

**ANABOLIC ACTIVITY OF ALCOHOLIC EXTRACT OF  
*PIPER GUINEENSE* IN IMMATURE CASTRATED RAT**

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**Summary**

The present study was undertaken to investigate anabolic and androgenic activities of alcoholic dry fruit extract of *Piper guineense* in immature rats. Two control groups; uncastrated and castrated wistar albino rats were orally treated each with distilled water (10 mL/kg, n=5). Four castrated groups received *per os* *P. guineense* extract (40, 80, 120 and 160 mg/kg, n=5/doses). One uncastrated group received orally the plant extract (160 mg/kg, n=5). For comparison, one castrated group received intra muscular injection of Testosterone enanthate (9.09 mg/kg, n=5). All animals were treated for 16 consecutive days. Androgenic activity was evaluated by the ability of this extract to increase seminal vesicle, ventral prostate and penis weights. Meanwhile the increase of *levator ani* muscle (LAM) weight and amount of red blood cells were used as indices of anabolic activity. Results showed that *P. guineense* alcoholic extract failed to increased seminal vesicle, ventral prostate and penis weights when compared with castrated control group. However, castrated extract treated rats with 120 and 160 mg/kg showed a significant

( $p < 0.05$ ) increase in LAM weight and red blood cells amount as compared to castrated control. Accessory sex organs and LAM weights of 160 mg/kg uncastrated treated rats were significantly ( $p < 0.05$ ) increased when compared with uncastrated control group. *P. guineense* extract effect was less potent than T. enanthate in androgenic and anabolic activities. The present results indicated that the alcoholic dry fruit extract of *P. guineense* possesses anabolic activity in immature rat. Its effect was tissue-selective in that, it stimulated the anabolic organ more than the androgenic organs.

**Keys words:** *Piper guineense*, tissue-selective, androgenic activity, anabolic activity, *levator ani*.

### Introduction

Many medicinal plants such as *Hibiscus macranthus*, *Basella alba*, and *Cucumis trigonus* have been shown to exhibit anabolizing and androgenic effects in male rat (1, 2). *Piper guineense* Schum and Thonn (Piperaceae) is widely used in traditional medicine for male sexual weakness treatment and to prevent abortion (3, 4). Previous phytochemical studies of *P. guineense* extract revealed the presence of various substances such as alkaloid, sterols, lignans and amides (5) Pharmacological and physiological studies of *P. guineense* extract showed depolarizing neuromuscular blocking action (6), insecticidal properties (7), sexual behavioural effect (8) and antifungal activity (9). Our laboratory have shown that, eight days treatment of uncastrated mature male rats with 40, 80 and 120 mg/kg of the alcoholic dry fruit extract of *P. guineense*, significantly increased penile erection index, mount frequency, intromission frequency, ejaculation frequency, body weight and seminal vesicle secretion (10). These parameters are controlled by androgens, which have anabolic and androgenic effect on different body tissues (11), including increase of haemoglobin levels and red blood cells volume (12). In this study, we have attempted to ascertain whether the alcoholic dry fruit extract of *P. guineense* has anabolic or androgenic activities.

## Materials and Methods

### 1- Preparation of the plant alcoholic extract.

Fruits of *P. guineense* were collected in the peripheral region of Yaounde in the Centre Province of Cameroon and identified at the National Herbarium, Yaounde, where a voucher specimen (43129-HNC) was deposited. The fruits were dried at 34°C using an oven type P-SELECTA and crushed. The dried powder (1 kg) was macerated in 5 L of 90 % methanol with occasional stirring every day for one week. The resultant extract was filtered and the filtrate was evaporated at 70 °C. The yield of the extraction was 30 % (w/w in term of dried material). This extract was administered orally at four different doses of 40, 80, 120 and 160 mg/kg of body weight, once per day, for 16 days. These doses were chosen based on previous results of our laboratory.

### 2- Treatment of animals

Male Wistar rats (Animal Physiology Laboratory, University of Yaounde I, Cameroon), one-month of age, approximately 60 g, were used in this study. 30 bilaterally castrated and 10 uncastrated immature rats were randomly distributed in 8 groups of 5 rats each. Group I: control bilaterally castrated rats received orally distilled water (10 mL/kg). Groups: II, III, IV and V-bilaterally castrated rats received plants extract at doses of 40, 80, 120, and 160 mg/kg respectively.

Group VI: bilaterally castrated rats, received an intramuscular injection of Testosterone enanthate (Androstardy<sup>R</sup> purchased from Schering AG Laboratory, Germany) at the dose of 9.09 mg/kg. Group VII: control uncastrated rats received orally distilled water (10 mL/kg) Group VIII: uncastrated; rats received orally plant extract at dose of 160 mg/kg. All animals had free access to food and water.

### 3- Estimation of red blood cells

Red blood cells number was evaluated at the beginning and at the end of the treatment period. 10 µL of blood were collected under antiseptic conditions from the tail of each rat and diluted with Macarno liquid. The blood cells were counted using protocol described by (13).

### 4- Biochemical analyses and organ weights

24 h after the last administration, all rats were weighed and sacrificed by cervical dislocation; aorta blood was collected into heparinized tubes then centrifuged to obtain the plasma. Plasma protein level was evaluated using the protocol described by (14). The animals underwent laparotomy for removal and weighing of seminal vesicle (SV), ventral prostate (VP), penis (P) and *Levator ani Muscle* (LAM). The removal of the LAM was done following a protocol described by (15).

Relative weights of these organs were then calculated. Increase in SV, VP and P relative weights were used as indices for evaluation of androgenic activity, while increase in LAM weight and red blood cells accounted for anabolic activity (15, 16).

### 5- Statistical analyses

The data are expressed as mean  $\pm$  SEM. Statistical analysis was carried out by one-way analysis of variance (ANOVA) and the comparison between the controls and experimental groups was done using the Dunnet test; groups were considered to be significant for  $p < 0.05$ .

## Results

### 1- Effect of *P. guineense* extract on body and organs weights

Rats exposed to the plant extract and T. enanthate showed no increase in body weight after 16 days of treatment when compared with castrated control group. As expected, our results showed significant weights decreases ( $p < 0.01$ ) of seminal vesicles (SV), ventral prostate (VP), penis and *levator ani muscle* (LAM) of castrated control group as compared to uncastrated control (Table 1). *P. guineense* alcoholic extract at doses of 40, 80, 120 and 160 mg/kg failed to increase SV relative weight when compared with castrated control group. 16 days of treatment with 40, 80 and 120 mg/kg doses also failed to increase VP and penis weight as compared to castrated control. On the other hand, plant extract at doses of 120 and 160 mg/kg significantly ( $p < 0.01$ ) increase LAM weight by 114.37 and 123.6 % respectively, as compared to castrated control. The ratio of myotrophic to androgenic activity was significantly high (6.8) with the dose of 80 mg/kg and (7.7) with 120 mg/kg treated extract rats when compared with castrated control group.

Intramuscular injection of T. enanthate for 16 days to immature castrated rats significantly ( $p < 0.01$ ) increase relative weight of SV, VP, penis and LAM as compared to castrated control. In this study, we observed a significant ( $p < 0.05$ ) increase in P and LAM relative weights in uncastrated rats treated with 160 mg/kg extract when compared with uncastrated control group.

### 2- Effect of *P. guineense* alcoholic extract in plasma protein

As T.enanthate, *P. guineense* alcoholic extract had no significant effect on plasma protein level after 16 days of treatment (Table 2).

### 3- Effect of *P. guineense* alcoholic extract in red blood cells level

Changes after 16 days of treatment with *P. guineense* extract are outlined in (Table 2). *P. guineense* extract with the doses of 120 and 160

mg/kg significantly ( $p < 0.01$ ) increased red blood cells level by 109.5 and 80.9 % respectively as compared to castrated control. A significant increase in red blood cell level was also registered with the dose of 160 mg/kg in *P. guineense*- treated uncastrated group when compared with uncastrated control group.

**Table 1: Effects of *P. guineense* alcoholic extract on body and relative organs weight**

Groups And treatments	Body Weight gain (%)	Seminal vesicle (SV) (mg/100g body weight)	Ventral prostate (VP) (mg/100g body weight)	Penis (P) (mg/100g body weight)	Levator ani muscle (LAM) (mg/100g body weight)	Ratio $\frac{LAM}{VP}$
castrated control distilled water (10 mL/kg)	42.3 ± 4.6	13.0 ± 0.9 <sup>ΦΦ</sup>	9.9 ± 1.3 <sup>ΦΦ</sup>	55.1 ± 3.0 <sup>ΦΦ</sup>	19.2 ± 2.3 <sup>ΦΦ</sup>	2.0
<i>P. guineense</i> (40 mg/kg)	57.1 ± 6.3	7.8 ± 1.0**	9.4 ± 2.1	42.6 ± 3.9	23.9 ± 2.0	3.3
<i>P. guineense</i> (80 mg/kg)	46.3 ± 4.4	6.3 ± 1.1**	7.3 ± 3.6	42.0 ± 93.6	26.4 ± 2.2	6.8 <sup>*b</sup>
<i>P. guineense</i> (120 mg/kg)	53.4 ± 9.8	6.0 ± 0.7**	5.9 ± 1.1	45.9 ± 4.3	41.1 ± 4.2* *	7.7 <sup>**b</sup>
<i>P. guineense</i> (160 mg/kg)	53.1 ± 8.8	9.4 ± 0.4*	8.7 ± 0.7	51.3 ± 2.1	42.9 ± 3.3* *	5.0 <sup>**b</sup>
T. enanthate (9.09 mg/kg)	59.4 ± 5.5	686.3 ± 3.3***	220.0 ± 4.9***	163.7 ± 8.7**	261.1 ± 9.5***	1.2
Uncastrated control distilled water (10 mL/kg)	40.0 ± 6.0	28.8 ± 2.6	28.5 ± 4.8	86.0 ± 5.1	57.2 ± 5.2	2.5
Uncastrated <i>P. guineense</i> (160 mg/kg)	40.8 ± 4.6	39.0 ± 4.4	47.8 ± 11.7	99.6 ± 4.2 <sup>Φ</sup>	84.3 ± 8.5 <sup>Φ</sup>	2.1

Values are mean ± SEM. N=5, \* $p < 0.05$  Vs castrated control rats, \*\* $p < 0.01$  Vs castrated control rats, \*\*\* $p < 0.001$  Vs castrated control rats, <sup>Φ</sup> $p < 0.05$  Vs uncastrated control rats, <sup>ΦΦ</sup> $p < 0.01$  Vs uncastrated control rats <sup>b</sup> $p < 0.05$  Vs T. enanthate treated rats. LAM/VP ratio values are means of each group.

**Table 2: Effect of *P. guineense* alcoholic extract on red blood cells and plasma protein level.**

Groups and treatments	Amount of red blood cells/mm <sup>3</sup> ( $\times 10^6$ )		Percentage of variation $\frac{\text{day 16} - \text{day 1}}{\text{day 1}}$ (%)	Plasma Protein level (g/L)
	day 1	day 16		
castrated control distilled water (10 mL/kg)	4.07 $\pm$ 0.60	2.08 $\pm$ 0.47	-1.99	81.60 $\pm$ 3.55
<i>P. guineense</i> (40 mg/kg)	4.52 $\pm$ 0.59	3.70 $\pm$ 1.09	-18.14	91.20 $\pm$ 7.19
<i>P. guineense</i> (80 mg/kg)	3.99 $\pm$ 1.27	2.48 $\pm$ 0.10	-37.80	89.10 $\pm$ 4.16
<i>P. guineense</i> (120 mg/kg)	4.43 $\pm$ 0.76	9.28 $\pm$ 0.59	+ 109.40 * *	87.90 $\pm$ 5.40
<i>P. guineense</i> (160 mg/kg)	4.66 $\pm$ 0.42	8.43 $\pm$ 0.61	+ 80.90 * *	87.70 $\pm$ 6.98
T. enanthate (9.09 mg/kg)	4.75 $\pm$ 0.92	7.36 $\pm$ 0.20	+ 54.90*	89.80 $\pm$ 4.26
Uncastrated control. distilled water (10 mL/kg).	4.74 $\pm$ 0.58	4.76 $\pm$ 1.05	+ 0.42	86.00 $\pm$ 8.08
Uncastrated <i>P. guineense</i> (160 mg/kg)	2.39 $\pm$ 1.07	5.03 $\pm$ 1.00	+ 110.46 $\Phi\Phi$	90.00 $\pm$ 3.35

Values are mean  $\pm$  SEM. N=5. \*p<0.05 Vs castrated control rats, \*\*p<0.01 Vs castrated control rats,  $\Phi\Phi$ p<0.05 Vs uncastrated control rats.

### Discussion

As expected, the present experimental study showed a significant decrease in seminal vesicle (SV), ventral prostate (VP) and LAM weights in castrated animals. This reduction in weight of the androgen-targeted organs in castrated control rat is the result of ablation of

endogenous androgen production. It shows that castration produces atrophy of the rat LAM and accessory sex organs. The administration of androgenic hormone to castrated animal results in hypertrophy in these androgen-targeted organs (17, 18). *P. guineense* extract at the doses of 120 and 160 mg/kg produced a significant increase in LAM weight as well as myotrophic / androgenic activities ratio. On other hand, 16 days of treatment of immature castrated rats with *P. guineense* (120 and 160 mg/kg) produced a significant increase in red blood cells level.

These results suggest the anabolic activity of the plant extract in immature rats. Some authors reported that, increase in LAM weight and red blood cells level are used as index of anabolic activity whereas increase in accessory sex organs weight is used as index of androgenic activity (12, 15, 16). The findings revealed that *P. guineense* extract failed to increased SV, VP and P weights but increase LAM weight in castrated treated animals. These results are in line with those of (1) which revealed that, alcoholic extract of *Cucumis trigonus* had no significant effect in VP weight but significantly increased LAM weight in treated rats. These results denoted tissue selective activity of component of the extract. The distinction in functional activities of this extract in immature castrated rats could be caused by difference in numerous factors including intrinsic activity and the absence of testicular secretions. Our results also showed significant increase only in red blood cell level and LAM after 16 days of *P. guineense* treatment. These finding suggest that the extract compounds were acting as nonsteroidal ligands. Such a nonsteroidal androgen, with equivalent activity to testosterone on bone and muscle would likely have less activity on ventral prostate or other accessory reproductive organ than testosterone (16). *P. guineense*-treated uncastrated immature rats showed a significant increase in SV and VP weights as compared to uncastrated control. On other hand, plasma protein was not significantly increase when compared to uncastrated group. These results are in line with those of (10). This plant extract significantly increase SV and VP weights and has no significant effect on plasma protein in uncastrated mature rats. Our results suggest that *P. guineense* alcoholic extract could serve as adjuvant for enhancing SV and PV weight.

In conclusion, the present findings show that alcoholic fruit extract of *P. guineense* had tissue-selective effect and possesses anabolic activity in immature castrated rats, but others parameters of anabolic activities such as wound healing effect and retention of nitrogen will be carried out to give more light on the anabolic effect of alcoholic extract of *P. guineense*.

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