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Morphometric and Histopathological Studies on the Effects of Some Chromatographic Fractions of *Phyllanthus amarus* and *Euphorbia hirta* on the Male Reproductive Organs of Rats

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Abstract

The aqueous crude extracts of *P. amarus* and *E. hirta* were administered to thirty eight-week old sexually mature male albino to determine the effects of these extracts on the male reproductive organs of these animals. The results from this study revealed that the aqueous crude extracts of *P. amarus* and *E. hirta* caused varying degrees of testicular degeneration as well as reduction in the mean seminiferous tubular diameter (STD) in the treated rats. It thus shows that the aqueous crude extracts of *P. amarus* and *E. hirta* have potentially deleterious effects on the testes and accessory organs of rats. Great caution should therefore be exercised in the use of these plants for medicinal purpose.

Key words: morphometry, histopathology, *Phyllanthus amarus*, *E. hirta*, epididymis, testes

Introduction

Phyllanthus amarus is a herb, common from Sierra Leone to Southern Nigeria and Equatorial Guinea, and widespread elsewhere in tropical Africa. It is a plant of general medicinal application. *Phyllanthus amarus* possesses activity against Hepatitis B virus and related hepadnaviruses. This plant is known to have the inhibitory effect on endogenous hepadnavirus DNA polymerase [13, 16, 20, 28, 29, 30, 31]. Recent biochemical, pharmacological and clinical studies have confirmed and also extended the medicinal uses of species of genus *Phyllanthus* in traditional medicine [6, 18, 24]. Potent analgesic from the methanolic extracts of callus culture in-vitro obtained from *P. tenellus*, *P. corcovadensis*, and *P. niruri* (*amarus*) has been demonstrated [23].

On the other hand, *Euphorbia hirta* is a herb, decumbent or erect to 40 cm tall, occupying open waster spaces, a weed of cultivation, road-side, path-sides and a diversity of situations, occurring widespread throughout West Africa, and dispersed pan-tropically and sub-tropically around the world [7].

Euphorbia hirta contains relatively abundant white latex. The plant has a diuretic and purgative action. It is also known to have a remedy for inflammation of the respiratory tract, and for asthma it has a special reputation for causing bronchial relaxation. The plant shows antibiotic activity. A number of substances have been detected in the plant; tannins, gallic acid, quercetin, phenols, phyto-sterols, alcohols, and alkaloids, etc. [8, 14].

The 2 plants belong to the family Euphorbiaceae which is a large family, trees, shrubs and herbs, of rainforest, Guinean, Soudanian, and xereophylactic habitats. Most members of this family are poisonous. Some are economically important [7, 10]. The genera Euphorbia and Phyllanthus grow wild around Ibadan and in Nigeria generally where they are commonly used for fencing, as arrow poisons and in traditional medicine [11, 12].

This study was carried out morphologically to provide insight into the activities of these extracts on the testes and seminiferous tubules of rats, especially as it is thought that these plants are not known to be toxic [22, 26, 27].

Materials and Methods

Animals, groupings and experimental design

Thirty 8-week old sexually matured male albino rats, bred and maintained at the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan were used in this study. They were divided into 3 groups of 10 animals per group. Two groups correspond to 2 aqueous crude extracts of *P. amarus* and *E. hirta* while the 3rd group served as control for the study.

Preparation of the extracts of *P. amarus* and *E. hirta*

Fresh leaves of the 2 plants were collected within the

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campus of the University of Ibadan and were identified at the Department of Botany and Microbiology, University of Ibadan. 100 g each of the fresh leaves of the 2 plants were macerated in 500 ml of distilled water and filtered. The filtrate served as the stock solution.

Administration of the extracts

The 2 plant extracts were administered orally at a dose of 400 mg/kg body weight for 14 days using an oral canula. The animals in the control group received only distilled water.

Sample collection

The animals were exsanguinated and the testes and other accessory organs were removed as described [19].

Histological and Histopathological procedures

The samples collected were fixed in Bouins fluid and processed by the usual method for paraffin embedding and stained with haematoxylin and eosin (H&E) as described [1]. The slides of these organs were evaluated for pathological changes with light microscope.

Morphometry

The slides were examined under the microscope and seminiferous tubular diameter was taken. ten measurements were made per section using a calibrated eye-piece micrometer (Graticules Ltd., Toubridge, Kent). The means of the measurements of parameter in each section were recorded for each animal.

Statistical analysis

All data were expressed as means with standard error. The data were subjected to the pooled variance *t*-test for comparison and Duncan Multiple Range Test (DMRT) as described [25].

Results

Morphometry

Table 1 shows the DMRT for the means of seminiferous tubular diameter (U) for control rats as well as *E. hirta* and *P. amarus* treated rats. The results show general reduction in the mean seminiferous tubular diameter (STD) in the treated groups compared with the control group. However, there was no significant difference between the means of seminiferous tubular diameter of the control and *E. hirta* treated rats. On the other hand, the mean STD in rats treated with the extract of *P. amarus* differed significantly ($p<0.05$) from that of the control rats. *P. amarus* treated rats had lower ($p<0.05$) STD (234.41+6.08 ㎛) than the control rats (265.2 +8.51㎛). It is also noteworthy that the mean STD in *E. hirta* treated rats differed significantly ($p<0.05$) from the *P. amarus* treated rats.

Table 1. Means of groups for the means of seminiferous tubular diameter (U) for control, *E. hirta* and *P. amarus* test rats (n = 10)

Parameter	Control	*E. hirta*	*P. amarus*
Seminiferous tubular diameter(㎛)	265.20+8.51[a]	264.0+10.17[a]	231.41+6.08[b]

Means with different superscripts within the row are significantly different at $P<0.05$
Means are expressed as means + S.E.
DMRT= Duncan multiple range test.

Histopathology

Rats treated with the fresh leaf extracts of *E. hirta* and *P. amarus* showed varying degree of testicular degeneration (Figs. 1-7) when compared with testicular tissue from the control rats (Fig. 8).

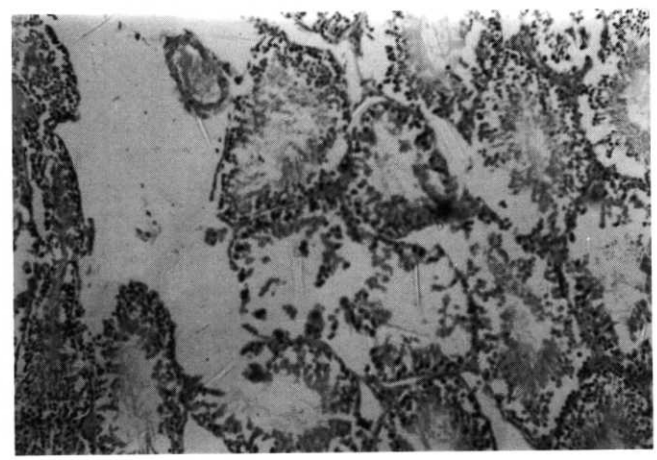

Fig. 1. Effect of *P. amarus* on the testes of rats showing testicular degeneration (×250).

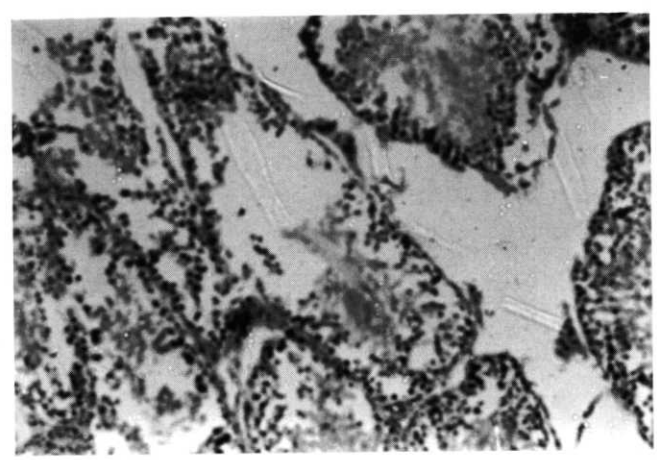

Fig. 2. Effect of *P. amarus* on the testes of rats showing testicular degeneration (×400).

Fig. 3. Effect of *P. amarus* on the testes of rats showing mild testicular degeneration with desquamation of the tubules (×250).

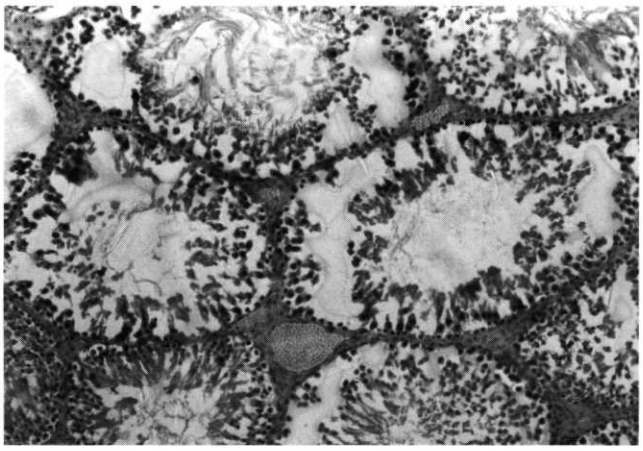

Fig. 4. Effect of *P. amarus* on the testes of rats showing mild testicular degeneration with desquamation of the tubules (×400).

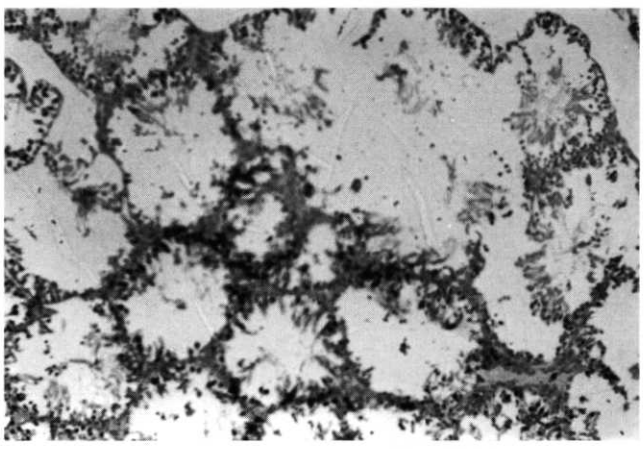

Fig. 5. Effect of *P. amarus* on the testes of rats showing severe testicular degeneration (×250).

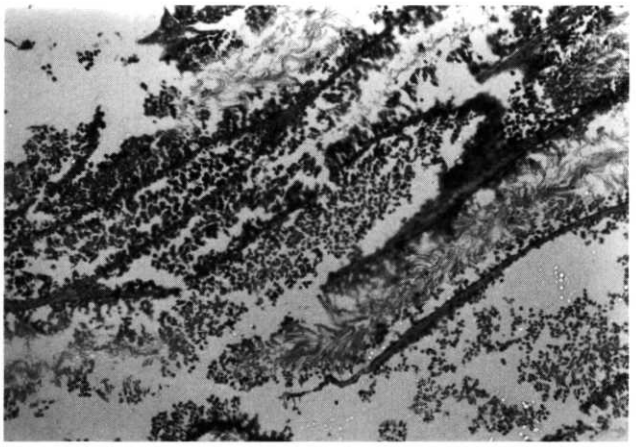

Fig. 6. Effect of *E. hirta* on the testes of rats showing mild testicular degeneration (×250).

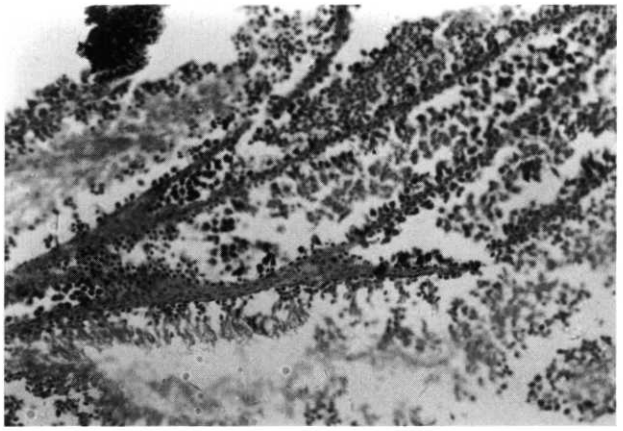

Fig. 7. Effect of *E. hirta* on the testes of rats showing mild testicular degeneration (×400).

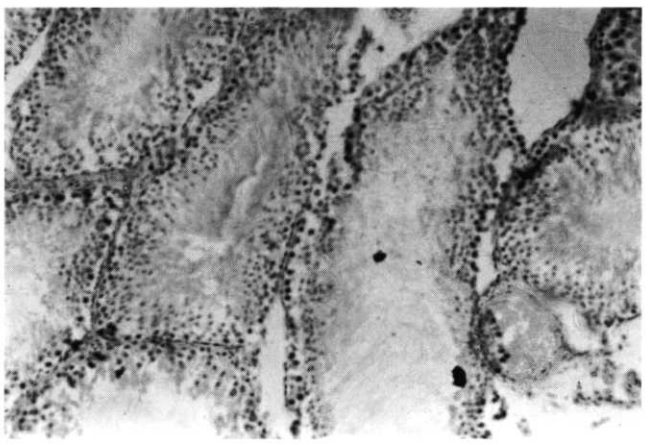

Fig. 8. Normal testis of rats.(control).

Note: Figs. 1 & 2 are from the same animal.
Figs. 3 & 4 are from the same animal.
Figs. 6 & 7 are from the same animal.

DISCUSSION

E. hirta and *P. amarus* have been reported to have numerous medicinal and economically important properties. However, they belong to the family Euphorbiaceae which is a large family of trees, shrubs and herbs which are said to be poisonous [7, 10, 11, 12].

In this study, the histopathology showed that the 2 plants caused varying degrees of testicular lesions which was more severe with the extract of *P. amarus* than that of *E. hirta*. In addition, significant reductions were observed in the seminiferous tubular diameter of the testes of treated rats (especially *P. amarus*) in comparison with the control rats.

The pattern of cellular damage observed in this study is consistent with the effects of phoxim [4], *Oestradiol valerate* [15] and *Curcuma comosa* extract [21]. The histological changes observed in the testes of rats in this study may be due to the presence of cardiac glycoside in *E. hirta* [5] and *P. amarus* [17] which was incriminated in pathological and ultra structural changes in the kidney tubules of Wistar rats [3]. As the interstitium was observed to be devoid of Leydig cells, the histological changes observed may also be due to decreased production of testosterone known to be responsible for normal testicular architecture [9].

The pattern of effects of *P. amarus* extract on the STD observed in this study is similar with the effect of *Calotropis procera* on the male reproductive organs of rats [2]. The general reduction observed in the STD in the treated rats in comparison with the control suggests that seminiferous tubular volume is lower in the treated rats than in the control rats. This might invariably affect the sperm volume negatively. *P. amarus* extract was however shown to cause reduction of STD more than the extract of *E. hirta*.

This study has therefore shown that these plants, *P. amarus* and *E. hirta* have potential toxic effects on animals hence caution should be exercised in their use as medicinal agents. On the other hand, in the quest for the search for male contraceptive more research light may be focused on *P. amarus* in order to discover its full potential. This might mean that appropriate dose that will not expose the testes to toxic injury should be determined. Further searchlight should be shed on the effect of these plants on the testosterone.

References

1. **Akinloye, A. K., Igharha, O. O., Olaniyi, M. O., Alaka, O. O. and Oke, B. O.** Preliminary investigation on the effects of bitter kola (*Garcinia kola*) extract on rabbit testes and epidydymis. Trop. Vet. 2000, **18(1 & 2)**, 49-54.
2. **Akinloye, A. K., Abatan, M. O., Alaka, O. O. and Oke, B. O.** Histomorphometric and histopathological studies on the effect of *Calotropis procera* (Giant milk weed) on the reproductive organs of wistar rats. African Journal of Biomedical Research. 2001, **4**, 41-45.
3. **Al-Robai, A. A., Abo-Khatwa, A. N. and Jamal, Z. A.** Toxicological studies on the latex of usher plant *Calotropis procera* (Ait) in Saudi Arabia. V. Seasonal variation of total cardiac glycosides in the usher plant and in various tissues of the usher hopper, *Poekilocerous bufonius* Klug. Arab-Gulf J. Scientific Res. 1997, **16(1)**, 129-144.
4. **Atef, M. Noussef, S. A. H., Ramadam, A. Nawito, M. F., El-Sayed, M. K. and El-Rahman, H. A.** Influence of phoxim on testicular and seminal vesicle organs, testosterone and cholinesterase level and its tissue residues in male rats. Deutsche-Tierarzttliche Wochenschrift 1995, **102(8)**, 301-305.
5. **Blanc, P; Bertrand, P. de Saqui Sanner, G. and Ane, M.** Identification par chromatographie et etude spectral de quelque acides phenols, acides ellaqique, gallique, chologenique, cafeique dans une *Euphorbiaceous exotique*; *Euphorbia hirta*, L. Annales des Pharmacie Francaises 1972, **30**, 720-721.
6. **Blumberg, B. S., Millman, I. Venka teswaran, P. S. and Thyagarajan, S. P.** Hepatitis B virus and hepatocellular carcinoma-treatment of HBV carries with *Phyllanthus amarus*. Cancer Detect Prevent. 1989, **1**, 195-201.
7. **Burkill, H. M.** The useful plants of West Tropical Africa. Royal Botanical Gardens, Kew. 1994, **2**, 21-150.
8. **Dalziel, J. M.** The useful plants of West Tropical Africa. Crown Agents for the Colonies, London, 1937.
9. **Elk-Nes, K. B.** Synthesis and secretion of androstetiedione and testosterone. *In* the Androgens of the Testis K.B. Eik-Nes, (ed), London, pp. 1-11.
10. **Garner, R. J.** Veterinary Toxicology. 1st ed. Balliere Tindale, London. 1957, 328-332.
11. **Hartwell, J. L.** Plants used against cancer; a survey, Lloydia 1969, **32**, 153-205.
12. **Hutchinson, J. and Dalziel, J. M.** Flora of West Tropical Africa. Vol. I, Part 1. 2nd ed. Crown Agents, London, pp. 1-11, 1954.
13. **Irvine, F. R.** Plants of the Gold Coast. Oxford University Press, London, 1930, pp. 29-35.
14. **Kerharo, J. and Adam, J. G.** La Pharmacopie Senegalese traditionelle. Plants medicinales et toxique, Vigot Freres, Paris, 1974, pp. 9-12.
15. **Kohler-Samouilidis, G., Papioannou, N., Kotsaki-Kovatsi, V. P. and Vadarakis, A.** The effect of oestradiol valerate on the male reproductive organs and different semen parameters. Frats. Berlinerund Munchener. Tirear/ Ztlich-Wochenscrift. 1998, **111(1)**, 1-17.
16. **Morton, J. F.** In: Thomas, C. C. (ed.) Atlas of Medicinal Plants in Middle America. 1st ed. Springfield, 1981, pp. 458-462.
17. **Odetola, A. A. and Akojenu, S. M.** Antidiarrhoeal and gastro-intestinal potentials of the aqueous extract of *Phyllanthus amarus* (Euphorbiaceae). Afr. J. Med. ed.

Sci. 2000, **29**, 119-122.

18. **Ogata, T., Higuchi, H., Mochida, S. Matsumoto, H., Kato, A., Endo, T., Kaji A. and Kaji, H.** HIV-1 reverse transcriptase inhibitor from *Phyllanthus niruri*. AIDS Research Human Retroviruses, 1992, **8**, 1937-1944.
19. **Oke, B.O.** Some aspects of the reproductive biology of the male African giant rat (*Cricetomys gambianus*). PhD Thesis University of Ibadan. 1988.
20. **Oliver-Bever, B.** Medicinal plants in tropical West African III. Anti-infection therapy with higher plants. J. Ethnopharmacol. 1983, **9**, 1-83.
21. **Piyacchaturawati, P., Timinkul, A. and Suksamran, A.** Growth suppressing effect of *Curcuma comosa* extract on male reproductive organs. Reproductive Biology 1998, **36(1)**, 44-49.
22. Raphael, K.R. Anti-mutagenic activity of *Phyllanthus amarus* [Schum & Thonn] *in vitro* as well as *in vivo*. Teratog. Carcinog. Mutagen. 2002, **22(4)**, 285-291.
23. **Santos, A. R. S. Filho, V. C., Niero, R., Viana, A. M., Moreno, F. N., Campos, M. M., Yunes, R. A. and Calixto, J. B.** Analgesic effects of callus cultured extracts from selected species of phyllanthus in mice. J. Pharm. Pharmacol. 1994, **46**, 755-759.
24. **Shead A., Vickery, K., Pajkos, A., Medhurst, R. J., Dixon, R. and Cozzart, T.** Effects of Freiman phyllanthus plant extracts on duck hepatitis B *in vitro* and *in vivo*. Antiviral Res. 1992, **18**, 127-138.
25. **Steel, R. G. D. and Torrie, J. I. I.** Principles and procedure of Statistics. A Biometric Approach. 2nd ed. McGraw Hill, New York, 1986.
26. **Taylor, L.** Herbal secrets of the rain forests. 2nd edition. 2002.
27. **Thyagarajan, S. P., Sudramanian, S. and Thirunalasundar, T.** Effect of *Phyllanthus amarus* on chronic carriers of hepatitis B virus. Lancet 1988, **2(8614)**, 764-766.
28. **Unander, D. W., Webster, G. L. and Blumberg, B. S.** Records of usage or assays in Phyllanthus (Euphorbiaceae) I. Subgenera Isocladus, Kirganelia, Cica and Emblica. J. Ethnopharmacol. 1990, **30**, 233.
29. **Unander, D. W., Webster, G. L. and Blumberg, B. S.** Uses and bioassays in phyllanthus (Euphorbiaceae): a compilation II. The subgenus phyllanthus. J. Ethnopharmacol. 1991, **34**, 97-133.
30. **Unander, D. W., Webster, G. L. and Blumberg, B. S.** Usage and bioassays in phyllanthus (Euphorbiaceae): a compilation III. The subgenera Eriococcus, Corami, Gomphidium, Botryanthys, Xylophylla and Phyllanthodendron, and a complete list of the species cited in the three part series. J. Ethnopharmacol. 1992, **36**, 103-112.
31. **Unander, D. W.; Bryan, H. H.; Larce, C. J. and McMillan, R. T. (Jr.).** Cultivation of *P. amarus* and evaluation of variables potentially affecting yield and the inhibitors of viral DNA polymerase. Economic Botany 1993, **47(1)**, 79-88.