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*Original article*

# The comparative effects of aqueous extract of *Tetracarpidium conophorum* seeds and Proviron on the sperm parameters of male guinea pigs

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**Abstract**

**Objective**: To investigation the comparative effects of the aqueous extracts of the seeds of *Tetracarpidium conophorum* (*T. conophorum*) and Proviron on the sperm parameters of male guinea pigs, and screen phytochemical constituents of the seeds of *T. conophorum*. **Methods**: The sperm count, motility and morphology of the male guinea pigs were done using Neubauer chamber (Haemocytometer), light microscope and dilute carbol Fuchsin (1: 20) method, respectively. Phytochemical screening was done by standard procedures. **Results**: The aqueous extract of the *T. conophorum* seeds (100 – 400 mg/kg) caused statistically significant increase ($P < 0.05$, ANOVA) in the sperm count and motility, from 67.7 ± 2.10 and 56.5 ± 2.40 to 78.0 ± 3.5 and 75.0 ± 2.8, respectively. The highest effect was obtained at 300 mg/kg of *T. conophoru*. At this dose, the sperm count and motility of the male guinea pigs administered with *T. conophorum* were the same with the group administered 12.5 mg/kg Proviron. The values were 78.0 ± 3.50 and 75.0 ± 2.80, respectively for sperm count and motility ($P < 0.001$, ANOVA). At 400 mg/kg, *T. conophorum* caused a slight decline in sperm count and motility. These effects were dose-dependent and comparable to the observed effects of Proviron (12.5 mg/kg) on sperm parameters of male guinea pigs. In time-dependent study, the observed effect of *T. conophorum* (300 mg/kg) and Proviron on the values of sperm count and motility at 14th day were almost the same. These values are 58.0 ± 1.80 and 70.0 ± 2.60, respectively for sperm motility and count. However, on the 7th day of treatment, *T. conophorum* exhibited highest effect which was higher than that of Proviron. These effects decreased progressively from the 14th to the 28th day. But Proviron showed the highest effect on the 28th day. These effects were all time-dependent and statistically significant at $P < 0.05$ (ANOVA). Finally, the phytochemical screening of the seeds of *T. conophorum* revealed the presence of flavonoids, tannins, alkaloids, terpenoids, carbohydrates, volatile oils, steroids, saponins and cardiac glycosides. **Conclusion**: This study shows that the seeds of *T. conophorum* possess some active principles that can contribute positively on male fertility. This therefore, supports the claims on the use of the seeds of this plant by traditional medicine practitioners to increase/improve libido in men. However, further studies need to be done to investigate the mechanism of this action and also to isolate and characterize the active principles responsible for this effect in the extracts of this plant.

**Keywords**: *Tetracarpidium conophorum*; Sperm parameters; Proviron; Male guinea pigs; Phytochemical screening

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## 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] (Dalziel). *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 [3-5]. Two isolectins, Agglutin Ⅰ and Ⅱ were characterized from the seeds extract [6]. The presence of oxalate, phytates, tannins, proteins, fibre, oil and carbohydrate in African Walnut seed has been reported[7,8].

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 to back up this claim or ascertain the efficacy of this plant as a fertility agent. It is in the light of this, that this study seeks to establish for the first time, scientific based information on the use of *T. conophorum* as male fertility agent.

## MATERIALS AND METHODS

### 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.

### Preparation and extraction of plant sample

The plant seeds collected were dried under the sun for 48 hrs. 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.

### Animals

Adult male guinea pigs of average weight 300 – 600g 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 7ds. The animals were properly feed on elephant grass throughout the experimental period.

### Phytochemical screening

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

### Sperm count

Semen was diluted at 1∶ 2 using micropipette in test tube with diluting fluid to immobilize and preserve the sperm. The Neubauer chamber (Heamocytometer) was prepared and charged with the diluted seminal fluid and allowed to stand in a moist chamber for 15 – 20 mins to allow spermatozoa to sediment to the grid of the counting chamber and counted with 40 × objective of light microscope.

### Motility

A drop of undiluted liquefied and well mixed semen was placed on a clean slide and covered with a cover slide and then examined under a light microscope to stimulate both quantitative and qualitative motility. Sperm motility was determined by counting all motile and immotile spermatozoa, in randomly chosen fields using a 40 × objective of light microscope.

### Morphology

Smears of semen suspension were made on clean slide and quickly fixed with 95 % alcohol. The smear was then stained with dilute Carbol Fuchsin (1∶ 20) and examined under the oil immersion objective. 100 – 300 cells were counted and the v/v of abnormal forms was noted. Semen was also examined

for the presence of particulate debris. Excessive contamination of the seminal fluid sample by epithelial cells, red blood cell, white blood cells and immature germ cells were observed and quantified.

## RESULTS

In does-dependent study, *T. conophorum* (100 – 400 mg/kg) caused statistically significant increases in sperm count and motility of male guinea pigs ($P < 0.05$, Table 1, Figure 1 and 2). These effects were also comparable to the observed effects with 12.5 mg/kg Proviron (as standard) on the sperm parameters of male guinea pigs. The highest effects were obtained with 300 mg/kg *T. conophorum* and these were 78.0 ± 3.50 and 75.0 ± 2.80 for sperm count and motility, respectively (Table 1, Figure 1 and 2).

Furthermore, in time-dependent study, *T. conophorum* (300 mg/kg) exhibited highest effects on sperm count and motility on the 7th day of post exposure period ($P < 0.001$, ANOVA). These values were 75.0 ± 2.90 and 75.0 ± 3.50 respectively for sperm count and motility (Table 2, Figures 3 and 4). These were also comparable to the observed effects of 12.5mg/kg Proviron on the sperm count and motility of the male guinea pigs. But Proviron showed highest effects on the 28th day, with $P < 0.001$ and $P < 0.05$ respectively for sperm motility and count (Table 2, Figures 3 and 4).

The effects of *T. conophorum* on the sperm morphology, debris quality and primordial cells of the male guinea pigs were also shown (Table 1 and 2). And the results of the phytochemical screening indicated the presence of flavonoids, tannis, alkaloids, terpenoids, saponins, and cardiac glycosides.

**Table 1** The dose-dependent comparative effects of *T. conophorum* and proviron on the sperm parameters of male guinea pigs.

| Group/Treatment(mg/kg) | Motility quality | Sperm count | Morphology of cells | Debris |
|---|---|---|---|---|
| 0.00 | 56.5 ± 2.4 | 67.7 ± 2.1 | 26.8 | 20.0 |
| 100Tc | 64.5 ± 2.5[a] | 70.0 ± 2.8[a] | 21.3 | 21.8 |
| 200Tc | 72.0 ± 2.1[b] | 75.0 ± 2.9[b] | 27.8 | 32.0 |
| 300Tc | 75.0 ± 2.8[b] | 78.0 ± 3.5[b] | 22.3 | 37.7 |
| 400Tc | 68.0 ± 1.8[a] | 67.6 ± 1.8 | 20.0 | 30.0 |
| 12.5Pv | 75.0 ± 3.1[b] | 70.7 ± 2.8[a] | 20.3 | 19.0 |

Values are expressed as mean ± SEM of five observations ($n = 5$). [a] represents significant values at $P < 0.05$, [b] significant values at $P < 0.001$ (ANOVA). Tc: *Tetracarpidium conophorum* seed extracts, Pv: Proviron.

**Table 2** The time-dependent comparative effects of *T. conophorum* (300 mg/kg) and Proviron (12.5 mg/kg) on sperm parameters of male guinea pigs.

| Group/Treatment (mg/kg) | Motility quality | Debris quality | Sperm count | Morphology of cells | Primordial cells | Duration(days) |
|---|---|---|---|---|---|---|
| Control | 56.50 ± 2.40 | 20.00 ± 0.70 | 67.70 ± 2.10 | 26.70 ± 0.70 | 19.00 ± 0.58 | 0 |
| Pv | 57.00 ± 2.00 | 20.00 ± 0.50 | 68.00 ± 1.90 | 26.00 ± 0.02 | 31.00 ± 0.10 | 7 |
| Tc | 75.00 ± 3.50[b] | 37.70 ± 1.70 | 75.00 ± 2.90[b] | 22.30 ± 0.58 | 28.60 ± 0.67 | 7 |
| Pv | 58.30 ± 1.70[a] | 31.00 ± 2.40 | 68.00 ± 1.90 | 20.00 ± 1.00 | 21.30 ± 0.95 | 14 |
| Tc | 58.00 ± 1.80[a] | 35.00 ± 1.70 | 70.00 ± 2.60[a] | 23.30 ± 4.40 | 29.70 ± 4.40 | 14 |
| Pv | 68.30 ± 2.60[a] | 19.00 ± 0.01 | 70.00 ± 2.80[a] | 20.00 ± 5.0 | 27.50 ± 5.50 | 21 |
| Tc | 55.00 ± 1.60 | 32.00 ± 0.02 | 67.00 ± 1.80 | 32.00 ± 0.01 | 31.00 ± 1.00 | 21 |
| Pv | 73.00 ± 3.00[b] | 25.00 ± 1.50 | 70.30 ± 2.70[a] | 28.70 ± 0.70 | 19.00 ± 1.70 | 28 |
| Tc | 55.00 ± 1.60 | 30.00 ± 0.01 | 66.00 ± 1.70 | 30.00 ± 0.02 | 20.00 ± 5.00 | 28 |

Values are expressed as mean ± SEM of five observations ($n = 5$). [a] represents significant values at $P < 0.05$, [b] significant values at $P < 0.001$ (ANOVA). Tc: *Tetracarpidium conophorum* seed extracts, Pv: Proviron.

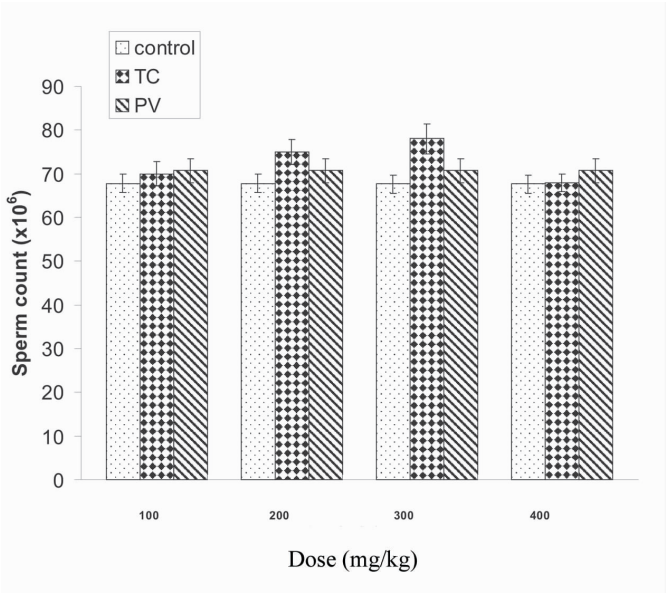


Fig 1 The dose-dependent effects of *T. conophorum* on the sperm count of male guimea pigs.
Tc: *T. conophorum*; Pv: Proviron; [a] $P < 0.05$, [b] $P < 0.001$ (ANOVA).

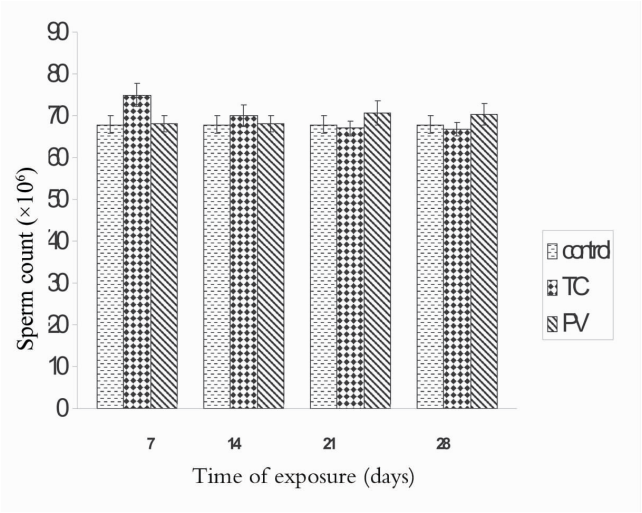


Fig 2 The time-dependent comparative effects of *T. conophorum* and proviron on the sperm count of male guimea pigs.
Tc: *T. conophorum*; Pv: Proviron; [a] $P < 0.05$, [b] $P < 0.001$ (ANOVA).

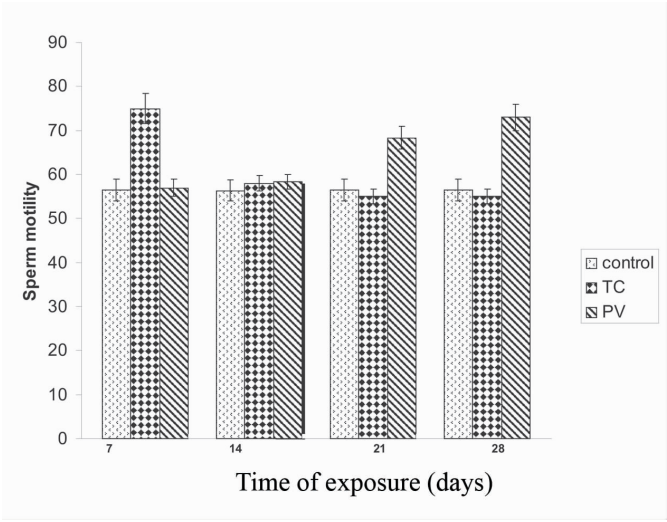


Fig 3 The time-dependent comparative effects of *T. conophorum* and proviron on the sperm motility of male guimea pigs.
Tc: *T. conophorum*; Pv: Proviron; [a] $P < 0.05$, [b] $P < 0.001$ (ANOVA).

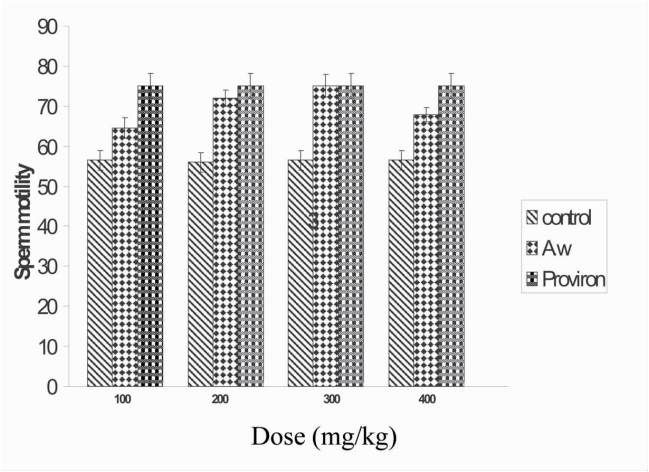


Fig 4 The dose-dependent effects of *T. conophorum* on the sperm motility of male guimea pigs.
A. W: *T. conophorum*; Pv: Proviron; [a] $P < 0.05$, [b] $P < 0.001$ (ANOVA).

## DISCUSSION

This study has shown that the aqueous extract of the seeds of *T. conophorum* (100 – 400 mg/kg) caused statistically significant increases on the sperm parameters of male guinea pigs (Table 1 and 2; Figure 1 – 4). The increases in the sperm count and motility were highest at 300mg/kg dose of the *T. conophorum* extract.

These effects were similar and very comparable to the observed effects of Proviron (12.5 mg/kg) on sperm parameters of male guinea pigs. These effects were also dose-dependent and significant at $P < 0.05$ (ANOVA).

In the time-dependent study, the observed effect of *T. conophorum* on sperm count, motility and cell morphology were similar to that of Proviron on the 21st day of treatment with the extract. However, *T. conophorum* exhibited maximum effect on the 7th day while Proviron exhibited maximum effects on the 28th day of post exposure. The values were also statistically significant at $P < 0.05$ (ANOVA). This implies that *T. conophorum* may have similar effects, properties and mechanisms of actions with Proviron

but with shorter duration of action; and with vitamin E, a known fertility agent and an antioxidant[13] with protein kinase C (PKC) inhibition as one of its mechanisms of actions[13].

*T. conophorum* is claimed by traditional medicine practitioners to possess fertility properties and this has also been supported by the report of its positive effect on hormonal parameters, especially testosterone (Obianime and Uche, in-press). Therefore, its positive hormonal effect properties may also contribute to its beneficial effect as a fertility agent (Obianime and Uche, in-press).

The phytochemical screening of the extract of the seeds of *T conophorum* revealed the presence of flavonoids, tannins, alkaloids, steroids, terpenoids, carbohydrate, volatile oils, saponins and glycosides. Flavonoids are antioxidants[14] and this may contribute to antioxidant and antimicrobial activities of this plant[2], hence its similar effect with vitamin E which is also a powerful antioxidant[13,15]. The steroids in this plant may be responsible for its positive effect on the hormone (testosterone) of male guinea pigs (Obianime and Uche in-press). This may because steroids are precursors in the synthesis of hormones.

In conclusion, this study shows that the seeds of *T. conophorum* increases sperm count and motility; therefore it can be used as a fertility agent. However, while further studies are on the way to investigate the exact mechanism of the action of the seeds of *T. conophorum* on male reproductive organ of male guinea pigs, there is need for further study to isolate, identify and characterize the active principles responsible for this effect in the seeds of *T. conophorum*.

## REFERENCES

1 **Dalziel JM**. *The useful plants of west tropical Africa*. London: Whitefrars Press;1937. 164.

2 **Ajaiyeoba EO**. Anti microbial potentials of extracts and fractions of the African walnut-*Tetracarpidium conophorum*. *Afr J Biotechnol* 2006;22(5): 2322-2325.

3 **Oke OL**, Fafunso MA. Lesser known oil seeds: The nutrient value of Conophor seeds in invitro. *Nutri Report Int* 1975; 12: 41-49.

4 **Adebona MB**, Ogunsua AO, Ologunde MO. Develoment of Conophor nut-based cereal snack food 1-biscuit. *J Food and Agric* 1988; 2: 123-136.

5 **Akpuaka MU**, Nwankwo E. Extraction, analysis and utilization of a drying oil from *Tetracarpidium conophorum*. *Biore Technol* 2000;73: 195-196.

6 **Animashaun T**, Togun RA, Hughas CR. Characterization of Isolectin in *Tetracarpidium conophorum* seeds (African walnut). *Glycoconjug J* 1994; 11:299-303.

7 **Enujugha VN**. Chemical and functional characteristics of conophor nut. *Paki J Nutri* 2003;2: 335-338.

8 **Enujiugha VN**, Ayodel-Oni. Evaluation of nutrient and antinutrient in lesser known under-utilized oil seeds. *Int J Food Sci Technol* 2003; 38: 525-528.

9 **Trease GE**, Evans WC. *Trease and evans pharmacognosy: A physician guide to herbal medicine*. 13th edition. London: Ballere tindal; 1989.

10 **Harborne JB.** *Phytochemical methods*. London: Chapman and Hall Ltd; 1978. 49-188.

11 **Sofowora A**. *Medicinal plants and traditional medicine in Africa*. New York: Wiley;1998.

12 **Odebedy O**, Sofowora A. Phytochemical screening of Nigerian medicinal plants. *Lloydia* 1978; 41: 41-234.

13 **Uche FI**, Obianime AW. The effects of Vitamin E on vanadium pentoxide toxicity of the biochemical parameters of male guinea pigs. *J Applied Sc Env Mgt* 2008; 12(3):101-105.

14 **Okwu DE**, Josiah C. Evaluation of the chemical composition of two Nigerian Medicinal plants. *Afr J Biotechnol* 2006; 5(4):357-361.

15 **Uche FI**, Obianime AW, Gogo-Abite M. Effects of Vanadium Pentoxide on the histological and sperm parameters of male guinea pigs. *J Applied Sc Env Mgt* 2008; 12(3):107-115.

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