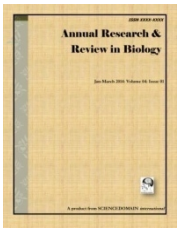

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Sperm Quality and Hormone Profile of Male Albino Rats FED with Seeds of African Walnut (*Tetracarpidium conophorum,* Mull)

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Herbal plants have been found to be effective in traditional medicine for healthcare. However, the full potentials of these natural resources are yet to be fully explored. This research was coined at investigating the effects of *Tetracarpidium conophorum* seeds on the hormone and sperm profile of male albino rats. Forty eight albino rats of about twelve weeks weighing between 130-180g each were divided into four groups (A, B, C and D) with twelve rats in each group. The test extract was obtained from the seeds and incorporated into the feed of the rats. Group A served as the control (without test substance) while groups B, C and D were fed with of 4, 8 and 12g/kg body weight (BW) of the test substance for the period of 63 days. Respectively the results obtained from hormonal analysis show that there are significant difference ($p<0.05$) in the serum level of follicle stimulating hormone (FSH) and luteinizing hormone (LH) of the rats between the different treatment groups while the serum levels of testosterone and estradiol were not significant ($p>0.05$). Results of semen quality show that there are significant differences ($p<0.05$) in the sperm count, sperm morphology, sperm viability and semen pH among rats between the different groups. Sperm motility and organ weights (testes and epididymides) were not significantly affected ($p>0.05$). The results indicate that the seeds of *T. conophorum* can enhance the

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production of reproductive hormones and may be used in the formulation of useful fertility drugs.

Keywords: Tetracarpidium conophorum; hormones; sperm.

1. INTRODUCTION

Traditional medicine has been used to complement conventional medicine in the healthcare system of the Federal Government of Nigeria. Herbal plants provide the major source of ingredient in the formulation and packaging of drugs. This is occasioned by their high efficacy with no or very little side effects [1]. According to [2], synthetic drug consumers in developed countries are becoming disillusioned and disenchanted with modern health-care and are therefore seeking for alternatives. This suggests a paradigm shift towards exploring, exploiting and optimizing our forest reservoir African walnut (*Tetracarpidium conophorum*) is a climbing shrub in the family of Euphorbiaceae [3]. The plant is locally cultivated mainly for the nuts which are cooked and consumed as snacks [4]. It is locally used by elderly people as a constipation treatment. The amino acid and fatty acid components of the nut are used for the treatment of prolonged and constant hiccups [5]. The seeds are also incorporated into livestock feed formulation [6]. They are used as tonic for the kidneys, strengthening of back and knees, and moistening of intestine [3]. The barks are used in coffee as laxative and also chewed for reducing toothache. The ascorbic acid present in the seeds can be used for the treatment of skin diseases such as eczema, pruritus, psoriasis, common cold, prostate cancer and male fertility booster [7,8,9,10]. Though there are various reports on the proximate, vitamins and phytochemical composition of the seeds of this plant, however, there are paucity of information about its effects on sperm and hormonal functions. Thus, the aim of the current research was to investigate the sperm and hormonal effects of the seed of *T. conophorum* on male albino rats in order to ascertain its possible usefulness as a fertility agent.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Materials

The seeds of African walnut (*T. conophorum*) were obtained from Watt Market Calabar, Cross River State, Nigeria and properly identified in the herbarium unit of the Department of Botany, University of Calabar. The seeds were chopped into pieces, sundried for 48hours and milled into fine powder using electric blender (Model: Lapriva 201).

2.2 Experimental Animals and Administration of Extract

Forty eight sexually matured male albino rats of about twelve weeks weighing between 130-180g were obtained from the animal house unit of the Department of Physiology, University of Calabar, Calabar. They were kept in a well ventilated conventional cage where they acclimatized for two weeks with water and feed *ad libitum* before commencement of treatment. Ethical care and handling of experimental animals was observed at all times and the study was approved by the University of Calabar ethical committee. The rats were then divided into four groups (A, B, C and D) using completely randomised design with twelve rats in each group. Room temperature range was maintained in each cage, the light cycle was set at day hours while the dark cycle was set at night hours respectively. Rats in group A

served as the control and were fed with normal commercial feed only, group B, C and D were fed with 4, 8 and 12g/kg BW of the powdered test material respectively via dietary inclusion for the period of 63 days. At the end of the treatment regime, the rats were anaesthetized using diethyl ether. The testes and epididymides were surgically removed and weighed. Blood samples, 15ml per each rat were collected through cardiac puncture into sterile tubes for hormonal analysis.

2.3 Hormone Assay

The blood samples collected were centrifuged at 2500rpm for 5min using Wisperfuge model 1384 centrifuge (Tamson, Holland) at 10-250C to obtain the serum sample which was analysed for testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol hormone level using enzyme linked immunoassay (ELISA) technique; using analytical grade reagents (Syntron Bioresearch Inc., USA) [11].

2.3.1 Test procedure for testosterone

Sufficient wells for calibrators, control and the test samples were properly placed in duplicate. 50µl of each calibrator, control and test samples was added to the corresponding labelled wells in duplicate followed by addition of 100µl of conjugate working solution. The solution was properly mixed and incubated on a plate shaker (approximately 200rpm) for 1hr at room temperature. Each well was washed properly with 300µl of diluted washed buffer and the plate firmly tapped against absorbent paper to ensure that it was dry. 150µl of tetramethylbenzidine (TMB) substrate was added to each well and incubated for 15 minutes at room temperature before the addition of 50µl stop solution. The absorbance was read at 450nm within 20 minutes after addition of the stop solution using spectrophotometer (model: Labtech: Advanced microprocessor uv-vis spectrophotometer single beam- 295).

2.3.2 Test procedure for follicle stimulating hormone (FSH)

Sufficient coated wells in a holder were placed to run 0.0MIU/ml, 25MIU/ml, 50MIU/ml and 100MIU/ml for FSH calibrators, control and the test samples in duplicate. 50µl of each sample was pipetted into the corresponding coated well with addition of 200µl of the enzyme antibody conjugate solution. The solution was properly mixed and incubated at room temperature for 45 minutes. The wells were properly washed with wash buffer and deionised water and decanted. 100µl substrate-chromogen solution was added to each well, mixed thoroughly and incubated for 15 minutes at room temperature. Immediately after incubation, 100µl of $1NH_2SO_4$ was added to each well and properly mixed. The absorbance of each well was read at 450nm spectrophotometerically. A graph of absorbance versus concentration of FSH was plotted to read off the actual amount of FSH in the test samples.

2.3.3 Test procedure for luteinizing hormone (LH)

Sufficient coated wells in a holder were placed to run 0.0MIU/ml, 25MIU/ml, 50MIU/ml and 100MIU/ml for LH calibrators, control and the test samples in duplicate. 50µl of the calibrators, control and the test samples was added to the corresponding coated well. 200ml of the enzyme antibody conjugate solution was added to all the wells, properly mixed and incubated at room temperature for 45 minutes. The wells were properly washed with wash buffer and deionised water and decanted. 100µl of substrate-chromogen solution was added to each well, mixed properly and incubated at room temperature for 15 minutes. This was followed by addition of 100µl of $1NH_2SO_4$ stop solution. The solution was properly mixed and

the absorbance was read at 450nm against water using spectrophotometer. A graph of absorbance versus LH concentration was plotted to obtain the actual amount of LH in the test samples.

2.4 Semen Analysis

2.4.1 Determination of epididymides and testes weight

The epididymides were dissected out and excess blood damped with cotton wool and placed in a clean weighing balance (Model: Mettle LA×214) to record the weight. (Grand G model: JJ 500ld = 0.01g).

2.4.2 Evaluation of sperm motility

Semen samples from the different treatment groups were dropped on a glass slide and viewed under the microscope. A minimum of five microscopic fields were assessed to evaluate sperm motility on at least 200 spermatozoa for each rat. The percentage of sperm motility was analyzed for progressive motile sperm (PMS), non-progressive motile sperm (NPMS) and non-motile sperm (NMS) distinguished by the movement of the sperm cells [12].

2.4.3 Estimation of mean sperm count

This was carried out according to the method of [13]. The epididymal content was obtained by macerating with fine scissors known weights of the caput and cauda epididymides in a glass petridish containing warmed buffered physiological saline in the ratio of 1:10w/v. After vigorous pipetting, the suspension was separated from tissue fragments by filtering it through an 80µm stainless mesh. A tissue-free aliquot was loaded into the Neubauer haemocytometer (Deep1/10, Labart, Germany). Five different counts were done for each sample, and the mean were taken as the mean count for each male rat.

$$\text{Sperm count} = \frac{\text{Total number of sperm cells in the cytometer.}}{\text{Mean value.}}$$

Where; mean value = Five.

2.4.4 Estimation of sperm viability

This was estimated using the improved one step eosin-nigrosin staining technique. A fraction of each suspension of the sperm samples was mixed with equal volume of eosin–nigrosin stain and air dried smears were prepared on glass slides for each samples according to [14]. The slides were coded randomly and examined under the microscope for percentage viability. Normal live sperm cells exuded the eosin–nigrosin while dead sperm cells took up the stain. Percentage viability was calculated based on the number of viable (live) sperm cells divided by the number of sperm cells within 30 minutes multiply by 100.

2.4.5 Estimation of semen pH

The pH of semen was measured using a specially treated calibrated paper blot that changes color according to the pH of the semen that it is exposed to [15].

2.4.6 Sperm head abnormalities

A fraction of each of the sperm suspension was examined by placing the solution (10:1) for 30min on a glass slide. The slide was examined for percentage abnormalities in every 200 spermatozoa observed on each slide and five air dried smear was prepared on glass slide for each sample according to [16].

2.5 Statistical Analysis

All data collected on sperm and hormonal assays were subjected to analysis of variance (ANOVA) using Predictive Analytics Software (PASW), version 18.0. Significant means were separated using the least significant difference at 5% probability level.

3. RESULTS

3.1 Effect of *T. conophorum* Seed Powder on Hormonal Profile

Our result show that the seed powder of *T. conophorum* had no significant effect ($p>0.05$) on testosterone and estradiol levels of rats in the various treatment groups Table 1. For Luteinizing hormone (LH), rats fed with 4, 8 and 12g/kg of the test substance were all higher (12.36, 14.28 and 16.10Mln/ml) than rats in the control group (8.32Mln/ml). However, for follicle stimulating hormone (FSH), the results indicate that there are significant differences ($p<0.05$) among rats in the different groups. The rats fed with 12g/kg BW had the highest level of FSH (2.2Mln/ml).

Table 1. Effects of the seed meal of *T. conophorum* on hormonal profile of male albino rats

Parameters	0	Concentration 4	(g)/kg BW 8	12
Testosterone (ng/ml)	4.8^{a}±0.02	4.92^{a}±0.01	5.62^{a}±0.01	5.98^{a}±0.04
Estradiol (ng/ml)	0.49^{a}±0.06	0.50^{a}±0.1	0.50^{a}±0.01	0.86^{a}±0.03
Follicle stimulating hormone (MIU/ml)	1.6^{c}±0.003	1.6^{c}±0.003	1.97^{b}±0.002	2.2^{a}±0.002
Luteinizing hormone (MIU/ml)	8.32^{b}±0.03	12.36^{a}±0.02	14.28^{a}±0.01	16.10^{a}±0.01

$^{(abc)}$ *means followed with different superscript along the same horizontal array indicate significant difference ($p<0.05$)*

3.2 Effect of *T. conophorum* Seed Powder on Sperm Profile

T. conophorum had no significant effect ($p>0.05$) on the weight of the testes, epididymides and sperm motility (both progressive motile sperm and non-progressive motile sperm) at the different treatment doses Table 2. However, results show that there is significant differences ($p<0.05$) in the sperm count, sperm head abnormalities and sperm viability which was dose-dependent. The sperm counts were increased as the concentration increased, rats fed with 4g/kg BW (72.25%) was significantly different from rats in the control (65.5%) while rats fed with 8g/kg BW (84.75%) also showed no significant different from rats fed with 12g/kg BW (99.25%). The results also indicated that the 12g/kg BW had the highest effect ($p<0.05$) on semen pH (7.00).

Table 2. Effects of the seed meal of *T. conophorum* on sperm profile of male albino rats

Parameters	Concentration (g)/kg BW			
	0	4	8	12
Sperm head abnormalities (%)	52.5 ^{c}±2.41	67.5 ^{b}±2.42	70.0 ^{b}±2.30	82.5 ^{a}±2.31
Sperm viability (%)	57.5 ^{c}±2.48	68.5 ^{b}±2.42	72.5 ^{b}±2.32	85.6 ^{a}±2.31
Progressive motile sperm (%)	47.5^{a}±1.86	51.25^{a}±1.60	55.0^{a}±1.58	61.25^{a}±1.21
Non progressive motile sperm (%)	17.5^{a}±1.44	20.0 ^{a}±1.42	17.5 ^{a}±1.44	13.75 ^{a}±1.82
Sperm count 10^{6}/ml	65.5 ^{b}±1.71	72.25 ^{b} ±1.63	84.75 ^{a}±1.60	99.25 ^{a}±1.50
Semen pH	6.0 ^{b}±0.002	6.80 ^{c}±0.02	6.91 ^{c}±0.004	7.0 ^{a}±0.001
Weight of testes (g)	1.14 ^{a}±0.002	1.01 ^{a} ±0.001	1.12 ^{a} ±0.002	1.19 ^{a}±001
Weight of epididymides (g)	0.31 ^{a}±0.001	0.31 ^{a} ±0.001	0.35 ^{a} ±0.001	0.36 ^{a}±0.001

$^{(abc)}$ means followed with different superscript along the same horizontal array indicate significant difference ($p<0.05$)

4. DISCUSSION

Plant based medication has been man's ultimate therapeutic agent over the years and is still in the frontline for improving human health [17]. *T. conophorum* is highly exploited in enthomedicine due to various reports on its therapeutic values. The administration of the seed powder of *T. conophorum* had a significant effect ($p<0.05$) on the FSH, which was dose-dependent. Tesosterone, estradiol and luteinizing hormone (LH) were not also significant among the rats in the different groups. It is interesting to mention the fact that the seeds of *T.conophorum* contains important bioactive component [3] whose effect can singly or synergistically enhance the biosynthetic processes underlying hormonal production. This presupposes that the said bioactive components in the seeds might have probably enhanced FSH in the rats. Though the seed extract of *T. conophorum* did not have significant effect on the testosterone, there was an observed difference on the sperm viability among rats in the different groups which could probably be as a result of the presence of vitamin E in the extract, a known male fertility agent [10]. At this point, it becomes imperative to mention the fact that testosterone cannot function alone in enhancing reproduction. It requires other reproductive hormones to function effectively. Thus, the dose-dependent enhancing ability of FSH by the test substance must have been responsible for the significant increase in the viability of the sperm cells, the level of testosterone notwithstanding. This could also be because FSH is indirectly linked with spermatogenesis [18].

Distortion in the cellular mechanism of testicular cells malign spermatogenesis [19], which apparently affect the quality and quantity of sperm cells. It thus suggests that the treatment had no significant distortion in the testicular cells of the rats that could lead to decrease in sperm count. According to [2,13,20], some medicinal plants lead to decrease in sperm count, sperm viability, sperm motility and reproductive hormones by disrupting spermatogenic pathways. This was contrary to our present result on sperm count as the treatment increased the sperm count of the rats dose-dependently. The sperm counts were increased from 65.5 x106/ml of the control to 99.25x106/ml at the highest concentration of 12g/kg BW Table 2. Although there were no significant difference in the level of testosterone among the rats, however, the level of this testosterone must have been enough to cause increase in the sperm count, its level of significant notwithstanding. It could be understood that FSH and LH work simultaneously with testosterone during spermatogenesis. This might be a contributing factor to enhance sperm count [18]. This increase in sperm count might also not be unconnected with the presence of bioactive component such as vitamin E and zinc in the extract [10]. It could be possible that the antioxidant potential of vitamin E in the

seeds must have played a major role in scavenging free radicals that might accumulate to reduce the number of sperm cells thereby leading to an increase in the sperm counts. There was also significant effect of the test substance on the sperm head abnormalities. Abnormalities such as pin heads, double heads and round heads were detected on the sperm cells of the treated rats. At this juncture one might expect a concomitant reduction in the sperm viability of the rats but this was contrariwise. This could therefore suggest that the level of morphological distortions on the sperm cells induced by the test substance was not enough to affect viable sperm cells. On the other hand, the administration of the seed powder of T. *conophorum* did not have any significant effect ($p>0.05$) on sperm motility (progressive motile sperm and non progressive motile sperm), testes and epididymides weight of the rats in the control and the different groups Table 2. According to [20], it could be possible that the inherent bioactive compounds in the seeds might have caused immobility or had weakening effects on the sperm cells. Understandably, it could be established that those bioactive compounds in the seeds of *T. conophorum* responsible for increased sperm count have a reverse effect on sperm motility, testes weight and epididymides weight of the male albino rats.

5. CONCLUSION

Undoubtedly, the consumption of *T. conophorum,* especially by the local people who may have little or no knowledge on the proper dosage could result in compromised reproductive pathways. Implicitly, administration of the seeds of *T. conophorum* caused an increase in the viability and sperm output of the male albino rats. Though this might suggest a possible incorporation of the seed in the formulation male fertility drugs, on the contrary, holistic measures should be taken regarding the observed effect on sperm head abnormalities of the rats. Further research similar to this work will be an additional premise to the current report.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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