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

A. A. Adedapo, M. O. Abatan, A. K. Akinloye, S. O. Idowu, and O. O. Olorunsogo

Departments of Veterinary Physiology and Pharmacology, 1Veterinary Anatomy, 2Pharmaceutical Chemistry, and 3Biochemistry, University of Ibadan, Ibadan, Nigeria

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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. corocadensis*, 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 wasteland 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 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, Soudarian, and xerophytic 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
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 cannula. 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 Bouin's 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 eyepiece micrometer (Graticules Ltd., Toubert, 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 (μm) 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+6.09) μm) than the control rats (235.2+8.51) μm). 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 (μm) for control, *E. hirta* and *P. amarus* test rats (n = 10)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th><em>E. hirta</em></th>
<th><em>P. amarus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Seminiferous tubular diameter(μm)</td>
<td>235.2+8.51°</td>
<td>234.0+10.17°</td>
<td>283.41+6.09°</td>
</tr>
</tbody>
</table>

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](image1.png)

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

![Fig. 2](image2.png)

**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


