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Original Article

Effects of Crude Aqueous Extract of *Cola Acuminata* Pods on the Reproductive System of Male Albino Rats

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ABSTRACT

Background and Aims: *Cola acuminata* pod extract (CAPE) is used in Nigerian traditional medicine to treat low sperm count in males. Hence, this study aimed to investigate the effects of CAPE on the reproductive system of male albino rats.

Materials and Methods: Preliminary acute toxicity testing, phytochemical screening, proximate analysis, and determination of vitamin E concentration were performed on the extract. Three groups (I, II, and III) of 12 rats were used in the study. Each group comprised three batches (A, B, and C) of 4 rats each for different periods (4, 6, and 8 weeks, respectively).

Results: Acute toxicity testing showed that CAPE had an oral lethal dose of 50% (LD_{50}) of >5 g/kg body weight (b.wt) in rats. Vitamin E concentration was 0.511 mg/l. The caudal epididymal sperm count increased marginally from 4 to 6 weeks for rats treated with 800 mg/kg b.wt., but reduced significantly at 8 weeks for both CAPE treatment groups (II and III) ($p<0.05$) compared with the control group (I). Significant reduction ($p<0.05$) of sperm motility and serum testosterone levels at 8 weeks were observed. The histology of the reproductive organs revealed evident histo-architectural changes.

Conclusions: These results suggest that the aqueous pod extract of *Cola acuminata* causes marked alterations in reproductive organs and shows antispermatogenic and antiandrogenic effects when administered orally over 8 weeks in mature male rats leading to contradicting its use as a traditional remedy for low sperm count in males.

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Introduction

Kolanut is the fruit of the Cola species and belongs to the family of Sterculiacaea [1]. In Nigeria, varieties of kola nut are *Cola acuminata (C. acuminata)*, *Cola nitida*, and *Garcinia cola* [2]. *C. acuminata* has high ceremonial values among the Igbo's and Yoruba's in Nigeria. Kolanut has a bitter taste when chewed and contains caffeine [2]. However, kola nut possesses medicinal and pharmacological values [3]. It is used for the treatment of whooping cough, malaria, fever, asthma, and acts as a bronchodilator because of caffeine [4, 5]. Also, kola nuts have antimicrobial [1], analeptic and lipolytic properties and can stimulate gastric juice secretion as well [6]. Traditionally, the leaves, tugs, bark, fruit follicles, and flowers were used in the treatment of dysentery, diarrhea, coughs, vomiting, and chest complaints [7]. Kola nuts have been linked with natural fertility regulation [8].Infertility is defined as the inability to achieve pregnancy after 12 months or more of unprotected sexual intercourse [9], which is a primary reproductive health concern in Nigeria [10], and women usually bear the blame. Most cases of infertility in Nigeria and Africa are due to infection [11, 12]. In males, causes of infertility are namely obstruction of the seminal tract, sexual disorder, inflammation, nutritional deficiencies, prolonged exposure to chemical, excessive heat, heavy metals, hormonal imbalance, lifestyle, abnormal sperm count or quality, and interference with spermatogenesis [13, 14]. Low levels of plasma testosterone, gonadotropins, or high levels of plasma estrogen are among hormonal disturbances that may lead to infertility [15, 16]. Environmental estrogenic agents such as phytoestrogen diets may reduce male fertility by disrupting the normal hormonal balance in the body [17, 18, 19]. Some studies have validated the use of plants either to enhance sexual desire or to improve spermatogenesis [20, 21, 22]. Many herbs used in the traditional remedies to treat male infertility include *Alframomum melegueta*, *Boerhaavia diffusa*, *Momordica charantia*, *Sphenocetum jolyanum*, *Telferia occidentalis*, *Phyllanthus amarus*, *Irvingia gabonensis*, *Landolphia dulci*, *Alliumm cepa*, and *Cissus populnea* [23, 24].

Materials and Methods

Experimental animals

A total of 36 adult male Albino Wistar rats weighing between 200-250 g were used in this study. These animals were procured from the animal house of the department of physiology, University of Nigeria, Enugu Campus, and housed in the animal house of the college of medicine, university of Nigeria, Enugu Campus. They were allowed to acclimatize for two weeks and fed with commercially available rat feed. Ethical approval of this study was obtained from the Ethics Committee of the University of Nigeria, Nsukka.

Plant material collection

The pods of kola nut were obtained from Ukehe town, Igbo-Etiti Local Government, Enugu State, and authenticated by the Botany Department, University of Nigeria, Nsukka.

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Plant aqueous extraction

2000 g of air-dried *C. acuminata* pods were macerated in 400 ml of distilled water, sieved with a muslin cloth, and kept in a refrigerator until required. The extractive value of the extract was determined and was given a value of 180 mg/ml.

Phytochemical screening

The phytochemical constituents of *C. acuminata* were determined with the method described by Trease and Evans [25] in the department of pharmacognosy, faculty of pharmaceutical sciences, university of Nigeria, Nsukka.

Proximate analysis

The method described by Pearson [26] was used to determine the percent of crude protein, moisture, crude fiber, fat, carbohydrate, and ash content in the pods of *C. acuminata*. The method of AOAC [27] was used to determine the mineral contents in the plant material. Vitamin E was also estimated using the technique of Emmerie and Engel [28].

Preliminary acute toxicity testing

The acute toxicity testing (LD_{50} determination) was conducted using the variation method of Lorke [29] with slight modification. Graded doses of 10, 100, 1000, 2000, 5000 mg/kg body weight (b.wt.) were administered to groups A, B, C, D, and E, respectively of 4 rats. Each animal was given a single oral dose of *Cola acuminata* pod extract (CAPE) after 24 hours quickly in the respective groups. After the drug administration, clinical observations were done hourly for 24 hours to record mortality or clinical signs of toxicity.

Experimental design

The animals were divided into groups (I, II, and III), including 12 rats. Each group comprised three batches (A, B, and C) with the following respective duration of treatment 4, 6, and 8 weeks of 4 rats each. The choice of up to 8 weeks of administration of the plant material intended to cover the spermatogenic cycle in rats.

The treatments were as follows:

Group I: This group served as the control group and would receive water by oral gavage.

Group II: Rats were treated with 400 mg/kg b.wt. of CAPE.

Group III: Rats were treated with 800 mg/kg b.wt. of CAPE.

All treatments were administered by oral gavage via an oral cannula daily for 4, 6, and 8 weeks for the individual batches (A, B, and C) in the three groups. The animals were at the end of each treatment period when sacrificed under chloroform anesthesia and one of the testes and its associated cauda epididymis, seminal vesicle and vas deferens were excised and preserved in 10% formal saline for further histological processing with light microscopy. The other cauda epididymis was removed for further sperm parameter analyses.

Epididymal sperm count and motility

Several incisions (1 mm) were made on other excised cauda epididymis, which was suspended in 1 ml of Ham's F-10 solution (Sigma Aldrich Chemical Co., U.S.A.). After 10 minutes of incubation at 37°C, sperm concentration and motility were determined by hemocytometer method [30].

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Histological processing

The tissues were cut up into smaller pieces (about 3 mm thick), then they were processed with the automatic tissue processor and sectioned at 5µm using the Rotary Microtome (Heitz 150 Rotary Microtome, Cambridge model). Sections were stained according to Hematoxylin and Eosin technique by Baker et al. [31].

Microscopy and photomicrography

The sections were examined using an Olympus Binocular Microscope with an in-built lighting system. The sections were photographed using a digital microscope camera (Hewlett Packard® attached to an eyepiece of an Olympus Binocular Microscope.

Testosterone assay

Blood samples were obtained from the retro-orbital sinus of the rats in each study group before the sacrifice. They were spun at 2500 revolution per minute for 10 minutes in an angle-head desktop centrifuge at room temperature. Subsequently, serum samples obtained were assayed for testosterone in batches with the control sera at both physiological and pathological levels by standard quantitative enzyme-linked immunosorbent assay technique with microwell kits from Syntro Bioresearch Inc. California, U.S.A.

Statistical analysis

Data obtained were analyzed using the SPSS software. All data were expressed, where appropriate, as Mean±Standard error of the mean. The difference between the mean scores was determined with the student's t-test and one-way analysis of variance (ANOVA). Finally, the results were considered significant at $p<0.05$.

Results

Acute toxicity testing

No toxic symptoms or mortality were observed in the treated animals, which lived up to 14 days after administering CAPE at a single dose level of 5000 mg/kg of body weight. The behavioral patterns of animals were observed first for 2 hours, followed by 6 hours and then 14 hours after the administration and the animals in all treated groups were healthy and did not display significant changes in behavior, skin effects, breathing, impairment in food intake and water consumption, postural abnormalities and hair loss.

Phytochemical screening

Table 1 shows the result of the phytochemical screening of CAPE. The plant material contains an abundant amount of saponins, a moderate amount of proteins, the presence of terpenoids, tannins, and resins, trace amounts of flavonoids, alkaloids, reducing sugars, fats, and oil, steroids and glycosides.

Proximate analysis

The result of the proximate composition of the dried *C. acuminata* pods is displayed in Table 2. It showed lower amounts of protein (2.14%) and fat (1.84%) but a larger amount of carbohydrates (24.54%). The crude fiber content was found to be 35.18% and an ash content of 20.20%.

Vitamin E determination

A value of 0.511 mg/L was obtained from Vitamin E determination of the pods.

Cauda epididymal sperm count and motility

Daily oral administration of the CAPE for 4 and 6 weeks slightly increased the epididymal sperm counts in CAPE-treated groups (Fig. 1), but this

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change was not statistically significant ($p>0.05$) when compared with the values obtained in the control group (Table 3). However, after 8 weeks of CAPE treatment, a significant reduction ($p<0.05$) was observed in the treatment groups compared with their corresponding control group. The results of the sperm motility analysis revealed no significant change after 4 and 6 weeks of treatment with CAPE (Fig. 2). Nevertheless, there was a significant reduction ($p<0.05$) in the treated groups when compared with the corresponding control group after 8 weeks, as shown in Table 3.

Serum testosterone levels

A significant decrease ($p<0.05$) in the mean testosterone levels were observed in rats treated with 400 mg/kg CAPE (groups II) and 800 mg/kg CAPE (group III) at 6 weeks and 8 weeks, respectively when compared with the corresponding control group (Table 4 and Fig. 3).

Histological findings

Administration of 400 mg/kg and 800 mg/kg CAPE for 6 and 8 weeks caused visible lesions upon microscopical evaluation within the seminiferous tubules (Fig. 4), epididymis (Fig. 5), vas deferens (Fig. 6) and seminal vesicles (Fig. 7) of treated rats. A summary of the histological findings in these tissues is provided in table 5.

Table 1. Phystochemical constituents of *C. acuminata* pods

Constituents	Inference
Flavonoids	Trace amounts (-)
Alkaloids	Trace amounts (-)
Saponins	Abundant amounts (+++)
Tannins	Present (+)
Resins	Present (+)
Proteins	Moderate amounts (++)
Reducing Sugars	Trace amounts (-)
Fats and oil	Trace amounts (-)
Steroids	Trace amounts (-)
Terpenoids	Present (+)
Glycosides	Trace amounts (-)
Acidic compounds	Neutral

Table 2. Proximate composition of dried *C. acuminata* pods

Sample Identification	Parameters (%)					
Kola Nut Pod	Moisture	Ash	Crude Fibre	Protein	Fat	Carbohy-Drate
	16.10	20.20	35.18	2.14	1.84	24.54

Table 3. Effects of CAPE on spermatozoa indices in male albino rats

Group	Dose of CAPE mg/kg body weight	4 Weeks		6 Weeks		8 Weeks	
		Motility (%)	Counts ($x10^6$/ml)	Motility (%)	Counts ($x10^6$/ml)	Motility (%)	Counts ($x10^6$/ml)
I (Control)	0	78.25±2.32	64.25±6.26	80.00±2.32	61.50±23.29	78.25±1.55	71.75±4.09
II	400	74.75±2.95	98.20±14.07	75.75±2.17	81.50±15.13	48.75±1.75*	18.50±3.12*
III	800	74.50±3.30	76.00±9.11	71.75±3.35	106.25±16.75	28.25±6.43*	20.25±7.15*

* $P<0.05$; CAPE= *C. acuminata* pod extract

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Table 4. Effects of CAPE extract on serum testosterone levels in male albino rats

Group	Dose of CAPE mg/kg body weight	Serum testosterone levels (ng/ml)		
		4 weeks	6 weeks	8 weeks
I (Control)	0	1.95±0.34	3.52±1.56	1.10±0.12
II	400	5.75±3.18	0.95±0.17[b]	0.75±0.10
III	800	2.92±0.90	2.27±0.31	0.60±0.04[a]

a: Statistically significant; and b: Significant when compared with the control group (I) and high dose group (III), respectively; CAPE= *C. acuminata* pod extract

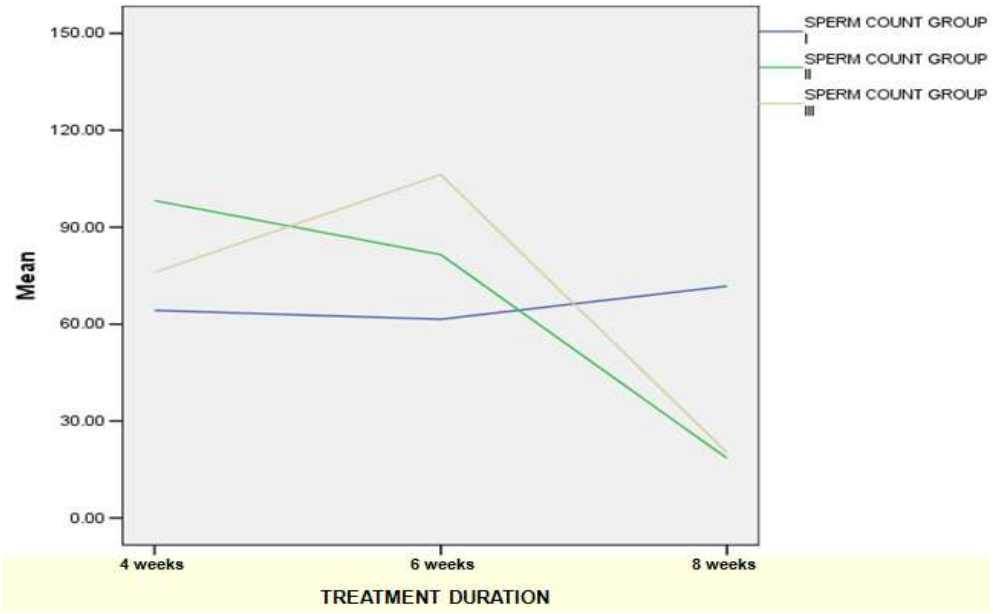

Fig. 1. Line graph illustrating the effects of *C. acuminata* pod extract on cauda epididymal sperm density [million per ml]

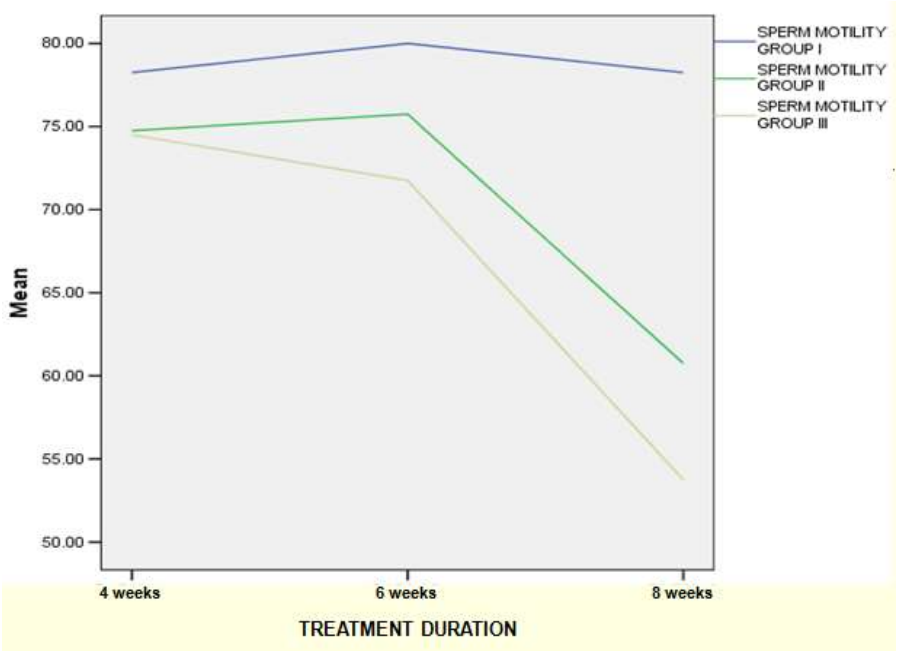

Fig. 2. Line graph illustrating the effects of *C. acuminata* pod extract on sperm motility [%]

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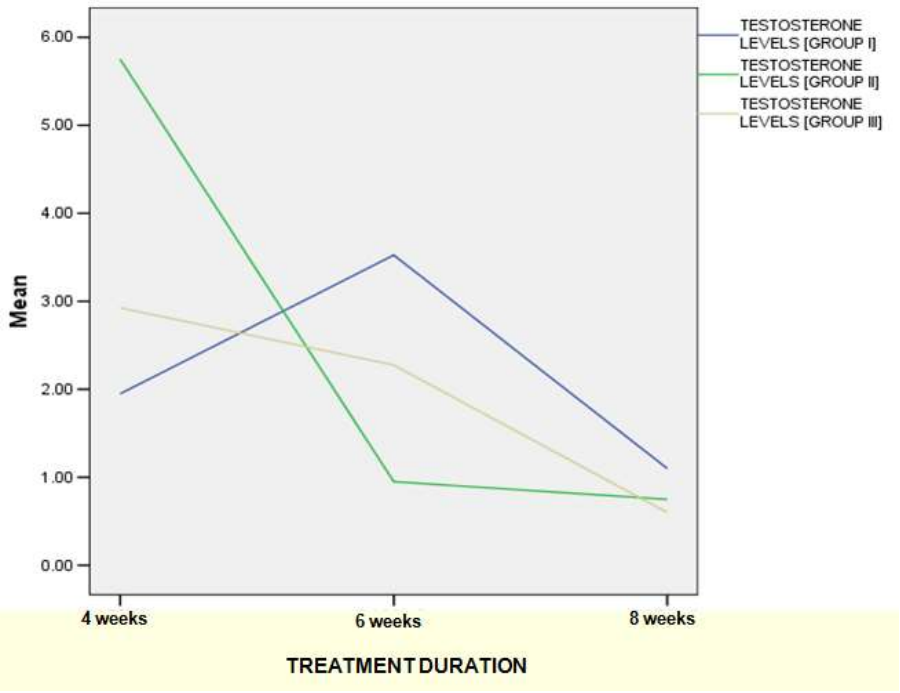

Fig. 3. Line graph illustrating the effects of *C. acuminata* pod extract on serum testosterone levels [ng/ml]

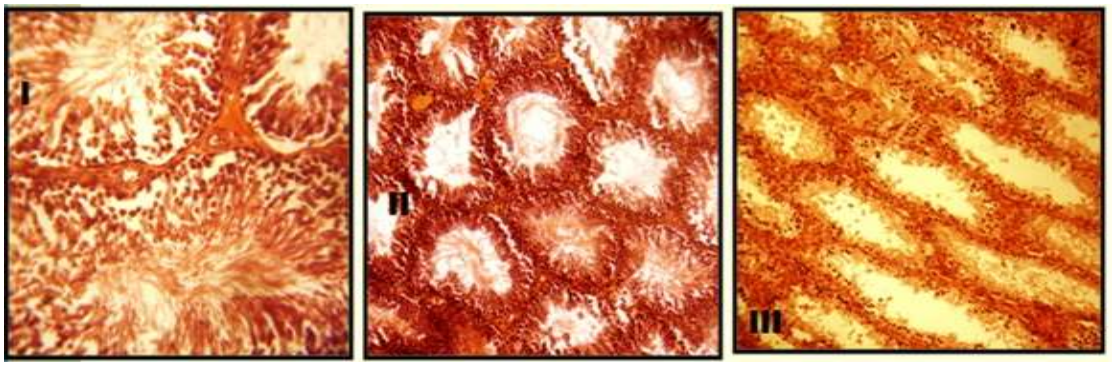

Fig. 4. Light photomicrograph of the testis from the control rat (I) and *C. acuminata* pod extract treated rat group (II and III) (H&E: Magx100). Seminiferous tubules of control rat group (I) showed a clear organization of various stages of spermatogenesis. *C. acuminata* pod extract treated rats (II and III) showed disorganization of the seminiferous tubules; fewer spermatozoa and azoospermic seminiferous tubules; and reduced leydig cell number.

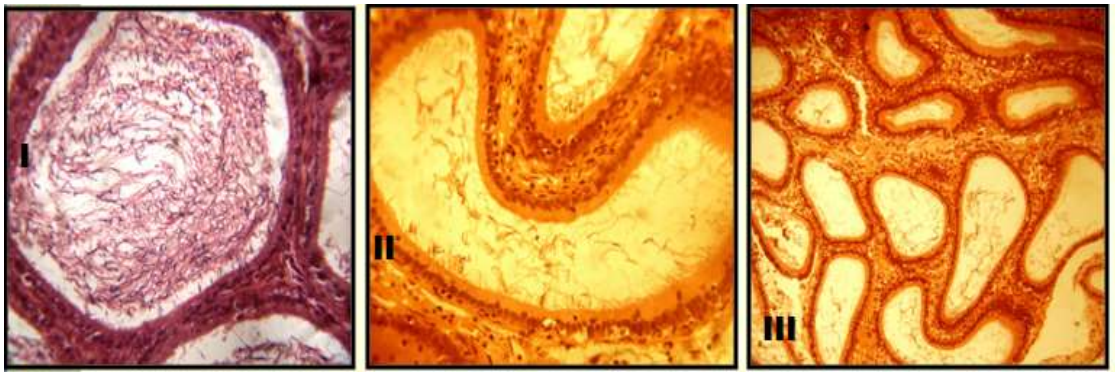

Fig. 5. Light photomicrograph of epididymis from the control rat (I) and *C. acuminata* pod extract treated rats (II and III) (H&E: Magx100). Control rat (I) showed normal tubules and epithelial lining, and numerous spermatozoa were present in the lumen. *C. acuminata* pod extract treated rats (II and III) showed fewer spermatozoa in the lumen of tubules, hyperplasia of epithelial lining.

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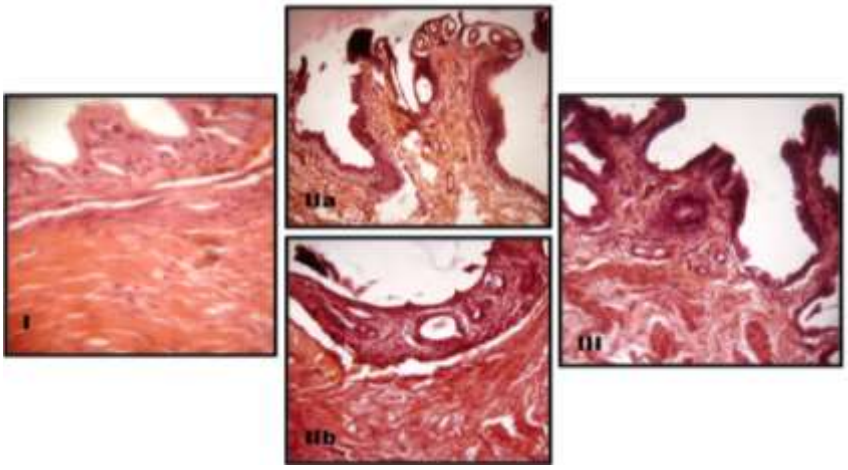

Fig. 6. Light photomicrograph of vas deferens from control rat (I) and *C. acuminata* pod extract treated rats (II and III) (H&E: Magx100). Control rat (I) showed normal honey-comb histoarchitecture with fluid in the lumen. *CAPE* treated rats (II and III) showed epithelial lining thickening and degeneration.

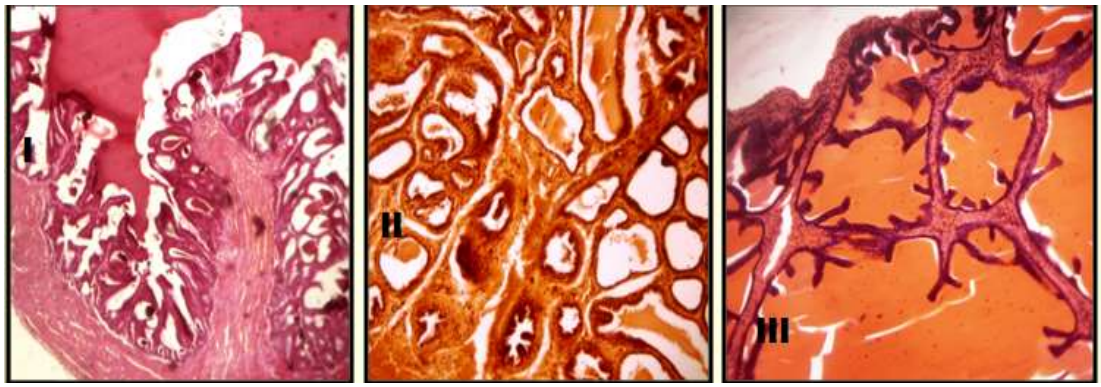

Fig. 7. Light photomicrograph of seminal vesicles from control rat (I) and *Cola acuminata* pod extract treated rats (II and III) (H&E: Magx100). Control rat (I) showed normal epithelial lining, and layers muscular wall. *Cola acuminata* pod extract treated rats (II and III) showed necrotic and hyperplastic epithelial lining, disorganized muscular wall, and inflammatory cellular infiltration.

Discussion

This study demonstrates the effects of daily oral administration of CAPE for 8 weeks on the reproductive system of male albino Wistar rats. Preliminary acute toxicity testing of CAPE in this study revealed an oral LD50 of >5000 mg/kg body weight in rats. Hence, CAPE would be considered safe, according to Organisation for Economic Cooperation and Development (OECD) standards [32].

Preliminary phytochemical screening of CAPE in this study demonstrated the abundant amount of saponins, the moderate amount of proteins, and the presence of terpenoids, tannins, and resins, and the trace amount of flavonoids, alkaloids, reducing sugars, fats, oil, steroids, and glycosides. The observed effects in this study can be a direct or indirect action of a single or combination of the phytochemical principles.

However, the extract in Nigerian folklore medicine is used to improve sperm count in sub-fertile males [33]. On the contrary, the present study has shown that oral delivery of

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CAPE over 8 weeks results in a significant decrease in cauda epididymis sperm count and motility. Testis and epididymis undergo histoarchitectural changes. Besides, microscopical evaluation reveals that spermatogenic cells and sperm production are reduced. Determination of sperm count, degree of motility, and viability are parameters used to assess sperm quality and quantity [30].

Caudal epididymal sperm count and motility were significantly reduced after treatment with CAPE and thus may have some implications for fertility, which correlates with the work of Aprioku and Clement, who reported a significant decrease in sperm count and motility [34]. The decline in sperm count may suggest that CAPE interferes with spermatogenesis and may also interferes with seminiferous epithelium and metabolic processes associated with sperm motility. So, it may result in a decrease in sperm motility [35]. Orisakwe et al. documented that drugs or chemicals that affect testicular function can impair the quantity and quality of spermatozoa [36]. The marginal increase in sperm counts in both treatment groups at 6 weeks may be attributed to increased output of spermatozoa from the testis to the epididymis for storage and maturation, rather than increased spermatogenesis. Its effects on spermatogenesis are observed between 53-60 days of post-treatment in an experiment, which is equivalent to one spermatogenic cycle in rats at which the testicular results would be interpreted to epididymal events [37]. Thus, the reduced sperm count at 8 weeks (56 days) can be the resultant effect of CAPE on spermatogenesis.

Vitamin E, a fat-soluble antioxidant, helps to improve the quality and quantity of spermatozoa [38]. Insufficient vitamins and low natural antioxidant intake can cause deleterious effects on spermatogenesis and producing healthy sperm, while sufficient consumption can protect sperm DNA from oxidative stress leading to the improvement of male fertility [39]. However, the amount of vitamin E in CAPE (0.511mg/l) can be considered low to exert the fertility benefits. Abbas and Luma reported that vitamin E had a protective effect on the reproductive system of male rats [40]. Also, Sukmawati et al. reported that vitamin E had an ameliorative effect on testicular histological features of allethrin treated rats [41].

The serum testosterone levels decreased marginally and significantly at 8 weeks in groups B and C when compared with their respective control groups. It may be inferred that CAPE exerts antiandrogenic effects in male rats, and this may be through the hypothalamic-pituitary –axis or by a direct effect on the testis. Thus, the observed testosterone deficiency in the present study may also explain the significantly reduced sperm characteristics. This finding is similar to the Aprioku, and Clement, who reported a reduction in serum testosterone concentration at 50 and 100 mg/kg extract exposed rats [34]. Similarly, Oyedeji et al. reported that aqueous extract of *Cola nitida* caused a significant decrease in testosterone levels of male albino rats [42].

The extent of spermatogenesis is determined by intratesticular testosterone concentration and not the serum peripheral levels [43]. It suggests that there may be changes in the rate of

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spermatogenesis when peripheral testosterone levels are unaffected, which may be via some mechanism that obstructs the availability of the hormone at the testicular level to impair spermatogenesis. Previous studies have shown that plant extracts can deplete testicular testosterone while sparing the peripheral testosterone levels to a large extent [44, 45].

Histologically, the study of the various reproductive organs (testis, epididymis, seminal vesicles, and vas deferens) revealed multiple degrees of derangement, a disturbance in spermatogenic cells and few or no spermatozoa in the seminiferous tubules of the testes. The reduced Leydig cells population observed would account for the reduced number of primary spermatocytes. Interstitial cells of the leydig are known to secrete the testicular testosterone necessary for the division of germ cells [46]. Thus, it suggests that CAPE contains antispermatogenic agents. Some plant extracts such as *Mondia whitei*, *Eurycoma longifolia*, *Tinospora cordifolia*, *Leptadenia hastate* have been observed to have a direct effect on spermatogenesis [47 -49].

The microscopical examination of the accessory organs showed some evidence of hyperplasia. These effects may be attributed to direct action of CAPE principles or by the depleted levels of androgens. Circulating androgens help to maintain a healthy reproductive accessory organ structure and its function [50]. The derangement in the histoarchitecture of these organs would alter their function. Besides, the impaired function of the reproductive organs has been shown to decrease sperm motility [51, 52]. These findings are also similar to the research carried out by Aprioku and Clement, who reported dose-dependent histological changes in the testis of rats treated with *C. acuminata* seed extract [34].

Conclusion

C. acuminata pod causes marked alterations in reproductive organs and has antispermatogenic and antiandrogenic effects when administered orally over 8 weeks in mature male rats, thus contradicting its use as a traditional remedy for low sperm count in males.

Conflict of Interest

The authors declare no conflict of interest.

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