

Research Article

Aqueous extract of *Tetracarpidium conophorum* increases FSH and LH plasma levels and impairs sperm indices in albino wistar rats

Okon Uduak Akpan* and Atai Akaninyene Anietie

Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria

*Correspondence Info:

Okon Uduak Akpan (PhD)

Department of Physiology,

Faculty of Basic Medical Sciences,

College of Health Sciences,

University of Uyo, Uyo, Akwa Ibom State, Nigeria

E-mail: chairmo2013@gmail.com

Abstract

Objective: This study sought to investigate the effects of *Tetracarpidium conophorum* on male fertility indices.

Materials and methods: Eighteen albino wistar rats weighing between 178g to 241g were randomly assigned into 3 groups. Group 1 was control group and oral gavaged 5ml of distilled water, group 2 and group 3 were administered medium dose (14.14mg/kg) and high dose (21.21mg/kg) of *Tetracarpidium conophorum* for 21 days. Acute toxicity study was done and the LD₅₀ was 1581.14mg/kg body weight. Animals were sacrificed on the 22nd day, blood samples were obtained by cardiac puncture and the serum was for hormonal assay. The testes were harvested for sperm analysis.

Results: Significant increases were observed for FSH and LH in both the medium and high doses group compared to the control group. A significant decrease in fast progressive movement in medium dose and high dose compared to control group with a significant dose dependent decrease in high dose group compared to medium dose group. There was a significant decrease in percentage sperm concentration in medium dose and high dose compared to the control group and a significant increase in high dose compared to the medium dose group. In percentage normal sperm morphology, there was a significant decrease in medium dose group and high dose compared to control group with significant increase in high dose compared to medium dose.

Conclusion: *Tetracarpidium conophorum* adversely affect sperm characteristics; though it appears to promote biosynthesis of FSH and LH. Its potential to enhance fertility is therefore in doubt.

Keywords: *Tetracarpidium conophorum*, sperm characteristics, male fertility hormones

1. Introduction

Tetracarpidium conophorum is an economic plant cultivated for its nut production widely used as nutritional delicacy. It is commonly found in the temperate, sub-saharan and tropical regions of the world¹. Its common parlance is African Walnut, reputed for its very wide and multiple uses both nutritionally and economically².

Phytochemical constituents of *Tetracarpidium conophorum* include quinines, oils, carbohydrates, tannins, proteins, vitamins and minerals^{3,4}. The major constituents of the oil are triglycerides, fatty acids, diglycerides, sterols and esters⁵. The vitamin content include thiamine, riboflavin, niacin, folate, pantothenic acid, folic acid, gallic acid, vanillic acid and phenolic acid³.

There have been few reports that walnut has the potential to boost male fertility⁶. Other studies have also demonstrated that *Tetracarpidium conophorum* have a significant antioxidant activity⁷. This property appears to be directly linked to the phenolic content of *Tetracarpidium conophorum* as many researchers have reported positive correlation between free radical scavenging activity and total phenolic compound content⁸.

Reactive oxygen species (ROS) has been implicated in the mechanism of fragmentation of spermatozoa DNA^{9,10}. Fertility capacity and attainment is hinged on the ability to produce a viable male reproductive gamete, the spermatozoa. The processes involved in the development and maturation of spermatozoa is principally directed and regulated by the relevant reproductive hormonal component. Briefly, GnRH act through cAMP as second messenger to stimulate FSH and LH secretion from the anterior pituitary. FSH and LH inturn act on the sertoli and leydig cells to trigger spermatogenesis and testosterone production respectively. This is a component of the intratesticular paracrine mechanism in the regulation of testicular activity. Both the sperm quantity and quality is secured by the optimal secretion and activity of FSH, LH and prolactin. Moderate level of prolactin is reported to potentiate the action of testosterone while hyperprolactinaemia is known to impair fertility in the male¹¹⁻¹³.

There is WHO approved normal ranges for each of the sperm parameters commonly analysed in the assessment of male fertility status. These include sperm count or concentration, motility, morphology, volume, fructose level and pH. Sperm count or concentration measures the concentration of sperm in an ejaculate while total sperm count is the sperm count multiplied by volume¹⁴. The sperm count and motility are the most critical determinant of its viability¹⁵.

Male fertility status evaluation usually involved analysis of both reproductive hormones (GnRH), FSH, LH, Testosterone, prolactin) and sperm characteristics. At the moment, there appears to be paucity of literature on the comprehensive assessment of the hormonal and sperm indices in the same experiment setting, vis-à-vis the effect of *Tetracarpidium conophorum* on them; this study was therefore undertaken to investigate the overall effect of *Tetracarpidium conophorum* on the reproductive hormones and sperm characteristics of male albino wistar rats.

2. Methods and Material

2.1 Plant Material

Tetracarpidium conophorum seeds (walnuts) were gotten from the cementary market in Aba, Abia State of Nigeria. They were gotten in large quantities, fresh and uncooked. The seeds were later identified and authenticated by a senior technologist in the Department of Botany and Ecological

Studies of the University of Uyo, Akwa Ibom State, Nigeria. The fruits were cooked for about two and a half hours, after which they were removed from the water, allowed to cool for about thirty minutes. A light hammer was then used to break up the nut shell of the fruits. Boiling was done to reduce its toxic effect as fresh walnut can be corrosive to the mouth. The edible part were put into an electric blender and blended into powder. 200ml of distilled water was added to it and allowed for 24 hours. After 24 hours it was filtered and the residue was recovered and filtrate obtained, stored in a cork sealed container and put into the refrigerator for use.

2.2 Animal Preparation, Experimental Groupings and Treatment

Eighteen male albino wistar rats were used for this study. The animals were randomly assigned to one of three groups such that each group had six (6) animals. After fourteen days of acclimatization, oral administration of *Tetracarpidium conophorum* extract to groups 2 and 3 commenced. Group 1 served as the control group fed with normal rat chow (feed) and 5ml/kg of distilled water. Group 2 was treated orally with a medium dose of *Tetracarpidium conophorum* extract (14.14mg/kg). Group 3 was treated orally with a high dose of *Tetracarpidium conophorum* (21.21mg/kg). With an oral cannula, these doses were administered twice daily for 21 days. All groups had access to water ad libitum. The animals were sacrificed on day 22. As a preliminary procedure, the medium lethal dose (LD₅₀) of the plant extraction was determined by method of Lorke (1983)¹⁶ and found to be toxic at 500mg/kg. All experiments were examined and approved by the ethical committee of the University of Uyo on Animal Research and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

2.3 Sperm Cell Concentration

Testes were crushed into pieces, diluted in 5mls of normal saline and allowed for 5 - 10minutes to enable the spermatozoa to spread out into the diluents solution. 1ml of supernatant was diluted in 100mls solution e.g 0.1 of solution 1 + 5mls of solution. 0.01 of the suspension was loaded into a charged Neubauer counting chamber and cover slipped. It was allowed to rest for 10minutes and observed microscopically. Number of cells were counted in millions/ml.

2.4 Sperm Morphology

1ml of seminal fluid was diluted with 20mls of buffered formol saline and then 0.01ml of the solution was loaded on a grease free slide with cover slip and viewed under a microscope and the following were observed: tail defect, neck defect, mid-piece defect, head defect and percentage of normal morphology.

2.5 Sperm Motility

1ml of seminal fluid was diluted with 20mls of buffered formol saline and 0.01ml of the solution was loaded on a grease free slide and covered with a cover slip and observed microscopically.

2.6 Serum FSH, LH and Prolactin Measurements

The FSH-RT, LH-RT and PROLACTIN-RT each is a one-step immunoassay, based on the principle of sandwich method. The assay system utilizes a high affinity and specificity monoclonal antibody (enzyme conjugated and immobilized) directed against a distinct antigenic determinant on the intact FSH, LH and prolactin molecule. The test sample is allowed to react simultaneously with two antibodies, resulting in the FSH, LH and prolactin molecules being sandwiched between the solid phase and enzyme- linked antibodies.

After incubation, the wells are washed with washing solution to remove unburned labeled antibodies. Tetra methyl benzidine substrate is added and incubated, resulting in the development of a blue colour. The colour development is stopped with addition of stopping reagent, changing the colour to yellow. The concentration of FSH, LH and prolactin is directly proportional to the colour intensity of the test sample. Absorbance is measured spectrophotometrically at 450nm.

2.7 Serum Testosterone Measurement

Testosterone level is determined using competitive microplate enzyme immunoassay. Plates are coated with anti-testosterone antibodies. Calibrator specimen is first added to microplate well. Enzyme testosterone conjugate is added. Testosterone present in the sample competes with enzyme-testosterone conjugate for building with anti-testosterone counted microplate to form an antigen-antibody complex. Unbound conjugate is removed by washing. The enzyme activity in the antibody-bound fraction is inversely proportional to the native testosterone concentration. The enzyme activity is revealed by colour change in tetramethylbenzidine substrate solution.

2.8 Statistical Analysis

All results were presented as mean \pm standard error of mean. Three sets of data were analyzed using one way ANOVA, followed by the least significant difference (LSD) procedure for significant F values, ($P < .05$) was considered significant. Computer software SPSS and Excel Analyzer was used for the analysis.

3. Results

3.1 Comparisons of FSH levels ($\mu\text{mol/ml}$) in different experimental groups treated with aqueous extract of *Tetracarpidium conophorum*.

Mean values of FSH level in control; medium dose and high dose were, mean \pm SEM, 0.12 ± 0.02 , 0.18 ± 0.02 , and 0.40 ± 0.03 . There was a significant increase in FSH level in both medium and high dose. There was significant increase in medium dose compared to control ($p < 0.05$), there was significant increase in high dose ($p < 0.01$) compared to the control group. There was a significant increase in higher dose ($p < 0.05$) compared to medium dose.

3.2 Comparisons of LH levels ($\mu\text{mol/ml}$) in different experimental groups treated with aqueous extract of *Tetracarpidium conophorum*.

Mean values of LH in control, medium dose and high dose mean \pm SEM, 0.12 ± 0.02 , 0.18 ± 0.02 , 0.20 ± 0.04 respectively. There was a significant ($P < 0.05$) increase in LH levels in both medium and high dose groups when compared to the control group.

3.3 Comparisons of prolactin levels ($\mu\text{mol/ml}$) in different experimental groups treated with aqueous extract of *Tetracarpidium conophorum*.

Mean values of prolactin in control, medium dose and high dose were, mean \pm SEM, 0.15 ± 0.02 , 0.18 ± 0.02 , 0.20 ± 0.02 respectively. There was a marginal increase in prolactin levels in both medium when compared to the control group and significant increase in high dose groups when compared to the control group.

3.4 Comparisons of testosterone levels ($\mu\text{mol/ml}$) in different experimental groups treated with aqueous extract of *Tetracarpidium conophorum*.

Mean values of testosterone in control, medium dose and high dose were mean \pm SEM was 8.12 ± 2.01 , 9.85 ± 2.63 , 13.82 ± 2.74 respectively. It was observed to be statistically insignificant but there was a dose dependent marginal increment in testosterone level

3.5 Comparisons of fast progressive movement (sperm motility) ($\mu\text{mol/ml}$) in different experimental groups treated with aqueous extract of *Tetracarpidium conophorum*.

Mean values of fast progressive movement (sperm motility) in control, medium dose and high dose groups were mean \pm SEM, $71/67 \pm 2.79$, 60.00 ± 1.83 , 46.00 ± 1.32 respectively. There was a significant decrease in medium dose ($p < 0.01$) compared to the control group, there was a significant decrease in high dose ($p < 0.001$) compared to control. There was also a significant dose dependent decrease in high dose compared to the medium dose ($p < 0.001$).

3.6 Comparisons of percentage sperm concentration ($\mu\text{mol/ml}$) in different experimental groups treated with aqueous extract of *Tetracarpidium conophorum*.

Mean values of percentage sperm concentration in control, medium dose and high dose were mean, \pm SEM, 69.67 ± 2.08 , 38.67 ± 1.87 , 58.67 ± 1.38 respectively. There was a significant decrease in medium dose ($p < 0.001$) compared to control, there was also a significant decrease in high dose ($p < 0.01$) compared to control. There was also a significant increase in high dose ($p < 0.001$) compared to medium dose.

3.7 Comparisons of percentage normal sperm morphology levels ($\mu\text{mol/ml}$) in different experimental groups treated with aqueous extract of *Tetracarpidium conophorum*.

Mean values of percentage normal sperm morphology in control, medium dose and high dose were mean \pm SEM, 81.33 ± 0.56 , 59.00 ± 0.73 , 49.33 ± 0.92 respectively. There was a significant decrease in medium dose ($p < 0.001$) compared to control, a significant decrease in high dose ($p < 0.001$) compared to control and there was a significant decrease in high dose ($p < 0.001$) compared to medium dose.

Fig. 1: Comparison of follicle stimulating hormone (FSH) levels in different experimental groups treated with *Tetracarpidium conophorum*

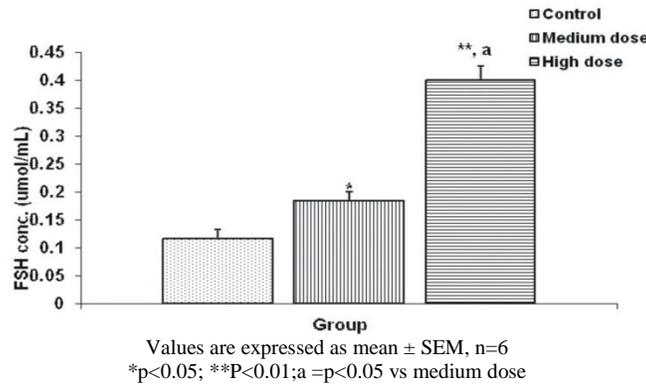


Fig. 2: Comparison of luteinizing hormone (LH) levels in different experimental groups treated with *Tetracarpidium conophorum*.

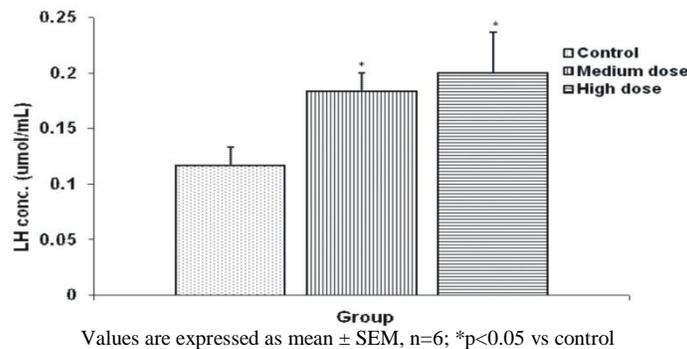


Fig. 3: Comparison of prolactin levels in different experimental groups treated with *Tetracarpidium conophorum*.

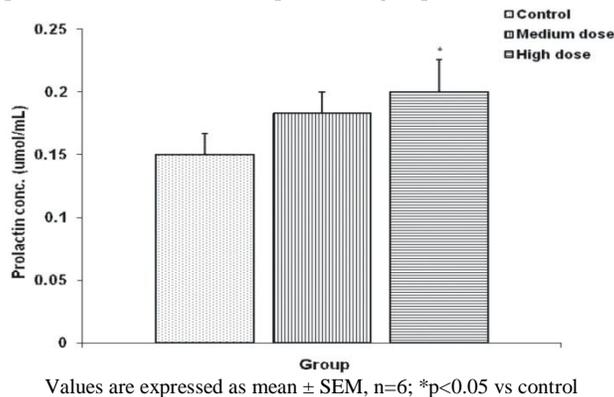


Fig. 4: Comparison of testosterone levels in different experimental groups treated with *Tetracarpidium conophorum*.

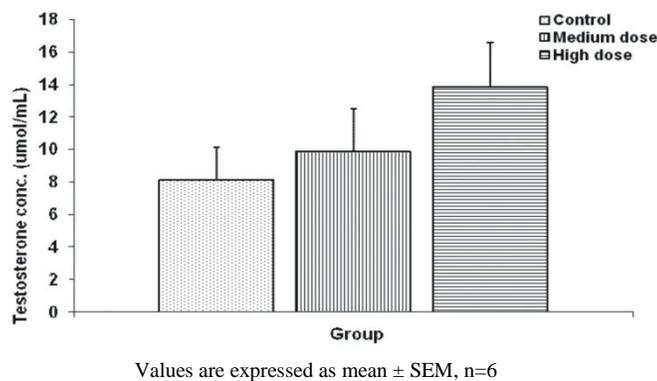
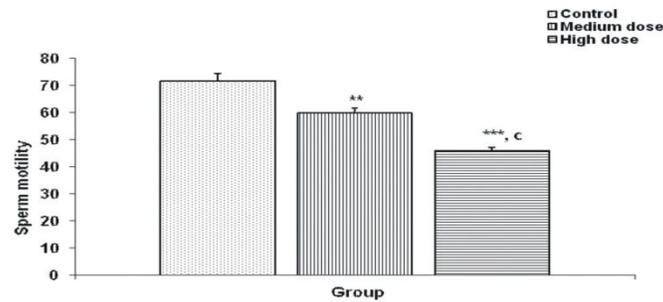
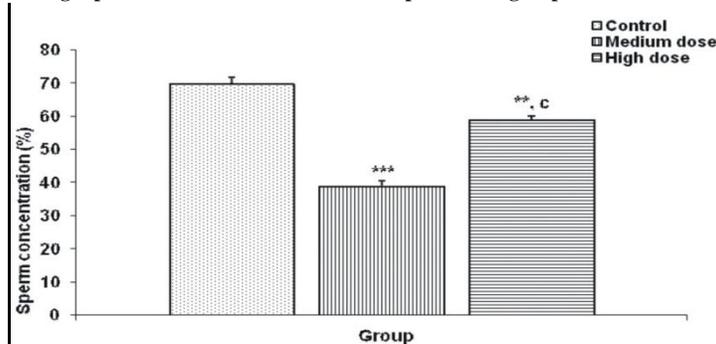
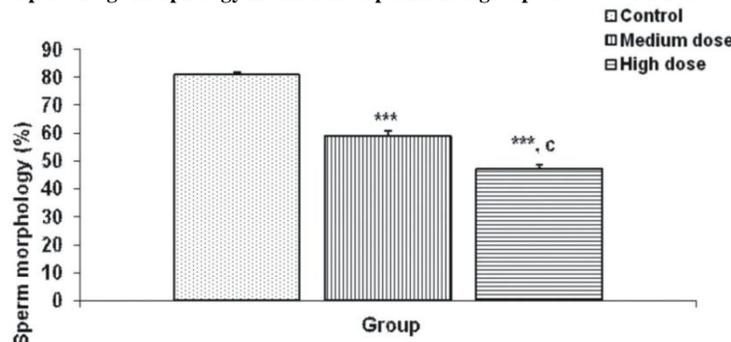


Fig. 5: Comparison of fast progressive movement of sperm in different experimental groups treated with *Tetracarpidium conophorum*.

Values are expressed as mean \pm SEM, n=6; **p<0.01; ***p<0.001 vs control; c = p<0.001 vs medium dose

Fig. 6: Comparison of percentage sperm concentration in different experimental groups treated with *Tetracarpidium conophorum*.

Values are expressed as mean \pm SEM, n=6; **p<0.01; ***p<0.001 vs control; c = p<0.001 vs medium dose

Fig. 7: Comparison of percentage morphology in different experimental groups treated with *Tetracarpidium conophorum*.

Values are expressed as mean \pm SEM, n=6; ***p<0.001 vs control; c = p<0.001 vs medium dose

4. Discussion

The gonadotrophins are principally responsible for the initiation, direction and regulation of reproductive activities. Their estimation is commonly employed in the diagnosis of infertility cases and the prognosis on treatment. Almost all the actions of the reproductive hormones are consummated on the development of a viable gamete capacitated to fertilize the ovum. Therefore the assessment of fertility parameters in the male must involve both hormonal and seminal indices. On the basis of this fact, this study was therefore undertaken to comprehensively determine the effect of *Tetracarpidium conophorum* on these indices. From the results it is evidenced that *Tetracarpidium conophorum* affect both the hormonal and seminal parameters estimated in this study in the positive and negative direction respectively.

Significant increases were observed for FSH and LH; a marginal increase for prolactin and testosterone; while the fast progressive movement of sperm (motility), percentage sperm concentration and percentage normal morphology were significantly reduced.

FSH is the key hormone involved in spermatogonia proliferation and also play supportive role in other steps of the spermatogenic processes. Therefore, an increase as obtained in this study should have, as expected promote spermatozoa maturation and viability. However, previous report had indicated that higher concentration of FSH is considered to be a reliable indicator of germinal epithelial damage and was shown to be associated with azoospermia¹⁷. This possibly implies that in this study, FSH might have been secreted in higher concentration due to the absent of the relevant negative feedback signal that would have been driven by an optimal testicular function. We therefore postulate that, some toxic phytochemical constituent of *Tetracarpidium conophorum* might have injured the testes. FSH is known to act through its receptors on the cells of Sertoli, coupled through a Gs protein to adenylate cyclase to generate cAMP which serves as a second messenger to activate protein kinase A (pkA) that stimulate spermatogonia proliferation and maturation through series of phosphorylation steps¹⁸. Inhibin secreted at the same time from the Sertoli cells conveys a negative feedback signal to inhibit further FSH release. Therefore both spermatogonia development and the negative feedback signal might have been impaired secondary to dysfunctional testicular tissues. FSH levels may therefore represent an imbalance in the gonadal pituitary feedback mechanism^{19, 20}.

LH receptors are located on the interstitial cells of Leydig of the testes. LH stimulates testosterone synthesis and secretion from the Leydig cells²¹. Testosterone on the other hand relays a negative feedback impulse to the hypothalamic nuclei that secrete GnRH and the gonadotrophs cells of the adenohypophysis that secrete LH, thus inhibiting its secretion. In the absence of a negative feedback impulse conferred by testosterone, again it implies that LH secretion will proceed unabated. From our findings it is likely that this negative feedback signal was lacking. Damage to the Leydig cells by the toxic component of *Tetracarpidium conophorum* again could account for this adverse occurrence. Another findings that support this proposition is the fact that testosterone levels were not significantly raised in this study, probably due to the non-responsiveness of the damage Leydig cells to LH stimulation.

A dose dependent increase was seen for prolactin. Moderate levels of prolactin had previously been reported to potentiate the action of testosterone¹¹⁻¹³. Two hypothetical mechanisms may elucidate this finding: i. A significant increase in prolactin levels could be provoked as a compensatory reaction to augment for the low activity of testosterone in direct relationship to its low levels. ii. The phytoconstituents of *Tetracarpidium conophorum* appears to promote the activity of all the secretory cells of anterior pituitary.

A disruption in spermatogenic process will lead to a decrease in sperm production²². This explains the reduction in the sperm count in our result. It has also been reported that suppression of spermatogenesis may result in production of immature sperm cell as seen in some pathologic conditions such as "Sertoli cell only syndrome" and spermatid arrest²³. Surprisingly, a decreased normal morphology point directly to the possibility of a fragmentation process⁹. This therefore indicates that the toxic phytoconstituent of *Tetracarpidium conophorum* over-rided this as previously reported in other studies⁷. A combination of this pathologic mechanism may therefore account for the reduction in motility, concentration and normal morphology of sperm.

Various medicinal plants have been reported to have adverse effect on sperm count, morphology, motility and its overall viability²⁴⁻²⁶. Moreover, some were observed to increase the hormonal component of the reproductive orchestra especially FSH²⁷. Identification and isolation of the specific toxic phytoconstituent responsible for this adverse effect on the testicular tissue should be aggressively undertaken by the scientific research community without further delay. This will avert the danger posed by the common practice of unregulated consumption and administration of most delicacies in their natural form and undermining their potential toxic unwittingly especially on the reproductive system, that may not present with immediate complaint which our present findings strongly support.

5. Conclusion

From the findings above, we therefore report that *Tetracarpidium conophorum* though it appears to promote the biosynthesis and secretion of fertility hormones in rats, also show a clear evidence of toxic damage to the spermatozoa. There may be need for caution on the excessive consumption of *Tetracarpidium conophorum* especially in males with fertility challenges, if these results in rats can be extrapolated to man.

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