

EVALUATION OF ANTIFERTILITY EFFECTS OF AQUEOUS EXTRACT OF PHYLLANTHUS AMARUS IN MALE WISTAR RATS

ABSTRACT

The present study was aimed at evaluating the antifertility effect of phyllanthus amarus extract in male wistar rats. The body and organ weights, cauda epididymal sperm parameters, serum levels of male reproductive hormones and fructose were assessed. Daily oral doses of the extract were administered to each animal in three different test groups for 28 consecutive days. Results show significant ($p < 0.05$) dose dependent decrease in the levels of testosterone, leuteinising hormone, sperm motility, sperm count and sperm viability of the treated animals when compared with the control; while follicle stimulating hormone significantly ($p < 0.05$) increased dose dependently. These findings suggest that the aqueous crude extract of phyllanthus amarus has anti-fertility activity.

Key words: antifertility, phyllanthus amarus, extract, reproductive hormones, sperm parameters.

INTRODUCTION

Phyllanthus amarus (kidney stone leaf), is a member of the Phyllanthus genus of the Euphorbiaceae family with over 300 genus and more than 1,500 species. P. amaratus is a small annual plant that grows 30-40cm in height and is found throughout the tropics and subtropics. It is a broad spectrum medicinal plant that has received worldwide recognition (Srividiya et al 1995).

Phytochemical analyses revealed that over 15 compounds was isolated from Phyllanthus amarus leaf products, including alkaloids, phenols, flavonoids, lignans, saponins, tannins, terpenes, resins, styeroid, carotenoids and essential oils, fatty acids (Sharma et al, 1993). The active ingredients in Phyllanthus amarus include the lignans, phyllanthine, phyllanthenol, phylochysine, phylltetralin and hypophyllathine (Thyagarajan et al, 1998).

Phyllanthus amarus has various uses in herbal medicine systems. It has reportedly been used traditionally to treat jaundice, diabetes, diarrhea, swelling, skin ulcer, gastrointestinal disturbances and blocks DNA, polymerase in the case of hepatitis B virus during reproduction (Oluwafemi and Debiri, 2008).

Earlier studies have greatly demonstrated its anti-oxidant activity, liver protective, anti-inflammatory, chemoprotective, hypolipidaemic, analgesic, hypotensive, antispasmodic, antimutagenic and hypoglycemic activities (Roa and Alice, 2001).

Furthermore, scientific investigation on the medicinal properties of plant extracts show that several plant extracts are used to alter different reproductive functions such as facilitation of parturition (Bacteria, 1994), inhibition of uterine contractio (Arcola and Munenge, 1998, Silver et al, 2000) for alleviation of menstrual cramps (Wenger et al, 1982. Halberstein, 2005).

There have been reports of decrease in male fertility potentials after treatment of animal with anti-malaria drugs resulting from impairments in sperm motility and viability (Nwanjo et al 2007). The impairment of male fertility has been reported with chloroquine and halofantrine treated rats (Adeeko and Daada 1998; Orisakwe et al 2003). Similar antifertility effects have also been reported in herbs that have antimalarial activity such as Alstonia boonei and Azaridichta indica (Oze et al.,2007; Raji et al., 2003). Phyllanthus amarus is greatly used in Asia and Africa for the treatment of malaria and studies have shown that it has anti-malaria properties (Adeneye, 2006; Aarthi, 2011).

However, there are scanty definite reports on the effect of Phyllanthus amarus on the male reproductive parameter of animals, hence the

purpose of this study. Results obtained from this study will be useful for researchers, medical practitioners and the general public on the arbitrary use of the plant.

MATERIALS AND METHODS

Preparation of Extract

The leaves of *Phyllanthus amarus* were collected from the local garden within the premises of University of Port Harcourt in November, 2012. The plant was identified and authenticated by a taxonomist in the department of Plant Science and Biotechnology (PSB), University of Port Harcourt. Voucher specimen was maintained at the herbarium. The fresh plant collected was washed and air-dried for 10 days until constant weight was recorded. The dried leaves were ground using a manual grinder. 100g of the grinded leaves of *Phyllanthus amarus* was thoroughly soaked in 600ml of distilled water in a beaker. The mixture was shaken and allowed to stand for 24hours before filtering with cheese cloth. The filtrate was evaporated using an oven at 50-60⁰c to produce the dry extract. An appropriate weight of the extract was dissolved in distilled water to obtain the various concentrations used for the experiment.

Experimental design

Male albino wister rats, weighing 180-200g, bred from the animal house of the department of Human Physiology, University of Port Harcourt were used for the study. The rats were maintained under hygienic conditions in well ventilated room and were fed with pelleted food; drinking water was available ad libitum. The rats were randomly categorised into four experimental groups of 12 animals each:

Group A (control): administered feed and water only

Group B: 150mg/kg/rat extract + feed and water

Group C: 250mg/kg/rat extract + feed and water

Group D: 350mg/kg/rat extract + feed and water

Daily oral dose of the extract in 2ml distilled water were administered to each animal in the different groups using oro-gastric canula, for a period of 28 consecutive days.

Body and Reproductive Organ Weight

Body weight of control and experimental animals were weighed at the beginning of the study and on the day of sacrifice. 24 hrs after the last dose of the extract, the rats were sacrificed by cervical dislocation. Testes and epididymis were dissected out, and cleared of fat and connective tissue. The testis were removed and weighed.

Hormonal Assay

After the animals were sacrificed 4ml of blood was collected by cardiac puncture. The blood sample was centrifuged at 2500rpm for 10min and serum used for testosterone(T), leutinizing hormone (LH) and follicle stimulating hormone (FSH) assay by the enzyme linked immunoassay technique.

Sperm Characteristics

Sperm analysis was performed on samples derived from the caudal epididymis. The distal cauda epididymides were minced with scissors to release the epididymal content into a Petri dish containing 2 mL phosphate buffer (0.1 M, pH 7.4). The sperm parameters were determined with a Neubauer Improved Haemocytometer as described for sperm count [Wang et al.1995] and motility [Lue et al.,1998]. Sperm viability was performed by the eosin nigrosin staining (WHO, 1999).

Statistical analysis

The data from all the groups were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test using

SPSS statistical software [version 17], at confidence level of $p < 0.05$. The obtained data were expressed as mean \pm standard error of mean (SEM) of animals in each group.

RESULTS

The results obtained from this study are summarized in tables. Table 1 shows that 28 days treatment of rats with aqueous extract of *Phyllanthus amarus* resulted to an insignificant ($p > 0.05$) increase in the body weight and organ weight of test animals when compared with the animals in the control group.

The results of the effect of the extract of *Phyllanthus amarus* on the male reproductive hormones showed a dose dependent decrease in leutinizing hormone (LH) and testosterone levels in animals in test group B ($p > 0.05$), group C ($p < 0.05$) and group ($p < 0.05$), when compared with animals in the control group. On the other hand, there was a progressive dose dependent increase in the level of follicle stimulating hormone (FSH), in animals in all the extract treated groups: A ($P > 0.05$), B ($P < 0.05$) and C ($P < 0.05$), when compared with those in the control group.

The results of the effect of the extract on sperm characteristics, presented in Table 3, shows that here was significant decrease ($P < 0.05$) in the sperm motility, sperm counts and sperm viability of rats treated with the extract when compared with that of the control.

DISCUSSION

Increase or decrease in weight of an organ after administration of a chemical agent has been reported to be an indication of a possible toxic effect of such agent (Simon et al., 1995). The non significant difference on the body and testicular weight shows that the extract may not confer a general toxic effect on the treated animals. The findings of the present study also showed that the aqueous extract of the leaves of *phyllanthus amarus* significantly altered the serum leuteinising hormone, follicle stimulating hormone and testosterone levels, as well as impairing the sperm parameters in the test rats. High levels of follicles stimulating hormone in the extract treated rats, with reduction in leuteinising hormone and testosterone indicates primary testicular failure. The increase in follicle stimulating hormone secretion may be principally due to a feedback signal from damaged seminiferous tubules. It was reported that in many states of testicular damage, the sertoli cells are found to produce less inhibin but more activin, a follicle stimulating hormone releasing factor (Lee et al, 1975). Under such situation, follicle stimulating hormone released from the anterior pituitary gland increased significantly, as observed in this study. The significant ($P < 0.05$) decrease in testosterone, especially in the higher test doses, can be attributed to the decrease in leuteinising hormone. However, this finding disagrees with that of Obianime et al., (2008) who reported that the extract caused an increase in the level of testosterone in Guinea pigs. The difference in the reports may arise from differences in the study designs.

Leuteinising hormone stimulates the testicular leydig cells to secrete testosterone and increase testicular protein synthesis, which are needed for spermatogenesis and sperm maturation. Therefore, the observed impairments in sperm parameters could be attributed to the reductions in Leuteinising hormone and testosterone by the extract.

CONCLUSION

The result of this study has shown that the aqueous extract of *Phyllanthus amarus* significantly altered the levels of reproductive hormones and sperm characteristics of albino rats, and therefore their fertility. Therefore, local care givers and traditional medical practitioners need to be educated on the adverse effects of this herb.

Group/Dose	Initial body weight (g)	Final body weight (g)	Change in body weight (g)	Testis weight (g)
A (control)	189.36 ± 44.08	188.41±15.51	0.95 ± 29	0.92±0.6
B (150mg/kg)	189.91± 091	190.61± 16.16	1.52 ± 16	1.06± 0.1
C (250mg/kg)	189.91± 0.22	188.88± 21.01	1.03± 21	1.19± 09
D(350mg/kg)	185.50± 13.9	184.61± 62.12	0.89± 48	1.31± 13

Table1: Effect of *Phyllanthus amarus* extract on body and organ weights of male wistar rats

Study Group/ Dose	Testosterone (ng/ml)	LH (m/U/ml)	FSH (m/U/ml)
A (control)	2.60 ± 0.4	3.51± 0.08	4.40± 0.05
B (150mg/kg)	1.90 ± 0.05	2.30 ± 0.15	5.70 ± 0.01
C (250mg/kg)	1.80 ± 0.11*	1.60 ± 0.03*	6.90 ± 0.10*
D(350mg/kg)	1.70 ± 0.12*	1.30 ± 0.07*	8.20 ± 0.09*

*significant difference (p<0.05)

Table 2: Effects of *Phyllanthus amarus* on serum concentrations of reproductive hormones in male wistar rats.

Study Group/ Dose	Sperm motility (%)	Sperm Count (10 ⁶)	Sperm Viability (%)
A (control)	78 ± 6.06	75 ± 4.02	94 ± 6.24
B (150mg/kg)	70 ± 6.54	72 ± 12.48	82 ± 8.55
C (250mg/kg)	73 ± 16.53	65 ± 3.96	79 ± 5.40
D(350mg/kg)	60 ± 3.81*	59 ± 5.40*	62 ± 11.01*

*significant difference (p<0.05)

Table 3. Effects of *Phyllanthus amarus* on sperm characteristics in male wistar rats.

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