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The effects of aqueous extracts of *Tetracarpidium conophorum* seeds on the hormonal parameters of male guinea pigs

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ABSTRACT

Objective: To investigate the effects of the aqueous extracts of the seeds of *Tetracarpidium conophorum* and the effect of Proviron (12.5 mg/kg) (as standard) on the hormonal parameters of male guinea pigs, compare the effects of the seeds of *Tetracarpidium conophorum* and Proviron, and screen the phytochemical constituents of the seeds of *Tetracarpidium conophorum*. **Methods**: The hormonal effects of the *Tetracarpidium conophorum* and Proviron were tested by hormonal assay, using enzyme immuno assay method. This was done by reaction of antibody with serum testosterone and testosterone label, magnetic solid phase separation and colour development step. Phytochemical screening was done using standard procedures. **Results**: The aqueous extract of the *Tetracarpidium conophorum* seeds (100–400 mg/kg) caused a statistically significant increase ($P<0.05$, ANOVA) in the level of testosterone of male guinea pigs, from (2.60±0.06) ng/mL to (3.40±0.05) ng/mL, (3.00±0.60) ng/mL and (3.30±0.45) ng/mL on the 7th, 14th and 21st day of the administration of the extracts, respectively. The highest increase was obtained after the 7th day of treatment [(3.40±0.05) ng/mL]. These effects were very comparable to the effects of Proviron on the testosterone of male guinea pigs, which were obtained to be (2.80±0.01) ng/mL, (2.90±0.16) ng/mL and (3.10±0.30) ng/mL on the 7th, 14th and 21st day, respectively. These effects were dose–and time–dependent. The optimum effect on testosterone level under dose–dependent study [(4.70±0.45) ng/mL] was obtained at 300 mg/kg of *Tetracarpidium conophorum* after 7 days treatment. Finally, the phytochemical screening of the seeds of *Tetracarpidium conophorum* revealed the presence of flavonoids, tannin, alkaloids, carbohydrate, volatile oils, terpenoids, saponins and cardiac glycosides. **Conclusion**: This study supports the claims on the use of the seeds of this plant by traditional medicine practitioners as a fertility agent. However, further studies need to be done to isolate and characterize the active principle(s) responsible for this activity in this plant.

1. Introduction

Tetracarpidium conophorum(*T. conophorum*) Mull (Arg), Family, Euphorbiaceae is commonly known as African Walnut, or Conophor. It is a west Equatorial perennial climber often found in the most forest zones of Sub-Saharan Africa. It is widely distributed in the southern part of Nigeria[1]. *T. conophorum* is known as Ukpa (Igbo) and awusa or asala (Yoruba) in the Littoral and Western Cameroon, Kaso or Ngak[1,2].

In southern Nigerian traditional medicine, *T. conophorum* is used as male fertility agent to improve fertility and increase libido in male[2]. The oil from the nut has been found useful in the formulation of wood vanish, stand oil, vulcanized oil for rubber and leather substitute.The stem, root, and leaves have been found to exert antimicrobial activities[2]. The seeds of *T.conophorum* are useful in the production of snacks and delicacy[3,4]. Two isolectins, Agglutin Ⅰ and Ⅱ were characterized from the seeds extract[5]. The presence of oxalate, phytates, tannins, proteins, fibre, oil and carbohydrate in African Walnut seed has been reported[6–8].

Although the *T.conophorum* is used by traditional medicine practitioners in Nigeria as a male fertility agent, to improve libido in males, there is no scientific based report or information backing up this claim or ascertain the efficacy of this plant as a fertility agent. In the light of this, this study seeks to establish scientific based information on the fertility effect of this plant and to ascertain the claims for the first time.

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2. Materials and methods

2.1. Plant materials

The seeds of *T. conophorum* were collected in June 2008 from the southern part of Nigeria (Osun state). The plant was authenticated by Edwin Wosu, a taxonomist at Botany Herbarium of University of Port Harcourt Nigeria, where voucher specimen was deposited.

All the chemicals used were of analytical grade.

2.2. Preparation and extraction of plant sample

Collected plant seeds were dried under the sun for 48 hours. The dried seeds were ground with hammer mill and the fine powdered crude drug was extracted using Soxhlet apparatus. The yields of the extract were obtained after removal of solvent by freeze-drying. The extracts were stored in the refrigerator for subsequent reconstitution and use.

2.3. Animals

Adult male guinea pigs of average weight 300-600 g were obtained from the animal house of University of Port Harcourt. They were housed in a cage of five animals per cage and were allowed to acclimatize with the new environment for 7 days. The animals were properly feed on elephant grass throughout the experimental period.

Chemical tests were carried out on the extracts and on the powdered specimens using standard procedures to identify the constituents[9,10] by characteristic colour changes as described by Sofowara[11, 12].

2.4. Hormonal assay

The animals from different groups were given diethyl ether anesthesia and dissected. Their respective blood samples were collected in lithium heparinized tubes.

2.5. Testosterone enzyme immuno assay

This was carried out in three stages namely: reaction of antibody with serum testosterone and testosterone label, Magnetic solid phase separation step and colour development step.

In the reaction of antiserum with serum testosterone and testosterone label, 50 μL of test blood sample was pip petted into different tubes. The testosterone blocking reagent, diluted testosterone label and testosterone antiserum (100 μL) were added to the test tube, covered, and mixed with vortex mixer.

3. Results

In time-dependent study, the *T.conophorum* (300 mg/kg) caused significant increase in the level of testosterone of the male guinea pigs (P<0.001 ANOVA) n the 7th and 21st day of post exposure period (Table 1 and Figure 1). These effects were very comparable to the observed effects of Proviron (12.5 mg/kg). But Proviron exhibited the highest effect on the 21st day which persisted with slight decrease on the 28th day. While *T. conophorum* declined significantly in action on 28th day after exhibiting optimum effects on the 7th and 21st days (Table 1 and Figure 1).

Furthermore, in dose-dependent study, *T.conophorum* (100-400 mg/kg) caused significant dose-dependent increases in the levels of testosterone at all doses: at 100 mg/kg, P<0.05, while at 200-400 mg/kg doses, P<0.001(ANONA). These effects were very comparable to the observed effects of the standard (Proviron) used (Table 2). However, there were no significant effects on the levels of Follicle stimulating hormone (FSH), Leutinizing hormone (LH) and estrogen levels. Although there were very slight increases noticed on the levels of these hormones, but they were not statistically significant (Figure 1, 2, 3 and 4) both in time- and dose- dependent studies (Table 1 and 2).

Table 1

The time-dependent comparative effects aqueous extracts of the seeds of *T. conophorum* and Proivron on the hormonal parameters of the male guinea pigs.

Treatment period (300 mg/kg)	Leutenizing hormone (mIU/L)	Follicle stimulating hormone(mIU/L)	Testosterone (ng/mL)	Estrogen (nmol/L)
Control	2.70± 0.22	1.40±1.50	2.60±0.06	0.20±0.01
Pv at 7 days	3.00±0.01	1.40±0.01	2.80±0.01	0.25±0.02
Tc at 7 days	3.10±0.15	1.50±0.02	3.40±0.05[b]	0.30±0.03
Pv at 14 days	3.10±0.16	1.50±0.14	2.90±0.16	0.33±0.03
Tc at 14 days	3.10±0.11	1.50±0.04	3.00±0.60[a]	0.31±0.02
Pv at 21 days	3.10±0.13	1.50±0.05	3.10±0.30[a]	0.26±0.05
Tc at 21 days	3.20±0.05	1.40±0.01	3.30±0.45[b]	0.25±0.04
Pv at 28 days	2.50±0.01	1.40±0.02	2.90±0.01	0.24±0.05
Tc at 28 days	3.00±0.03	1.40±0.03	2.60±0.50	0.31±0.04

Values are expressed as mean±SEM of five observations (n= 5).

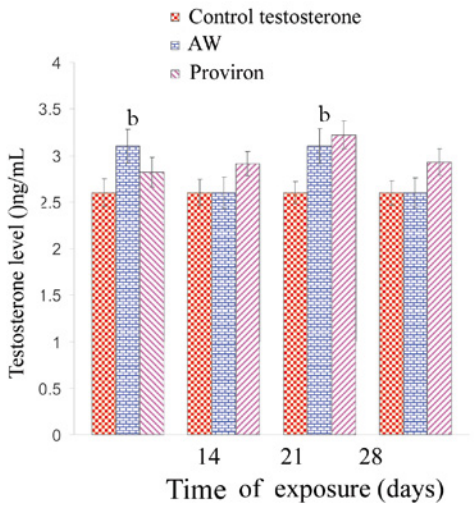
[a] represents significant value at P< 0.05 and [b] significant values at P < 0.001 (ANOVA).

Tc: *T.conophorum*, Pv: Proviron.

Table 2
The dose-dependent comparative effects aqueous extracts of the seeds of *T. conophorum* and Proivron on the hormonal parameters of the male guinea pigs.

Treatment (mg/kg)	Leutenizing hormone (mIU/L)	Follicle stimulating hormone(mIU/L)	Testosterone (ng/mL)	Estrogen (nmol/L)
Control	2.70±0.22	1.40±0.50	2.60±0.06	0.28±0.02
100	2.90±0.26	1.50±0.17	3.80±0.42[a]	0.30±0.05
200	3.00±0.23	1.40±0.01	4.00±0.30[b]	0.26±0.01
300	3.10±0.01	1.50±0.04	4.70±0.45[b]	0.27±0.03
400	3.00±0.13	1.50±0.02	4.30±0.48[b]	0.24±0.04
Proviron	2.70±0.01	1.40±0.01	3.00±0.01[a]	0.31±0.06

Values are expressed as mean±SEM of five observations (n= 5).
[a] represents significant value at P< 0.05 and [b] significant values at P < 0.001 (ANOVA).



[b] represents P<0.001 (ANOVA). AW represents *T. conophorum*

Figure 1. The comparative time-dependent effects of *T. conophorum* and proviron on testosterone of male guinea pigs.

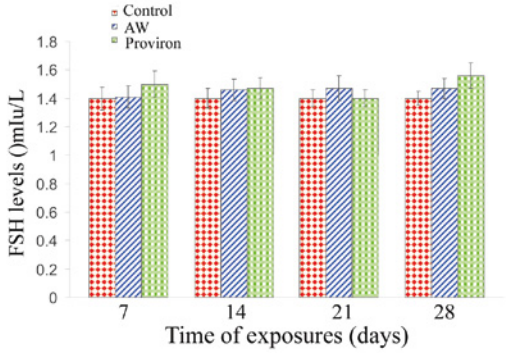


Figure 2. The comparative time-dependent effects of *T. conophorum* and proviron on FSH of male guinea pigs.

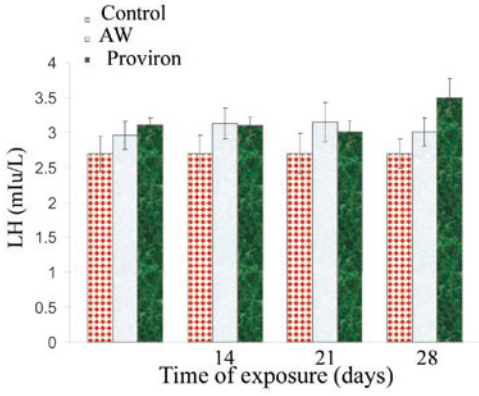


Figure 3. The comparative time-dependent effects of *T. conophorum* and proviron on LH of male guinea pigs.

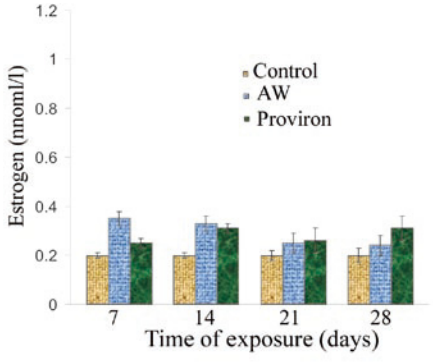


Figure 4. The comparative time-dependent effects of *T. conophorum* and proviron on estrogen of male guinea pigs.

4. Discussion

This study shows the effects of aqueous extract of the seeds of *T. conophorum* on the hormonal parameters of male guinea pigs. The results show that *T. conophorum* seeds extract causes increases in the level of testosterone but

has insignificant effect on the levels of FSH, estrogen and LH. The increase in the level of testosterone was found to be statistically significant at $P<0.05$ (ANOVA). The increase in the testosterone may be responsible for the effect of this plant as a libido enhancer or fertility agent as, claimed by traditional medicine practitioners. This is so because optimum level of testosterone is required for normal sex drive in adult male and the increase in the level of testosterone can lead to an increase in the spermatozoa[13-15].and hence the increase in male fertility[15].

The phytochemicals found present in the seeds of *T. conophorum* include: flavonoids, tannins, saponins, alkaloids, terpenoids, volatile oils, steroids and cardiac glycosides. Flavonoids present in this plant has been shown to possess many pharmacological properties such as: anti-oxidant activities, anti-inflammatory activities, anti- cancer activities and anti- microbial effects hence, flavonoids may have a contributory effect on its fertility properties and other pharmacological effects the plant possesses[13,14,16]. Favonoids as an anti-oxidant, has a rejuvenating effect on cells or tissues, it is anti-aging hence can contribute substantially on the fertility effect of this plant[13,14]. Alkaloids and tannins may also contribute to the plant´s effects as Antimicrobial agent[15,16].

Therefore, this study supports the claims on the folkloric use of this plant to improve libido and reproductive function in men. However, further study needs to be done to isolate, identify and characterize the active principle responsible for this effect.

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