JASEM ISSN 1119-8362

Full-text Available Online at www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage. *March*, 2009
Vol. 13(1) 37 - 41

The comparative effects of methanol extract of *Phyllanthus amarus* leaves and Vitamin E on the Sperm parameters of Male guinea pigs.

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ABSTRACT: The effects of the methanol extracts of the leaves of *Phyllanthus amarus* on the sperm parameters of male Guinea pigs were investigated. The effects of Vitamin E on the sperm parameters were also investigated and compared to that of *Phyllanthus amarus.* The phytochemical screening of the leaves of *Phyllanthus amarus* was also carried out. The methanol extract of the *Phyllanthus amarus* leaf (50-200mg/kg) caused a decrease in the sperm count and motility, from 65.0± 2.80 and 66.7±0.33 to 46.0± 2.10 and 37.7± 1.50 respectively. However, this effect was less pronounced at higher doses of *Phyllanthus amarus (400 and 800mg/kg).* At 400 and 800mg/kg, *P. amarus* caused a decrease in sperm motility and count from 65.0±2.50 and 66.7± 1.5 to 52.7± 0.50 and 56.7± 0.80 : to 43.3±1.70 and 48.3±1.70 respectively . These effects were dose- dependent and comparable to the observed effects of Vitamin E (500IU) on sperm parameters of male guinea pigs. These were significant at $P < 0.05$ (ANOVA). In time-dependent study, the observed effect of P. amarus (800mg/kg) and Vitamin E on the values of sperm count and motility at 28 day were almost the same. These values are 56.7± 3.30 and 48.3± 1.70 for vitamin E; 47.5±2.50 and 47.5± 2.50 for *P.amarus* respectively for sperm motility and count. These effects were time- dependent and statistically significant at $P < 0.05$ (ANOVA). Finally, the phytochemical screening of the leaves of *Phyllanthus amarus* revealed the presence of flavonoids, tannins, alkaloids, terpenoids, steroids, saponins and cardiac glycosides. This shows that the leaves of *P .amarus* do not largely contribute direct to the claims on the use of the aerial part of this plant by traditional medicine practitioners to increase/improve libido in men but it may have some indirect beneficial effects on this claim (like antioxidant activity) that mimic Vitamin E in both action and mechanism of action. However, further studies need to be done to investigate the contribution of the seeds of this plant in the improvement of libido in men and to isolate and characterize the active principles in the extracts of this plant. @ JASEM

Phyllanthus amarus Schum (Family Euphorbiaceae) is a widely distributed small erect, tropical annual herbal shrub whose stem has green capsule, and and grows upto10-50cm high and blooms with flowers with 5 white sepals and apical acute anther. The fruit has green capsules,and smooth and fruiting pedicels. The seeds are longitudinally rugose. It is locally called Iyin-olobe (Yoruba, south- west Nigeria) (Adeneye et al, 2006) or kidney stone plant. In Traditional medicine, it is used for its hepatoprotective , antidiabetic, antihypertensive, analgesic, anti-inflammatory and antimicrobial properties (Adeneye et al., 2006).The plant is also used in the treatment of stomach disorders, skin diseases and cold (Kokwaro, 1976, Iwu,1993). It has antidiarrheal effect (Odetola and Akojenu 2000). Its anti- viral activity against hepatitis B virus has been established (Thyagarajan et al., 1988; Meixa et al 1995); anticarcinogenic(Joy and kutten, 2000) and antimutagenic activities (Joy and Kutten, 1998). It also has anti- nociceptive and anti- inflammatory activities (kassuya et al, 2003), antidiabetic and anti-lipidemic potentials (Adeneye et al ,2006). So far, there is no report on the effects of *Phyllanthus amarus* on sperm parameters, such as sperm count, motility and morphology.

Also there is claim on the use of aerial part of *Phyllanthus amarus* to improve libido or fertility in men, by traditional medicine practitioners. Based on this claim, this study came up to establish, for the first time, a scientific information on the effectiveness of Phyllanthus amarus as fertility agent by its effects on the sperm parameters of male guinea pigs. And also to establish the contribution of the leaves of *Phyllanthus amarus* to this effect.

MATERIALS AND METHODS

All the chemicals used were of analar grade.

Plant Material

The leaves of *Phyllanthus amarus* were collected from the local garden within the premises of University of Port Harcourt in June 2008. The plant was identified and authenticated by Edwin Nwosu of department of Botany herbarium, University of Port Hacurt .Voucher specimen was maintained at the Herbarium.

The fresh leaves collected were air- dried for 10days, until a constant weight was attained.

Preparation of Extract

The dried leaves of *Phyllanthus amarus* were pulverized (100g). The crude drug was extracted with methanol using Soxhlet extraction method. The solid residue obtained was kept in a capped container in a refrigerator. Different concentrations of the extract were reconstituted from this stock.

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Experimental animals
The male guinea pigs were collected from the animal house of University of Port Harcourt. The weight of the animals ranges from 300-600g. The animals were allowed to acclimatize with the new environment (7) before the experiment. They were housed in a cage of five animals per cage and were adequately feed throughout the experiment.

METHOD

Phytochemical screening
Chemical tests were carried out on the methanolic extracts and on the powdered specimens using standard procedures to identify the constituents (Trease and Evans, 1989; Harborne, 1973) by characteristic colour changes as described by Sofowara, (1993); Odebedy and Sofowara, (1978).

Sperm count
Semen was diluted 1: 2 using micropipette in test tube with diluting fluid to immobilize and preserve the sperm. The Neubauer chamber (Heamocytometer) was prepared and charged with the diluted seminal fluid and allowed to stand in a moist chamber for 15-20 minutes to allow spermatozoa to sediment to the grid of the counting chamber and counted with 40x objective of light microscope.

Motility
A drop of undiluted liquefied and well mixed semen was placed on a clean slide and covered with a cover slide and then examined under a light microscope to stimulate both quantitative and qualitative motility. Sperm motility was determined by counting all motile and immotile spermatozoa, in randomly chosen fields using a 40x objective of light microscope.

Morphology
Smears of semen suspension were made on clean slide and quickly fixed with 95% alcohol. The smear was then stained with dilute (1 : 20) carbol. Fuchsin and examined under the oil immersion objective. 100-300 cells were counted and the v/v of abnormal forms were noted. Semen was also examined for the presence of particulate debris. Excessive contamination of the seminal fluid sample by epithelial cells, red blood cell, white blood cells and immature germ cells were observed and quantified.

RESULTS AND DISCUSSION

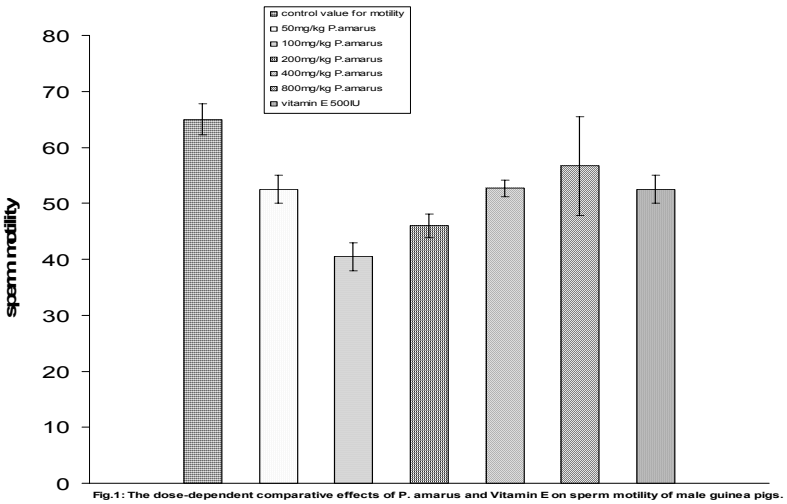

Fig.1: The dose-dependent comparative effects of P. amarus and Vitamin E on sperm motility of male guinea pigs.

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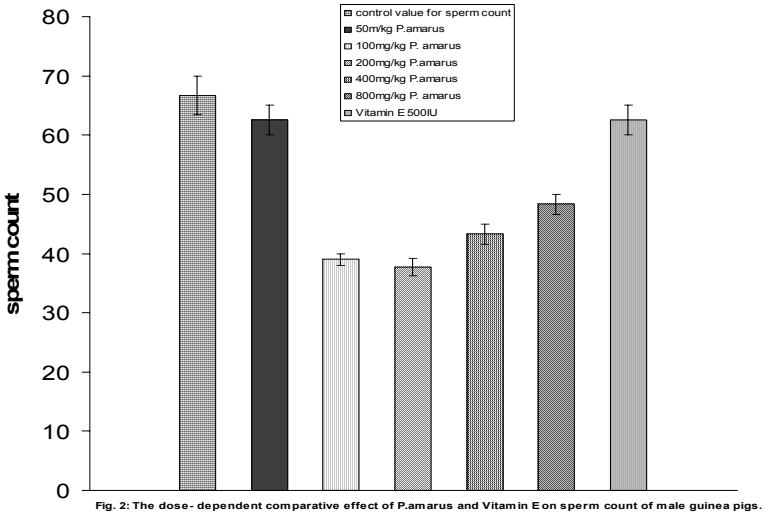

Fig. 2: The dose- dependent comparative effect of P.amarus and Vitamin E on sperm count of male guinea pigs.

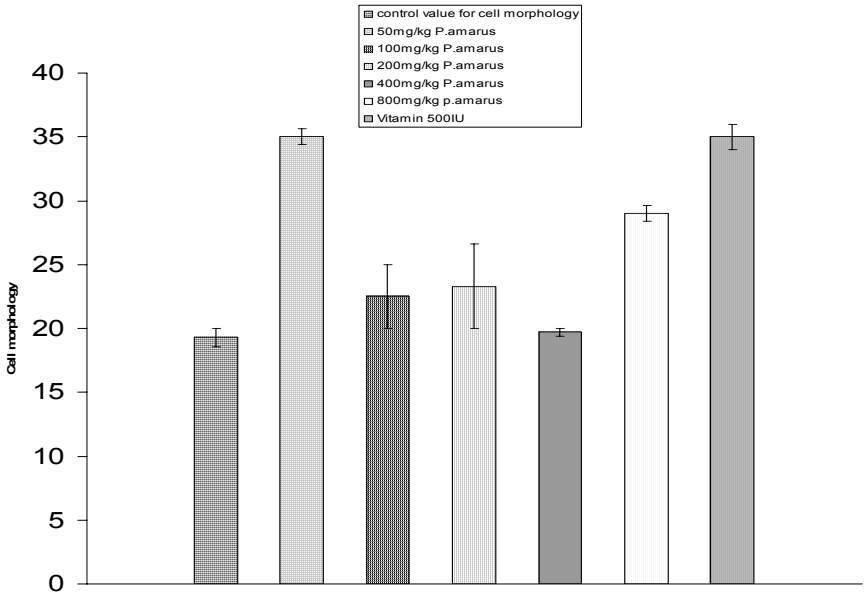

Fig 3: The dose- dependent comparative effect of *P. amarus* and Vitamin E on the cell morphology of male guinea pigs.

Table1: The time-dependent comparative effects of *Phyllanthus amarus* (800mg/kg) andVitamin E (500IU) on sperm parameters of male guinea pigs.

Group/Treatment	Motility quality	Debris quality	Sperm count	Morphology of cells	Primordial cells	Duration (days)
Control	65.0±2.90	18.7± 0.70	66.7±3.30	19.3±0.70	19.0±0.58	7
Vit E (500IU)	* 52.5±2.50	40.0±0.50	*62.5±2.50	35.0±0.02	31.0±0.10	7
P.a (800mg/kg)	*56.7±0.80	26.7±1.70	*48.3±1.70	29.0±0.58	28.6±0.67	7
Vit E (500IU)	* 43.8±9.40	26.3±2.40	*46.3±0.12	22.0±1.00	21.3±0.95	*14*
P.a (800mg/kg)	*#26.7*±3.30	43.3±1.70	# 20.3±0.33	29.7±4.40	29.7±4.40	14
Vit E (500IU)	*77.5±2.50	20.0±0.01	* 72.5±2.50	25.0±5.0	27.5±5.50	21
P.a (800mg/kg)	*50.0±0.01	40.0±0.02	# 23.5±1.50	30.0±0.01	31.0±1.00	21
Vit E (500IU)	* 56.7±3.30	27.3±1.50	*48.3±1.70	28.7±0.70	26.7±1.70	28
P.a (800mg/kg)	*47.5±2.50	30.0±0.01	*47.5±2.50	*30.0*±0.02	35.0±5.00	28

Values are expressed as mean ± SEM of five observations (n = 5).
* represents significant values at P<0.05 and # significant values at P<0.001 (ANOVA).
P.a represents *Phyllanthus amaru* leaf extracts.

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Table 2: Phytochemiacal Screening

Phytochemicals	*P.amarus*
Alkaloids	+
Flavonoids	+
Terpenoids	+
Saponins	+
Tannins	+
steriod	+
Resins	-
Cardiac glycosides	+

+ = present
- = absent

This study has shown that the methanol extract of the leaf of *Phyllanthus amarus* (50-800mg/kg) caused statistically significant decreases on the sperm parameters of male guinea pigs (fig.1, 2 and 3; table 2). The decreases in the sperm count, motility and cell morphology were less pronounced at higher doses of the *P. amarus* extract. These effects were similar and very comparable to the observed effects of vitamin E (500IU) on sperm parameters of male guinea pigs (Obianime and Uche in-press). These effects were also dose-dependent and significant at $P < 0.05$ (ANOVA).

In the time-dependent study, the observed effect of *P. amarus* on sperm count, motility and cell morphology were similar to that of vitamin E on the 28^{th} day of treatment with the extract. The values were also statistically significant at $P < 0.05$ (ANOVA). This implies that *P. amarus* may have similar effects, properties and mechanisms of actions with vitamin E, which is a known fertility agent and an antioxidant (Uche and Obianime, 2008) with protein kinase C (PKC) inhibition as one of its mechanisms of actions (Uche and Obianime, 2008).

The *P. amarus* which is claimed by traditional medical practitioners to possess fertility properties has also been reported to have antioxidant activity (Adeneye et al., 2006).

Therefore, its antioxidant activity property may contribute to its indirect beneficial effect as a fertility agent (Obianime and Uche, in- press).

This study also shows that P. amarus and vitamin E do not have direct positive effect on sperm parameters at 50-800mg/kg and 500IU respectively. However, since Vitamin E and P. amarus are known to be fertility agents, their beneficial effects on reproductive system could be through positive hormonal effect (Obianime and Uche in – press) or due to their antioxidant activity (Adeneye et at., 2006; Obianime and Uche, in-press).

The phytochemical screening of the extract of the leaves of *P.amarus* revealed the presence of flavonoids, tannins, alkaloids, steroids, terpenoids, saponins and glycosides.

Flavonoids are antioxidants (Okwu and Josiah, 2006) and this may contribute to antioxidant activity of this plant (Adeneye et al., 2006), hence its similar effect with vitamin E which is also a powerful antioxidant. The steroids in this plant may be responsible for its positive effect on the hormone (testosterone) of male guinea pigs (Obianime and Uche in-press). This may be so because steroids are precursors in the synthesis of hormones.

In conclusion, this study shows that the leaves of P.amarus do not significantly contribute directly to the claim by traditional medical practitioner on the use of the aerial part of this plant to increase libido and as a fertility agent. Although it may have some indirect beneficial effect to this claim; such as antioxidant activity or hormone booster effect which mimics vitamin E in both effects and mechanism of action. However, while further studies are on the way to investigate the effects of the seeds of P. amarus on male reproductive organ of guinea pigs, there is need for further study to isolate, identify and characterize the active principles in the leaf extract of *P. amarus*.

REFERENCE

Adeneye, A.A; Benebo, A.S; Agbaje, E.O (2006). Protective effect of the Aqueous Leaf and seed Extract of Phyllanthus amarus on Alcohol – induced hepatotoxity in rats. West Afr. J. Pharmacol. Drug Res. 22&23 :42-50

Adeneye A. A; Amole, O.O; Adeneye, A. K (2006). The hypoglycemic and hypocholesterolemic activities of the aqueous leaf and seed extracts of Phyllanthus amarus in mice. Fitoterapia. 77: 511-514.

Iwu, M.M (1993). Modalities of drug administration. In : Hand book of African Medicinal Plants. CRC Press Inc. Florida. 309- 330.

Joy, K.L; Kuttan, R (1998) Inhibition by Phyllanthus amarus of hepatocarcinogenesis induced by N-Nitrosodiethylamine. J. Bioch. Nutr. 24: 133-139.

Kassuya, C.A; Silerstre, A.A; Rehder, V; Calixto J.B (2003). Anti allodynic and antioedematogeni properties of the lignan from Phyllanthus amarus in models of persistent inflammatory and neuropathic pain Eur. J Pharm . 478: 145-153.

Kokwaro, J.O (1976). Medicinal Plants of East Africa Literature Bureau. 95

* Corresponding author: [1]Obianime, A.W.

Meixa, W; Haowei, C ; Yanji, L (1995). Herbs of the genus phyllanthus amarus in the treatment of chronic hepatitis B: Observation with three preparations from different geographical sites. J. Lab. Clin. Med.126:350-352

Odebedy O and Sofowora (1978). Phytochemical screening of Nigerian medicinal plants Lloydia 41: 41-234.

Odetola, A.A; Akkojenu S.M (2000). Antidiarrhoeal and gastrointestinal potentials of the aqueous extracts of Phyllanthus amarus (Euphorbiaceae). Afri. J.Med. Sci.29: 119-122.

Sofowora, A (1993). Medicinal Plants and Traditional Medicine in Africa. Spetrum books.

Uche, F. I; Obianime A.W (2008). The Effects of Vitamin E on Vanadium Pentoxide toxicity in Biochemical Parameters of Male Guinea Pigs. J. of Applied Sci. and Environmental Management. Vol 12 (3); 101-105.

Obianime A.W; Uche F. I . The Phytochemical screening and the effects of methanolic extract of the leaf of *Phyllanthus amarus* on hormonal parameters of male guinea pigs in- press.

Okwu, D.E; Josiah, C (2006).Evaluation of the chemical composition of two

Nigerian Medicinal plants. African journal of Biotechnolgy, 5 (4) : 357-361.

Thyagarajan S.P; Subramanian, S; Thirunalasundar, T (1988). Effects of Phyllanthus amarus on the chronic carriers of hepatitis B virus. Lancet 2: 764-766.

Trease, G.E; Evans W.C (1989). Trease and Evans Pharmacognosy. A physician guide to Herbal medicine 13th edition: Ballere tindal London

* Corresponding author: [1]Obianime, A.W.