

EFFECTS OF METHANOLIC LEAF EXTRACT OF *PIPER GUINEENSE* ON SOME REPRODUCTIVE PARAMETERS OF MALE ALBINO RATS (*RATTUS NORVEGICUS*)

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ABSTRACT

This study investigated the effects of the administration of methanolic leaf extract of Piper guineense on serum concentration of testosterone, cauda epididymal sperm reserves (CESR), gonadosomatic weights of the testes and epididymides as well as testicular histomorphology of male albino rats. Twenty four adult male rats of 12 weeks of age were used for the study. Commercial pelleted feed and water were provided ad libitum. The rats were randomly selected into 4 groups of six rats each designated A, B, C and D. Group A served as untreated control and received only distilled water; while groups B, C and D received 50, 100 and 200 mg/kg body weight of the methanolic leaf extract of Piper guineense respectively for 30 days. Oral administration of the plant leaf extract was done every other day with the aid of orogastric cannula. There was no significant variation ($p>0.05$) in the mean serum testosterone concentration and cauda epididymal sperm reserves across the different groups. However, the mean gonadosomatic weights of the testes of the different treatment groups and the mean gonadosomatic weight of the epididymis of only group C (100 mg/kg body weight) were significantly higher ($p<0.05$) than that of the control. There was no obvious histomorphologic lesion in testes of the rats of the different groups. It was concluded that oral administration of methanolic seed extract of P. guineense at 50, 100 and 200 mg/kg body weight every other day for 30 days did not affect testosterone concentration, CESR and testicular histomorphology but caused an increase in the allometric weight of the testes.

Keywords: *Piper guineense*, Testis, Testosterone, Cauda epididymal sperm reserves, *Rattus norvegicus*

INTRODUCTION

Infertility is a major problem amongst human population and the use of phytomedicine is becoming increasingly popular for the treatment of reproductive health problems (Ekere *et al.*, 2013). The use of plant products forms an integral part of the Nigerian ethnomedical practice. Despite its successes, phytomedicine is fraught with drawbacks which include lack of dosage regimen and documented potential adverse effects (Omoja *et al.*, 2015). Yongabi (2004) listed *Piper guineense* in the Nigerian phytomedical pharmacopoeia. This plant

belongs to the family *Piperaceae* (Macmillan, 1984; Iwu, 2014) and its other common names include bush pepper, Benin pepper and Ashanti pepper. Among the Nigerian ethnic nationalities, it is known as *uziza* in Ibo, *masoro* in Hausa and *ota-iyere* in Yoruba (Iwu, 2014).

The plant leaves and fruits are used as flavouring for dishes. The leaves are used phytomedically as contraceptive, antipyretic, antiemetic, carminative, antibiotic, febrifuge, aphrodisiac amongst others (Iwu, 2014). The leaves of the plant have been demonstrated to exhibit cholinergic activity (Udoh *et al.*, 1996) and a depolarizing neuromuscular blocking

action (Udoh, 1999) which may be the bases for its repro-active effects.

This study investigated the effects of the sub-chronic administration of methanolic leaf extract of *P. guineense* on serum testosterone concentration, cauda epididymal sperm reserves, ganodosomatic weights of the testes and epididymides as well as testicular histomorphology of adult male albino rats.

MATERIALS AND METHODS

Animals: A total of 42, 12 weeks old male Sprague-Dawley albino rats weighing 200 ± 25 g were used for the study. The rats were procured from the Laboratory Animal Unit of the Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were acclimatized for two weeks. The rats were kept in stainless steel cages under standard environmental conditions (ambient temperature: 25 – 28°C; day light: approximately 12 hours natural light per day; humidity: 60 – 70 %). Commercial pelleted feed (Vital®, GCOML, Nigeria) containing 14.5 % crude protein and 2,500 Kcal/kg metabolizable energy; and potable water were provided *ad libitum*.

***Piper guineense*:** Fresh leaves of *Piper guineense* were sourced from Umunze town in Anambra State, Nigeria. The leaves were authenticated by a taxonomist. The leaves were then air-dried under room temperature. The dried leaves were pulverised and the weight was determined. Cold maceration method of extraction was done using 1.3 kg of the pulverised leaves with 5 litres of 80 % methanol for 48 hours with intermittent agitation. It was filtered using Whatman No. 1 filter paper. The filtrate was concentrated to obtain a dry crude extract. The percentage yield was determined, and the extract was preserved at 4° C throughout the duration of the study.

Toxicity: Eighteen of the rats were used for acute toxicity study of the plant extract using the method of Lorke (1983), to determine the LD₅₀. Furthermore, twenty four male rats were randomly assigned to four groups designated A,

B, C and D, and replicated thrice with each replicate having two albino rats were deployed for the sub-lethal study. Group A served as the control and received only equivalent volume of distilled water, while groups B, C and D were dosed with 50, 100 and 200 mg per kilogram body weight of the extract respectively. The rats were dosed orally every other day between 0900 – 1000 hours using a gavage needle for 30 days.

Samples and Analyses: Body weights of the rats were taken weekly. Blood samples were collected from the retrobulbar plexus of the eye of the rats (Stone, 1954). Serum testosterone concentrations were then assayed using testosterone Accubind™ Microplate enzyme immunoassay test kit (Monobind Inc., Lake Forest, USA). At the end of the study, the weight of the rats was recorded and orchietomy was performed on the rats under chloroform anaesthesia. The testes and epididymides that were resected were weighed, their allometric weights were determined as described by Amann (1970). The cauda epididymal sperm reserves (CESR) determination was by the method of Amann and Almquist (1961). The testicular and epididymal tissue samples were promptly fixed in Bouin's fluid and histological preparations were done as described by Drury and Wellington (1976), using Hematoxylin and Eosin (H and E) stains. Photomicrographs were captured using a Moticam digital camera (Motic China Group Company Limited., Xiamen, China).

Ethics: The management of the rats used in the study were duly conducted in accordance with the Ethics and Regulation Guiding the Use of Research Animals as approved by the University of Nigeria, Nsukka.

Data Analysis: Data generated were subjected to one way analysis of variance (ANOVA), and variant means were separated by the least significant difference (LSD) method. The statistical analyses were done using SPSS 16.0 (for Windows SPSS 16.0 Inc., Chicago, IL, USA).

Table 1: Serum testosterone concentration and caudal epididymal sperm reserves of rats administered different doses of methanolic leaf extract of *Piper guineense*

Treatments	Testosterone concentration (mg/ml)	CESR (x 10 ⁸)
Group A (control)	0.35 ± 0.21 ^{ab}	1.76 ± 0.48 ^a
Group B (50 mg/kg BW)	0.70 ± 0.42 ^a	1.07 ± 0.50 ^a
Group C (100 mg/kg BW)	0.25 ± 0.07 ^{ab}	1.50 ± 0.32 ^a
Group D (200 mg/kg BW)	0.20 ± 0.00 ^b	1.77 ± 0.52 ^a

Values with different superscript within a column are significantly different (p<0.05). CESR = cauda epididymal sperm reserves

Table 2: Testicular and epididymal allometric weights of rats administered different doses of methanolic leaf extract of *Piper guineense*

Treatments	Testes (g/100g body weight)	Epididymides (mg/100 g body weight)
Group A (control)	0.48 ± 0.01 ^a	78.67 ± 4.62 ^a
Group B (50 mg/kg BW)	0.55 ± 0.03 ^b	79.83 ± 5.80 ^a
Group C (100 mg/kg BW)	0.57 ± 0.02 ^b	99.00 ± 5.29 ^b
Group D (200 mg/kg BW)	0.53 ± 0.03 ^b	84.33 ± 4.04 ^a

Values with different superscript within a column are significantly different (p<0.05).

Significance was accepted at probability value of p<0.05. The results were presented as means ± standard deviation (SD).

RESULTS

The percentage yield of the extract was 8.39 % of the starting weight of the plant leaf. Acute toxicity test showed that the methanolic extract of the plant leaf was well tolerated at the dose of 5000 mg/kg body weight.

The mean serum testosterone concentrations of treatment groups B, C, and D did not differ significantly (p>0.05) from that of the group A (control). However, treatment group B value was significantly higher (p<0.05) than that of group D (Table 1). There was no significant variation (p>0.05) in the CESR across the groups (Table 1).

The mean testicular allometric weights of all the other treatment groups were significantly higher (p<0.05) than that of the control group; but there was no significant variation (p>0.05) within these treatment groups (A, B and D) (Table 2). The mean epididymal allometric weights of the different groups were significantly lower (p<0.05) than that of group C (Table 2). There were no significant variations (p>0.05) in weekly body weights of the rats across the groups (Figure 1).

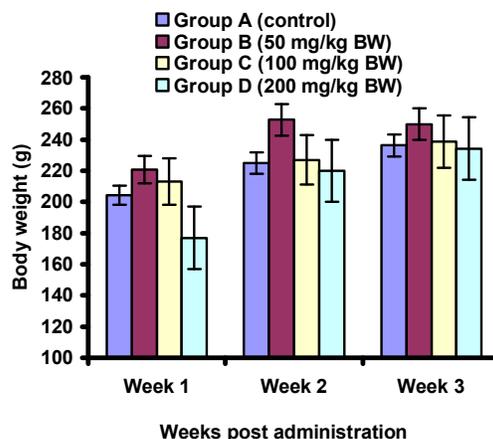


Figure 1: Body weights of rat given graded doses of methanol leaf extract of *Piper guineense* orally

Histomorphologic sections of the testes of the rats of the different groups showed normal spermatogenic seminiferous tubules and intact interstices (Figure 2).

DISCUSSION

The results of the acute toxicity tests showed that the rats tolerated up to 5000 mg/kg body weight dose without mortality or any obvious signs of toxicity. This may indicate that the leaf extract is unlikely to present acute hazard in normal use (WHO, 2001).

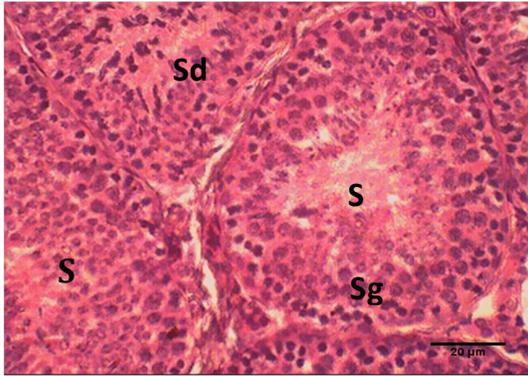


Figure 2: Photomicrograph of section of the testis of rat given 100 mg/kg body weight of methanol leaf extract of *Piper guineense* orally for 30 days, showing no obvious lesions. Note active seminiferous tubules (S) bearing spermatogonium (Sg), spermatids (Sd), and the interstitium (T); H and E, $\times 400$

The finding of no significant variation in the mean serum testosterone concentrations of the different treatment groups indicated that biosynthetic activity of the Leydig cells may not have been adversely affected following dosing with methanol leaf extract of *P. guineense*. Mbongue *et al.* (2005) had demonstrated testosterone biosynthetic enhancement property of this plant fruit extract in rats following dosing of 122.5 and 245 mg/kg body weight orally. Testosterone is the end product of the steroidogenesis of the hypothalamo-pituitary-testis regulatory axis which produces about 95 % of the serum testosterone compared to the 5 % produced by the adrenal glands (Urban, 1999). Spermatogenesis is totally dependent on testoids (Pakarainen *et al.*, 2005) and disruption of the neuro-endocrine regulatory process by food items and epithelial spermatogenic activity (Igwebuike *et al.* 2011).

The finding of no significant variation in the CESR of the other treatment groups when compared to the control group is in tandem with the no variation in the mean serum testosterone concentration across the groups. This however, differs from the report of Malini *et al.* (1999) who reported a decline in both caput and cauda epididymal sperm reserves of rats when piperine (an alkaloid present in the fruits of *P. guineense*) was administered at the doses of 5 and 10 mg/kg.

The absence of obvious histomorphologic lesions in the testes and lack of significant variations in the CESR across the groups indicated that this plant leaf extract does not adversely affect spermatogenic and steroidogenic activities of the testes. This however, is at variance with the report of Ekanem *et al.* (2010) which observed histopathological changes in the testicular cells of mouse fed graded doses of ethanol extract of the plant seed.

However, the mean testicular allometric weights of all the other treatment groups were significantly higher than that of the control group, while mean epididymal allometric weight of only group C (100 mg/kg body weight) was higher than that of the control group. This finding indicated that the plant leaf extract has androgenic properties because the mass and morphometry of these organs are positively affected by the testosterone concentration (Jockenovel and Swerdloff, 2000; Parkinson, 2001) and this therefore may reflect their bioactivity. This finding differ from the observations of Mbongue *et al.* (2005) which reported no significant difference in the allometric weights of the testes and epididymides of rats treated with aqueous extract of *P. guineense* fruits at the doses of 122.5 and 245 mg/kg body weight orally. Continued administration of exogenous androgens will alter the hypothalamo-pituitary-testicular axis resulting in decrease in the concentration of testosterone due to its negative feedback effect on the axis (Parkinson, 2001) consequently producing a negative effect on the testicular and epididymal tissues. The finding of no significant variation in the body weights across the groups through the study is consistent with the finding of no significant variations in the serum testosterone, CESR and absence of obvious histomorphologic lesions in the testes across the groups.

Conclusion: The present study has shown that even though the methanolic leaf extract of *P. guineense* has some androgenic properties which might have been responsible for the increase in testicular and epididymal allometric weights, the androgenic effect was not

observed in other tissues/organs or in the body weight gain. Thus its ethnomedical use to enhance male fertility may not be justified.

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