation aligns with the report of Nurudeen and Yakubu (2016), that documented a positive effect of the aqueous extract of *Phyllanthus amarus* on sexual competence in female Wistar rats at doses of 20, 40, and 80 mg/kg—also within a relatively low dose range. By contrast, Ataman and Sakpa (2017) reported antifertility effects at a single, much higher dose of 250 mg/kg. The inference, therefore, is that their study was primarily aimed at investigating the toxicological profile of *Phyllanthus amarus*, which likely influenced their choice of a dose five times greater than the highest dose used in our study.

Based on the results of this preliminary evaluation, it can be concluded that at lower dose ranges, the ethanol extract of the aerial parts of *Phyllanthus amarus* supports conception and exerts a positive influence on pregnancy outcomes in female Wistar rats.

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