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Subchronic *Cola acuminata* Seed Exposure: Effects on Body Weight and Male Reproductive Parameters in Rats

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Abstract: *Objectives*: To investigate the effects of 60 days oral administration of aqueous *Cola acuminata* seed extract on body weight, testosterone level, sperm parameters and testis histology. *Design*: This is an experimental animal study. *Setting:* This study was conducted at the Department of Pharmacology and Toxicology, Niger Delta University, Nigeria. *Methods*: Twenty-four Wistar albino rats weighing 100-120 g were randomly distributed into four equal groups (N=6). They were administered 0, 25, 50 or 100 mg/kg body weight of extract by oral gavage. *Results*: Phytochemical analysis of extract showed very high amounts of alkaloids and high amounts of saponins, tannins and steroids. There was increase in body weights of treated groups which was comparable to control. Serum testosterone concentration was reduced (P<0.05) in extract administered rats compared to control. Sperm motility and count were equally reduced (P<0.0001; P<0.01) in extract exposed rats. In addition, sperm morphology was altered; proportion of spermatozoa with normal morphology was reduced (P<0.0001) dose-dependently by extract, while the reverse was observed in sperms with abnormal morphology. Furthermore, there was dose-dependent distortion in the histoarchitecture of testes of rats that received 50 and 100 mg/kg extract. This was characterized by exudation or necrosis in interstitial tissue, loss of Leydig cell, as well as cytolysis, anisocytosis or atrophy of tubular cells. The results demonstrate that sub-chronic exposure of *Cola acuminata* seed does not affect body weight but altered sperm characteristics, reduced testosterone level and altered testicular histology of rats.

Key words: *Cola acuminata* · Kola Nut · Sperm Parameters · Testosterone

INTRODUCTION

Cola acuminata seed, commonly known as ‘‘kola nut’’ or ‘‘cola nut’’ is one of the most widely consumed plants in Africa. It belongs to the Family, Sterculiaceae and is indigenous to West Africa, particularly Sierra Leone, Liberia, Ivory Coast and Nigeria [1]. Other species of the Coca genus that are commonly used include, *C. nitida, C. ballayi, C. verticillata* and *C. sphaerocarpa*, but only *C. acuminata* and *C. nitida* are of significant economic importance [2]. It is a slender tree which grows to a height of 10-20 m and diameter of about 30 cm. It grows with many branches which are frequently divided, slender crooked and often close to the ground.

Cola acuminata is very popular in Nigeria and is identified by different local names such as ‘‘Guoro’’ in the north; ‘‘Oji’’ in the east; and ‘‘Obi’’ in the west. Generally, the plant is popular for its use as a stimulant. It produces euphoria and is also used to suppress appetite, increase alertness and energy [3]. These effects are basically due to the large content of caffeine and smaller amounts of theobromine, kolatin and glucose which act as stimulants and may also be mildly addictive [2, 4]. It is also effectively used in refreshing the mouth because of its characteristic bitter taste and the twigs are used for this purpose as chewing sticks to clean the teeth and gums [5]. Preparations of various parts of the plant are used as remedies for different conditions like dysentery, fatigue,

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cough, diarrhea, fever and chest pain [3, 6, 7]. Kola nut is also of great religious and socio-cultural significance in many African societies. Among the various cultures using it, kola nut plays important cultural roles in birth ceremonies, marriage ceremonies and death rites. The presentation of kola nut is used to express friendship, respect and hospitality and it is exchanged between parties during business dealings [1, 8]. Also, it is used in divination and other ritual activities [8].

A number of pharmacological studies have been done to demonstrate or authenticate perceived medicinal properties of kola nut [8, 9]. However, its influence on male reproductive biology has not been well documented. Kola nut increases physical energy and is therefore assumed to enhance sexual performance by some users. But high dose exposure of caffeine in pubertal rats has been reported to impair growth and function of the testis [10]. Also, long term caffeine administration in rats had been shown to induce testicular toxicity [11]. This raises concern of the reproductive effect of prolonged *Cola acuminate* consumption in the male animal. This motivated this study with the objective of investigating the effects of sub-chronic administration of aqueous extract of *Cola acuminate* seed on sperm indices, testosterone level and testis histology in Wistar albino rats. The study additional aims to evaluate whether or not continuous consumption of the plant would affect body weight in the experimental animals.

MATERIALS AND METHODS

Preparation of Plant Material and Phytochemical Screening: Fresh seeds of *Cola acuminate* were obtained from a green grocer (Ekuigbo Road, Ughelli, Nigeria) and authenticated by a botanist, Prof. Ajibesin Kola of the Department of Pharmacognosy and Herbal Medicine, Niger Delta University, Nigeria. Large quantities of the seeds were washed and reduced in size and air-dried for three weeks. The dried materials were ground using a blender to obtain smooth powder. Weighed portion (6 kg) of the pulverized sample was macerated and extracted with distilled water (1:3 weight/volume) for 72 h at room temperature. The resulting solution was then filtered using a clean wire gauze and sieve with tiny pores (0.25 mm). The filtered solution was concentrated under reduced pressure using a rotary evaporator to obtain a brownish pasty extract with a percentage yield of 5.6% of the starting material.

Phytochemical screening of the extract was done for presence of alkaloids, saponins, tannins, flavonoids, steroids, anthraquinones, cardenolides and carbohydrates using standard procedures [12].

Animals: Male Wistar albino rats weighing 100-120 g were used for the study. The animals were obtained from the Animal House of Department of Pharmacology and Toxicology, Niger Delta University, Nigeria. They were maintained with rodent feeds and water (given *ad libitum*) at room temperature of 26±4°C and 12 h light-dark cycle. Animals were handled according to standard international guidelines for care and use of laboratory animals [13]. All experimental procedures were approved by the Departmental Committee on the use and care of animals.

Experimental Design: Twenty-four rats were randomly divided into a control and three experimental groups (n=6 animals per group). Extract was gavaged to experimental groups at three dose levels (25, 50 and 100 mg/kg) daily for 60 days. Control group was given distilled water. At completion of extract administration, animals were sacrificed by cervical dislocation under deep diethylether anesthesia. Blood sample was collected from the jugular vein and serum testosterone level was quantified using ELISA technique with commercially available kits. Testis was removed and the caudal epididymis was isolated and lacerated from which sperm was gently squeezed out and analyzed. The testis was preserved in 10% formal saline and processed routinely for histologically evaluation. Animals' body weights were monitored biweekly using an electronic balance.

Sperm Analysis: Sperm fluid was emulsified with equal volume of 1% $NaHCO_3$ buffered Tyrodes Lactate solution on a clean dry glass slide and viewed under a light microscope (Surgifield Medicals, England) to analyze sperm motility, count and morphology using standard methods [14, 15]. Briefly, sperm motility was determined by counting both motile and non-motile spermatozoa in 10 randomly selected fields using 400 × magnification powers. Sperm motility was categorized as progressive (or active) motility, sluggish motility or immotile. Percentage of motile sperm was calculated from the mean percentage motility for all the fields counted. Sperm count was determined using the new improved Neubauer counting chamber. The chamber was prepared and charged with diluted seminal fluid (1:20) and allowed to stand in a moist

chamber for 15 Min. and complete morphologically mature sperm cells were counted using 400× magnification. For sperm morphology, sperm smears were prepared and properly stained with Walls and Ewas stain. The slides were then examined under 1000× magnification and oil immersion objective. About 100 spermatozoa were counted and the percentage of normal and abnormal forms was noted.

Tissue Processing for Histological Study: Tissues were processed for histological studies using the hematoxylin and eosin (H&E) staining method as described [16]. Briefly, fixed testis tissue was dehydrated using graded concentrations of ethanol and cleared in xylene. Tissue was then impregnated with soft paraffin wax at 58°C in three successions at an hour interval before it was embedded in molten paraffin wax and blocked out. Sections (5-7 µm thickness) were prepared from tissue block afterwards using a rotary microtone. The sections were stained with H&E stain and examined under a light microscope (Leica CMX, Germany). All alterations from the control slides were noted and photographed using 400×.

Statistical Analysis: Data are expressed as mean±standard error of mean (SEM). Comparison between control and experimental groups was done by one-way analysis of variance (ANOVA) and Dunnett's posttest using GraphPad Prism 5 statistical software. Values were considered statistically significant at $P<0.05$.

RESULTS

Phytochemical Profile of Extract: Phytochemical screening of extract showed the presence of alkaloids, flavonoids, tannins, steroids, cardenolides, anthraquinones, saponins and carbohydrates (Table 1). Alkaloids were most abundant, while tannins, saponins and steroids were moderately present. The other phytochemicals were present in low quantities (Table 1).

Effect of Extract on Body Weight: Mean body weight of animals in control significantly ($P<0.01$) increased at week 2 (134.70±5.18 g) compared to their initial body weight, 104.90±3.68 g (Figure 1). The body weights of control animals at other observation periods (weeks 4, 6 and 8) were higher ($P<0.0001$) than the corresponding weeks 0 and 2 body weights. Maximum body weight obtained in control group was 173.70±3.35 g (Figure 1). Body weights of extract treated groups were also increased over the

Table 1: Phytochemical constituents of aqueous *Cola acuminata* seed extract

Phytochemical	Result
Alkaloids	+++
Tannins	++
Saponins	++
Steroids	++
Phenols	+
Flavonoids	+
Carbohydrates	+
Glycosides	+

Key: + Present, ++ Moderately present, +++ Abundantly present

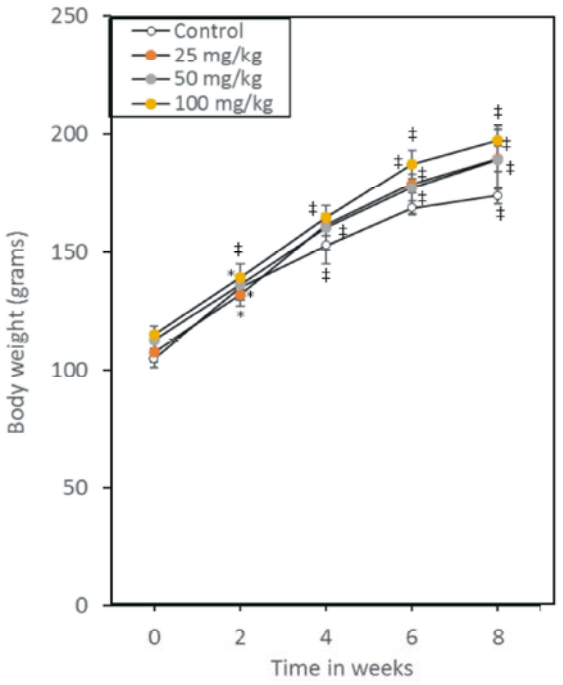

Fig. 1: Effect of aqueous *Cola acuminata* seed extract on body weight in Wistar albino rats Data are expressed as mean±SEM, n = 6 per group.
* Significant at $P<0.01$, ‡ Significant at $P<0.0001$

treatment period in similar fashion as control (Figure 1). Animals' body weights increased from 107.50±3.99 to 189.40±5.43 g; 112.60±3.47 to 189.10±12.66 g and 114.90±3.55 to 197.10±6.35 g in the treatment groups, respectively (Figure 1).

Effect of Extract on Serum Testosterone: Testosterone levels in all extract administered rats were lower compared to control, but only the values in rats that received both 50 and 100 mg/kg of extract were statistically significant, $P<0.05$ (Figure 2).

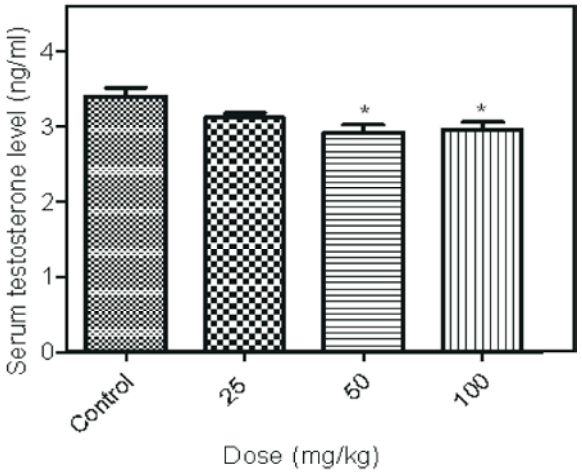

Fig. 2: Serum testosterone level decreases following 60 days oral administration of aqueous *Cola acuminata* seed extract in male Wistar albino rats Data are expressed as mean±SEM, n = 6 per group.
* Significant at $P<0.05$.

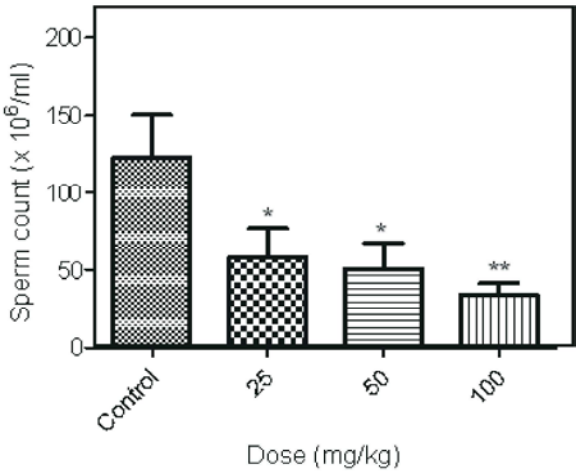

Fig. 4: Sperm count decreases following 60 days oral administration of aqueous *Cola acuminata* seed extract in maleWistar albino rats Data are expressed as mean±SEM, n = 6 per group.
* Significant $P<0.05$, ** Significant $P<0.01$.

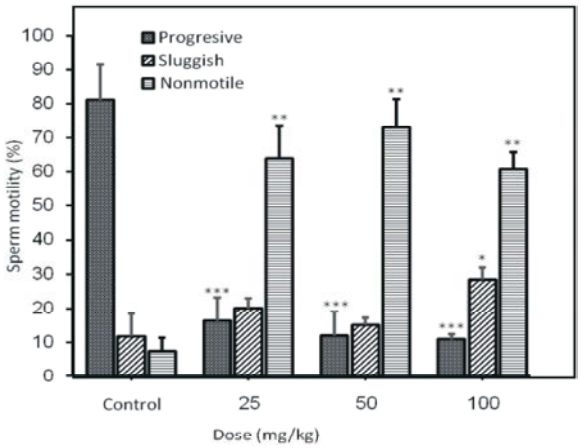

Fig. 3: Effects of 60 days oral administration of aqueous *Cola acuminata* seed extract on sperm motility (progressive sperm motility, sluggish sperm motility, and non-motility) in Wistar albino rats Data are expressed as mean±SEM, n = 6 per group.
* Significant at $P<0.05$, ** Significant at $P<0.01$, *** Significant at $P<0.0001$

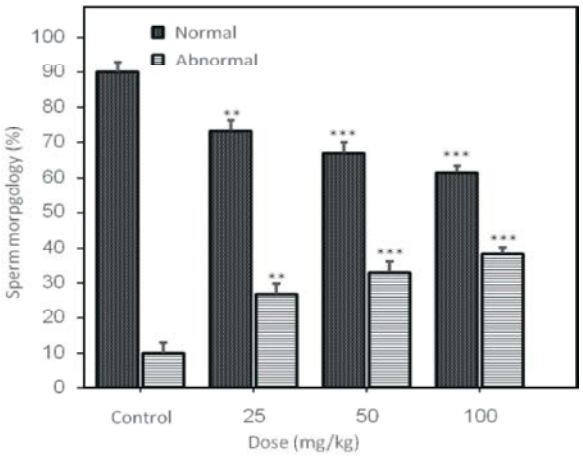

Fig. 5: Effects of 60 days oral administration of aqueous *Cola acuminata* seed extract on sperm morphology (normal and abnormal sperm morphology) in Wistar albino rats Data are expressed as mean±SEM, n = 6 per group.
** Significant at $P<0.01$, *** Significant at $P<0.0001$.

Effect of Extract on Sperm Parameters: Percentage of actively motile sperms was strongly reduced ($P<0.0001$) in all treated rats (Figure 3), whereas non-motile sperms population was high ($P<0.001$) compared to control (Figure 3). Spermatozoa with sluggish motility was reduced ($P<0.05$) only in rats that received the highest dose of extract (100 mg/kg), the values obtained in other treatment groups were not altered compared to control group (Figure 3). In addition, there was a dose-dependent reduction ($P<0.01$) in sperm count in all extract treated rats when compared with control (Figure 4). Further, morphology of sperms was altered in extract exposed rats. Percentage of spermatozoa with normal morphology

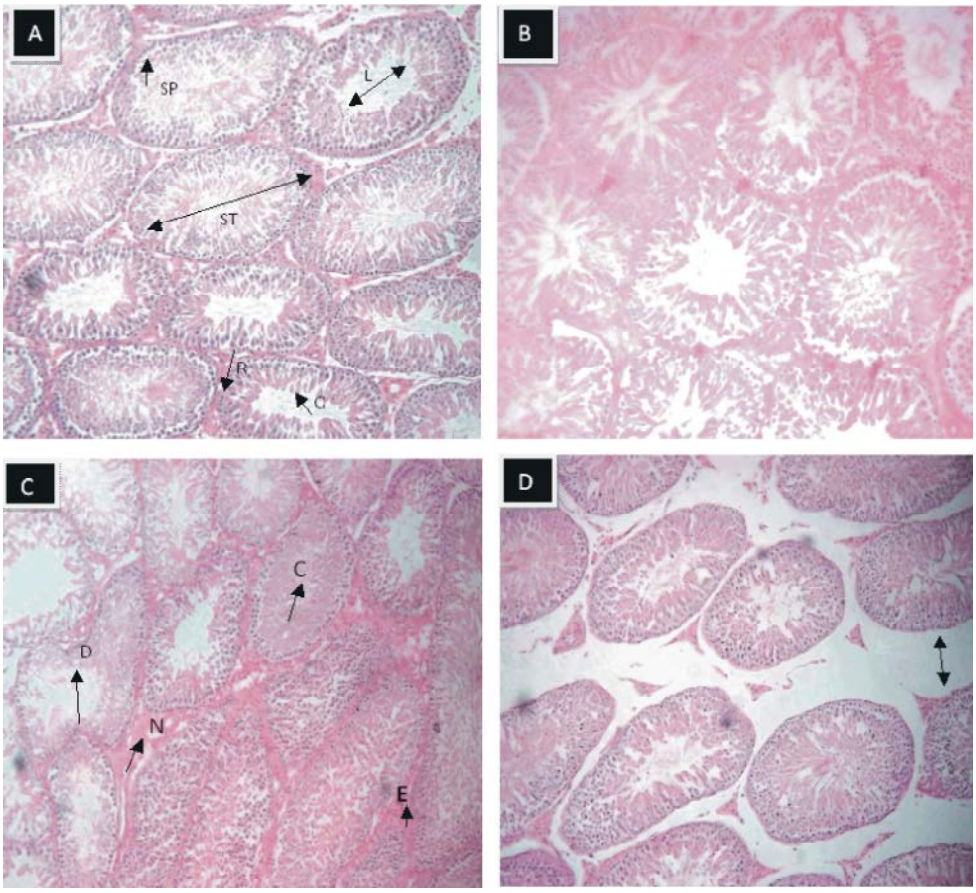

Fig. 6: Photomicrographs of testis following 60 days oral administration of aqueous Cola acuminata seed extract in Wistar albino rats (H&E staining, 400x)
A: Vehicle treated group (Control); showing normal histology of testis. Seminiferous tubules (ST) have lumen (L) and well outlined spermatogonia cells (SP) with intact spermatids (Q). Interstitial tissues housing the Leydig cells are also present in between the tubules.
B: 25 mg/kg treated group; showing normal testis histology. Seminiferous tubules have lumen and well outlined spermatogonia cells with intact spermatids. Also in between the tubules are interstitial tissues housing the Leydigcells.
C: 50 mg/kg treated group; showing abnormal histology of testis with interstitial necrosis (N) and exudation (E). There is sparse cytolysis (C) within the seminiferous tubules as well as seminiferous tubules anisocytosis (D).
D: 100 mg/kg treated group; showing atrophy of the seminiferous tubules. There is also loss of interstitial tissue and Leydig cells.

(normal sperm morphology) was reduced (P<0.0001) in extract administered rats in a dose-dependent manner, compared to control (Figure 5). The reverse was observed in sperms with abnormal morphology (abnormal sperm morphology), as the proportion was increased (P<0.0001) in extract treated animals dose-dependently (Figure 5).

Effect of Extract on Histology of Testis: Histological analysis of control rats showed normal histological features displaying seminiferous tubules with lumen, well outlined spermatogonia cells and intact spermatids (Figure 6A). There were no cyto-morphological changes in the testes of 25 mg/kg extract treated rats compared with control (Figures 6A and 6B). But there was dose-dependent alteration in the histo-architecture of the testes of rats that received 50 and 100 mg/kg extract. These alterations were characterized by interstitial exudation and necrosis; cytolysis, anisocytosis and atrophy in seminiferous tubules; and loss of interstitial tissue and Leydig cell (Figures 6A, 6C and 6D).

DISCUSSION

Cola acuminate seed is consumed popularly as a stimulant and is often taken habitually partly because of its mild addictive potential [2]. This study reports the effects of oral sub-chronic administration of aqueous extract of *Cola acuminate* seed (kola nut) on sperm indices, testosterone level and histology of the rat testis. The doses used in this study (25, 50 and 100 mg/kg) were based on our preliminary laboratory results [17].

The results of the present study showed that treatment with extract for 60 days does not affect body weight. This is because there was progressive body weight gain in extract treated animals over the period of experiment which was similar with observations in control. This observation indicated that the extract may not affect appetite or other nutrient utilization processes that can alter normal growth in the rat.

The results also showed that extract treatment caused alteration in the sperm parameters. Sperm count was reduced as well as sperm motility, but the inhibitory effect was higher on sperm motility. It was observed that proportion of spermatozoa with progressive (active) motility was strongly reduced, while immotile sperm population was simultaneously increased at all the doses. Additionally, the highest dose (100 mg/kg) increased sluggishly motile sperms population such that there were very few sperms with normal motility in extract administered rats. The extract induced a negative influence on sperm morphology by increasing the proportion of abnormal forms and decreasing the percentage of sperms with normal morphology. The negative alterations of sperm motility, count and morphology observed in this study indicate that the extract possibly interferes with seminiferous tubule function and the whole process of spermatogenesis [18]. Additionally, the marked reduction in sperm motility by the extract suggests that the extract may have effect on the microenvironment of the seminiferous epithelium possibly impairing availability or utilization of micronutrients (like calcium, fructose, etc.) that are necessary for sperm motility [19, 20].

Furthermore, the reduction of serum concentration of testosterone that was observed in 50 and 100 mg/kg extract exposed rats may refer to their negative impact on Leydig cells due to the importance of testosterone for the initiation and the maintenance of spermatogenesis [18, 21]. The androgen is essential for growth and division of germinal cells of the seminiferous tubules [21]. The secretion of testosterone by Leydig cells is controlled by anterior pituitary and hypothalamus [22]. From the histological analysis, it was observed that 25 mg/kg extract produced no effect, but 50 and 100 mg/kg extracts resulted in a dose-dependent histological changes in the testis. Exudation or necrosis in interstitial tissue, as well as loss of Leydig cell that were observed in the rats of the current study confirm the decrease in testosterone. The extract also induced sparse cytolysis, anisocytosis or atrophy in tubular cells which correlated with the increased populations of abnormal sperm and suggests that the extract may have direct toxicological influence on the testis. The histological effect of Leydig cell provides reason for decrease in testosterone, while tubular cell alteration may have contributed to poor spermatogenic indices that was obtained.

Similarly, the phytochemical screening of extract showed presence of very high amounts of alkaloids; and high amounts of saponins, tannins and steroids, while other compounds like glycosides, flavonoids and carbohydrates were present in low quantities. Alkaloids and saponins adversely affected testis function and may be partly responsible for the negative testicular influence of extract that was observed [23-25]. Additionally, saponins interfered with some enzyme activities in the spermatozoon like e.g. hyaluronidase and acrosin which are necessary for its motility which may account for their strong inhibitory effect on sperm motility [23].

These findings are of importance because the effect of kola nut on male fertility has been mostly speculative and no sufficient data were existed. Our preliminary laboratory findings before commencement of this study showed that short term exposure of *Cola acuminata* seed (100-200 mg/kg) to rats exhibited reduction in sperm count and motility [17]. The data presented in the present study further demonstrate that long term consumption of *Cola acuminata* is associated with adverse effects on sperm characteristics even at relatively low amounts. Sperm quality is a critical determinant of male fertility [26, 27]. As reduced sperm count, motility or abnormal changes in sperm morphology negatively affects sperm quality, it is logical to suggest that the extract can affect fertility in male rats.

CONCLUSION

This study shows that sub-chronic administration of *Cola acuminata* seed extract does not affect body weight but altered sperm characteristics, reduced testosterone and altered testicular histology in rats. Thus, the habitual consumption of kola nut may not be healthy in males of reproductive age.

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