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Modifications in sperm quality of Wister Albino Rats by Ethanol Extract of *Phyllanthus amarus* (Schum. and Thonn)

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Abstract
The effect of a well known herb, *Phyllanthus amarus* on the sperm characteristics in male albino rats was studied. This was an investigation of the age-long claim by the locales in the rural communities in the southern states of Nigeria where this plant is consumed religiously that it affects sperm quality, hence sexual potency in males. Ethanol extract of *P.amarus* in graded doses of 70mg/kg, 140mg/kg and 210mg/kg BW were administered by oral gavage to the experimental animals for 14 days. Epididymal sperm was collected and analyzed using standard procedures. Sperm analyses involved sperm count, sperm morphology test and sperm motility test. At the doses administered, *P. amarus* extract affected the sperm number, morphology and motility of treated animals. Epididymal sperm count and motility were significantly reduced ($P<0.05$). Compared to the control mean sperm count of 6.39 x 10^6, groups II, III and IV rats had mean sperm counts of 4.56 x 10^6, 3.67 x 10^6 and 2.5 x 10^6 respectively. Sperm motility scores were 75.0%, 71.67%, 66.67% and 50.0% for group I (control), groups II ,III and IV respectively. Modified sperm morphologies were observed. The present investigation shows that at high doses, ethanol extract of *P. amarus* modifies sperm characteristic qualities in albino rats thus altering the reproductive functioning of the sperm cells, conferring antifertility properties on the rats. This proves that the claim that *P. amarus* affects sexual potency in man may be true. Further research in this regard is recommended.

KEYWORDS: Sperm count, sperm motility, sperm morphology, *Phyllanthus amarus*
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Introduction

Phyllanthus amarus, the miracle plant, as it is fondly called in the northern parts of Cross River State is locally used as a cure for most ailments. The whole plant is usually soaked in hot water to make an infusion or tea and sometimes it is taken as an enema for the treatment of various ailments including jaundice, gonorrhea, diabetes, hepatitis and malaria (Rao and Alice, 2001). It is believed by the people that consume the herb that *P. amarus* reduces sperm quality and hence affect sexual potency in men. This study was thus conducted to investigate the modifications, and the extent to which this claim is true.

PLATE 1: *Phyllanthus amarus* (Source: Field work, 2010)

Materials and Methods

Twelve sexually matured male albino rats (*Rattus norvegicus*) of the wister strain were fed graded doses of ethanol extract of *P. amarus* by oral gavage. The animals were housed in four plastic cages in groups of three rats/cage. The cages were labelled A, B , C and D corresponding to the feeding regime with the following doses 0mg/kg, 70mg/kg, 140mg/kg and 210mg/kg respectively. The control group (group A) were given distilled water. Administration was done for 14 days. At the end of this period, the rats were anesthetized and dissected. Semen from the epidydymis was expelled and diluted with physiological saline. This suspension was placed on microscopic glass slides for further analyses. Sperm analyses involved sperm count, sperm morphology test and sperm motility tests. The Neubers' haemocytometer was used for the sperm count(Zaneveld and Polakoski,1977); sperm motility test was done using a wet mount((Rouge, 2004) while the sperm morphology test was done by staining air-dried and fixed smears with haematoxylin/eosin stain (Verma *et al.,* 2006).

The completely randomized design (CRD), with a one-way ANOVA, was used for the analysis of data. Means were separated using Least Significance Difference (LSD) test. All evaluations were done with the aid of the Genstat (7.2) statistical package.

Results

Results were obtained by viewing slides under a compound microscope at high magnifications. Photomicrographs were obtained using a microscopic digital camera. Table 1 shows the sperm analyses results after 14 days administration of the plant extract. Sperm count results were significantly ($P< 0.05$) different and dose- dependent. Sperm motility reduced significantly ($P< 0.05$) with increase in dosage of administered herb extract. Modified sperm morphologies were more at higher doses than at the lower dose. Plate 1 shows a normal rat sperm morphology, while Plates 2-4 show some of the modified sperm morphologies as a result of the administered extract.

Table1. Sperm analyses of rats treated with ethanol extract of *Phyllanthus amarus*

	0	70	140	210
Sperm count (x 10^6/ml)	$6.39^{bc} \pm 0.98$	$4.56^{b} \pm 0.12$	$3.67^{ab} \pm 1.36$	$2.5^{a} \pm 0.00$
Sperm motility (%)	$75.0^{abc} \pm 13.23$	$71.67^{ab} \pm 7.64$	$66.67^{a} \pm 5.77$	$50.0^{a} \pm 4.0$
Abnormal Sperm Morphology (%)	$4.0^{a} \pm 1.7$	$5.0^{a} \pm 0.0$	$6.67^{a} \pm 2.8$	$8.5^{ab} \pm 2.12$

^{abc} Means with different superscripts are significantly different ($P<0.05$). Source: Field work (2010)

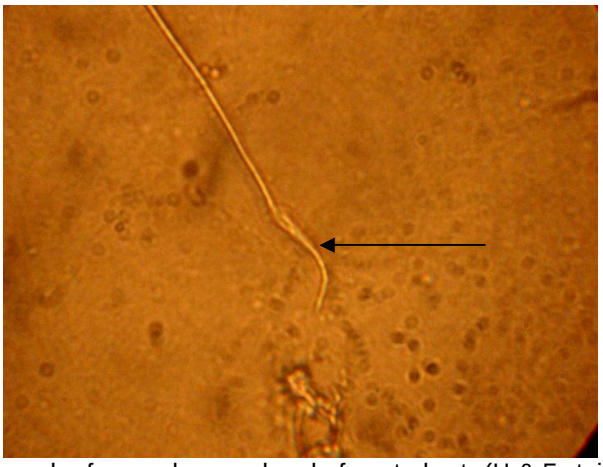

PLATE 1: Photomicrograph of normal sperm head of control rat. (H & E stain)(X 40)

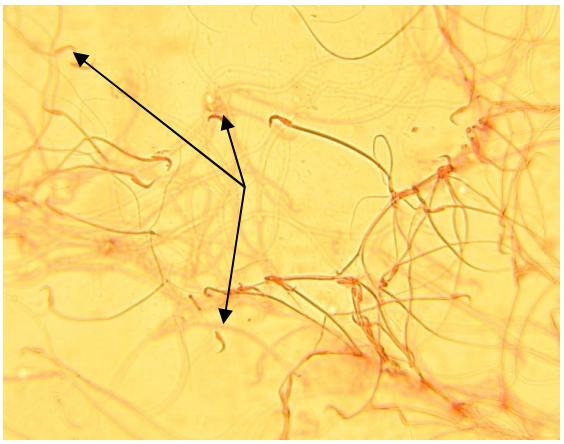

PLATE 2: Photomicrograph of detached sperm heads of rat treated with 70mg/kg of *P.amarus* (X 100)

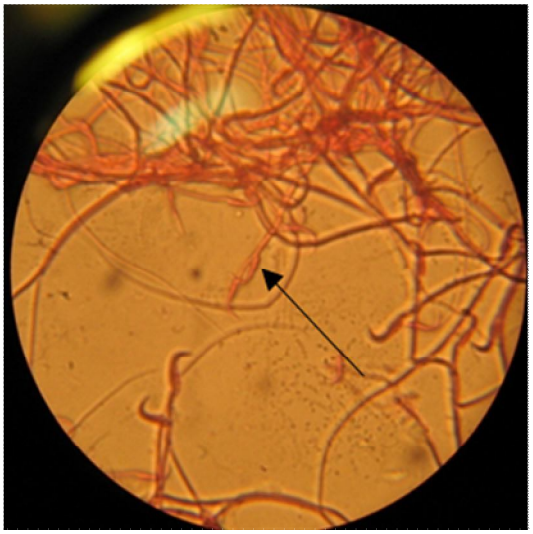

PLATE 3: Photomicrograph of rat treated with 140 mg/kg of *P.amarus* showing fusion sperm (two fused sperm heads). (X 100)

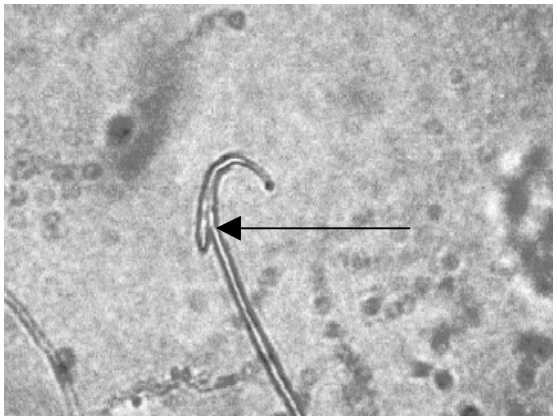

PLATE 4: Photomicrograph of rat fed with 210mg/kg *P. amarus* showing sperm with mis-shappen head (Hook at wrong angle)(X 40)

Discussion

Sperm tests provide a direct measure of the quality of sperm produced in chemically treated animals. According to Sarathchandiran *et al.* (2007), lack of motility, decrease sperm count, incident of sperm abnormalities strongly point to spermatotoxic effect of any herb extract via epididymis. Sperm

counts observed in treated animals decreased in a dose-dependent way. The control rat had the highest sperm count while the highest extract dose administered group, 210mg/kg, had the l The ANOVA results for sperm cell count in *P. amarus* treated male rats were significant ($P<0.05$) indicating that the ethanol extract of *P. amarus* affected the sperm cell counts differently. This could be attributed to the sperm entering the epididymis in a diluted form caused by lowered estrogen levels in the epididymis. These findings were similar to the report made by Ufearo *et al.* (1996),that low sperm count in rat's semen, was always responsible for infertility in the rats. Again, results obtained by Verma *et al.* (2006) on the toxicology of *Carica papaya* seed extracts in mice were similar to the results in this study. Gossypol acetic acid (GAA) reduced the sperm cell count in rats at the doses administered (Hadley *et al.*, 1981; Udoh *et al.*, 1992). Odeigah (1997) had similar results on the sperm count from formaldehyde administration in albino rats. The sperm morphology test for the *P.amarus* treated rats showed detached heads, fusion sperm, and mis - shappened heads with hook at wrong angle, at higher doses. These modified sperm morphologies agree with results observed by Edelman and Clarke, 1974 and Aduloju *et al.*, 2008 in two independent studies. These observed abnormalities could be due to the induction of point mutations in the early spermatocytes and spermatogonia at the premeiotic stages of spermatogenesis (Sarathchandiran *et al.*, 2007). The breaking away of head from flagellum of the sperm appears to occur due to impact of active constituents of *P.amarus* at the neck or connecting piece of flagellum (Yong and Cooper, 1983). Thus, it could be that a component in the *P.amarus* ethanolic extract, distrupts the protein and tubillin present in this region, causing the breaking away of the head from flagellum. It is reasonable to speculate that an active compound in the ethanol extract of *P.amarus* gained access into the epididymis and altered the epididymis in respect of its function towards initiation of spermatogenesis. Lack of motility, decrease sperm count, increase incident of sperm abnormalities strongly point to a spermatotoxic effect of *P. amarus* via epididymis (Sarathchandiran *et al.,*2007). The head malformations of the sperm, in particular, suggest some biochemical changes in the sperm surface and strongly indicates change in the genetic disposition of the sperm cell.

The arbitrary use and consumption of these herbs should also be curtailed as it is now established that, depending on the quantity consumed, these herbs can be deleterious in their effects especially in the male mammal. On the other hand, the antifertility property of this herb can be harnessed as a tool in developing natural, safe and affordable male contraceptives to help control child spacing and family size especially among the rural dwellers who tend to be more predisposed to consuming the herbs. Proper standardization of these extracts will also protect our people who consume this herb, for the treatment of several other ailments like malaria, Kidney stones and Diabetes.

References

Aduloju, R.K., Otubanjo, O. A. and Odeigah, P. G. C. (2008). An invivo assay of the mutagenic potential of praziquantel (PZQ) using sperm head abnormality test. *Journal of human Ecology*, *23* (1): 59-63.

Hadley, M., Linn, Y. C. and Martin, D. Y. M. (1981). Effects of gossypol on the reproductive systems of male rats. *Journal of Andrology, 2155* (4): 190-199.

Rao, M. V. and Alice, K. M. (2001). Contraceptive effects of *Phyllanthus amarus* in female mice. *Phytotherapy Research, 15* (3): 265-267.

Rouge, M. (2004). Sperm morphology. In M. Rouge (ed.), Hypertexts for biomedical sciences. Colorado: Colorado State University Press, 33-36.

Sarathchandiran, I., Manalavan, R., Akbarsha, Z. M., Kadalmani, Z. B. and Karar, K. (2007). Studies on spermatotoxic effects of ethanol extract of Capparis aphylla (Roth). Journal of Biological Sciences, 7 (3): 544-548.

Udoh, P. B., Patil, D. R. and Deshpande, M. K. (1992). Histopathological and biochemical effects of gossypol on pituitary donodal axis of male albino rats. *Contraception, 45*: 493-509.

Udoh, P. and Ekpenyong, J. (2001). Effects of *Mucuna uren*s (horse eye bean) on the gonads of male guinea pigs. *Phytotherapy Research, 15*: 99-102.

Yong, R. J. and Cooper, G. W. (1983). Stabilization *in vitro* and *in vivo* of the spermatozoon head and tail to detachment induced by primary amine, thiol and detergent. *Genetic Research*, *7*: 222-288.

Zaneveld, L. J. D. and Polakoski, K. L. (1977). Collection and physical examination of the ejaculate. In E. S. E. Hafez (Ed.), *Techniques of human andrology.* Armsterdam: North Holland Biomedical Press, 147-156.