

Effects of calcium carbide ripened banana on organ weight, histological architecture and semen profile on male albino Wistar rats

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World Journal of Advanced Research and Reviews, 2024, 21(03), 1323–1334

Publication history: Received on 31 January 2024; revised on 09 March 2024; accepted on 11 March 2024

Article DOI: <https://doi.org/10.30574/wjarr.2024.21.3.0798>

Abstract

Fruits play an important role in human diets, providing essential nutrients and helping to prevent disease. However, concerns arise regarding their impact on male reproductive health due to potential exposure to pesticides, heavy metals, and artificial ripening agents. This study investigates the acute and chronic effects of calcium carbide-ripened bananas on testicular weight and histological architecture, and semen profile in adult male Wistar rats. A 14-week experiment involved acclimatization, followed by acute (4 weeks) and chronic (12 weeks) phases, with rats divided into control and various banana-treated groups. Notably, in the chronic phase, significant decreases in relative testicular weight were observed in rats administered 200mg/kg of calcium carbide-ripened bananas compared with the control and with those treated with normal ripe banana respectively. Significant reductions in sperm motility and total sperm count were noted in group D in the acute phase when compared with the group treated with normal ripe banana. Increased levels of active spermatogonia and spermatogenesis were observed in the rats administered with normal ripened banana, with mild degeneration of germinal epithelium in the chronic phase, particularly in the calcium carbide-ripened banana-treated groups. These findings may suggest potential adverse effects of calcium carbide-ripened bananas on male reproductive health, warranting further investigation into their safety and regulation.

Keywords; Calcium carbide; Banana; Ripening; Male infertility; Semen quality; Testicular histology.

1. Introduction

Fruits are a crucial food item in human diets, rich in nutrients and known for defending against diseases (1), and consumption of fruits positively impacts on testicular functions. Mediterranean diets which are high in fruits, vegetables, fish, and whole grains, are associated with higher semen quality [2]. However, inconsistent results on dietary fiber intake and hormone levels affect male reproductive system and may be attributed to pesticide residues, heavy metals, artificial ripening agents, and nitrate.

Banana is a popular, nutrient-rich fruit crop grown in tropical and sub-tropical regions for local consumption and export [3]. Its low price, good taste, and high nutrient content make it a popular choice [4], Bananas are multipurpose plants, with parts used in various ways depending on the species. The edible fruit, ripe as a dessert, is the most important part [5], Nutritionally, bananas are rich in carbohydrates, vitamins A, B, and C, and potassium [6]. [7] and [8] have also reported that banana is also rich in bioactive compounds like carotenoids, flavonoids, phenolics, amines, vitamin C, and E, which have antioxidant activities, providing numerous health benefits, including lowering blood cholesterol levels [9].

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Fruit production is crucial for food and income security in producing countries [10], with agricultural research addressing diseases and climate issues. However, it appears that food security is no longer a concern, since quantity is now prioritized by consumers and retailer [11, 12]. Ensuring fruit safety is therefore challenging due to the use of chemicals and artificial ripening agents like calcium carbide, ethephon, ethrel, ethylene glycol, and formalin. These agents are used to ripen immature fruits quickly for an attractive color [13]. Similarly, high temperature and orthodox methods like ash and kerosine trigger fruit ripening before the season [14]. Calcium carbide (CaC₂) is the most common artificial ripening agent, and a strong reactive chemical with traces of arsenic and phosphorous hydride [15]. It is a popular ripening agent for bananas due to its affordability, availability in local markets, and 24-hour ripening effect [16], Calcium carbide has been reported to be hazardous to health, potentially causing cancer, skin irritation, diarrhea, liver, kidney, and gastrointestinal issues [17].

Infertility is a reproductive system disease defined by failure to achieve a pregnancy after unprotected sexual intercourse lasting 12 months or more [18]. It is a major cause of marital disharmony, separation, and personal misery [19]. Historically, it is considered a woman's fault, but medical evidence shows equal rates for both genders. Male infertility which is the inability of a male to conceive a fertile female for at least one year, contributes about 20% of all cases. It is also reported to be a significant factor in 30%-40% of infertility cases [20]. Factors influencing male fertility include age, medications, surgical history, environmental toxins, genetic problems, and systemic diseases [21]. Male infertility can be caused by erectile dysfunction, poor libido, and oxidative stress [22].

There is little or no information on the effects of calcium carbide on the male reproductive characteristics. The present study therefore aimed at investigating the acute and chronic effects of Calcium Carbide ripened banana on reproductive characteristics of male Wistar rats such as, relative testicular weight, testicular histology architecture and semen profile.

2. Materials and Methods

2.1. Fruit collection and preparation

Three bunches of unripe matured banana, planted in a private farm at Okofia, Nnewi was directly harvested from the farm for this procedure. One bunch together with 10g of calcium carbide was placed in a clean polythene bag and kept in a dark dry cupboard; another bunch was left to ripen naturally. Signs of ripening was monitored daily in the fruit which was indicated by color changes in their peels [23,24]. The ripening stages was assessed as described by Bafor et al., 2019 [25], on a ripening scale of 1-7.

The Calcium carbide and normal ripened banana were removed from its peel and the pulp were separately blended in an electric blender together with deionized water and the juice was filtered with a porcelain cloth and filter paper, the juices were put in a clean rubber bottle, well labeled and properly stored in the refrigerator for further usage [26].

2.2. Animal and Experimental Design

Forty-Five male wistar rats weighing 150-170g were obtained from the Animal House, Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, the ethical approval was obtained from Research and Ethical committee of Basic Medical Sciences, College of Health Sciences (NAU/CHS/NC/FMBS/480). All rats were used under standardized animal house condition (12 h light/12h dark, 25± 2 °C, standard water and pellet diet ad libitum).

The experiment lasted for 14 weeks (2 weeks of acclimatization, with 4 weeks of administration in the (acute phase) and 12 weeks of administration (chronic phase) respectively. The animals were divided into five groups, with five (5) rats per group as follows;

Group 1: Control (CG)

Group 2a: 500mg/kg of normal ripened banana (*Musa spp.*) (acute)

Group 2b: 500mg/kg of normal ripened banana (*Musa spp.*) (chronic)

Group 3a: 100mg/kg of calcium carbide ripened banana (*Musa spp.*) (acute)

Group 3b: 100mg/kg of calcium carbide ripened banana (*Musa spp.*) (chronic)

Group 4a: 200mg/kg of calcium carbide ripened banana (*Musa spp.*) (acute)

Group 4b: 200mg/kg of calcium carbide ripened banana (*Musa spp.*) (chronic)

Group 5a: 500mg/kg of calcium carbide ripened banana (*Musa spp.*) (acute)

Group 5b: 500mg/kg of calcium carbide ripened banana (*Musa spp.*) (chronic)

2.3. Sample collection

At the end of 4th and 12th week respectively, animals in the different groups were anesthetized using chloroform in an enclosed container after 24-hours of the last administered dose of the extract. Blood was collected from the animals using a heparinized capillary tube through ocular puncture as described by [27].

Semen was obtained from the caudal end epididymal gland immediately and fixed in a clean, labelled slide. The testis was removed from the animals, weighed and stored using 10% formal saline for proper fixation for histopathological studies [28].

2.4. Sperm Motility

Sperm Motility Sperm cells were obtained from the epididymis' caudal end, placed in a clean glass slide, and mixed with a physiological solution of 990 μL (sodium citrate) in the ratio of 1:20. About 5.0 μL of supernatant containing the sperm was placed between the slide and coverslip and observed at 100 x in a negative phase contrast microscopy (XSZ-107BN). The evaluation of the movement of the sperm was held in three different fields, and motility was expressed from the middle of the fields in the percentage of motile sperm of the total sperm counted (29).

2.5. Total Sperm Count

Approximately 10 μL of the diluted contents was transferred to hemocytometer (Neubauer chamber) and taken in light microscopy at 400 x. The pelleted cells were counted on the surface of the chamber. The sperm concentration calculation was performed according to the number of counted cells and hemocytometer dimensions. The concentration was expressed in millions of sperm per mL (29).

2.6. Sperm Morphology

About 20 μL of the sperm suspension was placed on the slides and swiped with 95% v/v ethanol for proper fixation for 5-10 minutes and was allowed to air-dry. The smear was washed with sodium bicarbonate solution (reagent no. 72) to remove any mucus, which may be present. The smear was rinsed with several changes of distilled water, thereafter, it was allowed to air-dry for two minutes and was covered with carbon fuchsin (1 in 20). It was allowed for staining for 3 minutes and wash off with distilled water. After that, the counterstain was done by covering the smear with dilute Loeffler's methylene blue (1 in 20) for 2 minutes; thereafter, it was allowed to dry and wash off with distilled water [29]

2.7. Statistical Analysis

Data was analyzed using SPSS version 25 and the results expressed as mean \pm SD. The statistical significance between the means was analyzed using one-way analysis of variance (ANOVA) with Least square significant difference tests to determine the levels of significance among groups. Statistical significance was set at $P < 0.05$ for comparative tests.

3. Results

Table 1 Effect of calcium carbide ripened *Musa sapiens* on sperm motility and total sperm count following acute intake

	Active motility (%)	Non-motile (%)	Total sperm count ($\times 10^6/\text{mls}$)
	MEAN \pm SD	MEAN \pm SD	MEAN \pm SD
Group A (control)	83.75 \pm 4.78	16.25 \pm 4.78	61.20 \pm 4.49
Group B (500 mg/kg of NRB)	91.67 \pm 5.77 ^a	8.33 \pm 5.77 ^a	73.40 \pm 5.32 [*]
Group C (100 mg/kg of CCRB)	81.67 \pm 2.88 ^a	18.33 \pm 2.88 ^a	66.36 \pm 3.74 ^a
Group D (200 mg/kg of CCRB)	75.00 \pm 8.66 ^a	25.00 \pm 8.66 ^a	63.07 \pm 2.29 ^a
Group E (500 mg/kg of CCRB)	85.00 \pm 8.66 ^a	15.00 \pm 8.66 ^a	65.50 \pm 5.67 ^a
F-ratio	2.63	2.63	3.50

Data was analyzed using ANOVA followed by post Hoc LSD and values were considered significant at $p < 0.05$, NRB; Normal ripened banana, CCRB; Calcium carbide ripened banana, *; significant, a; non-significant.

Table 1 result for active sperm motility indicated no significant difference in groups B, C, D and E compared to control group. The mean non-motile sperm result also indicated no significant difference in groups B, C, D and E when compared to the control. The total sperm count indicated no significant difference in groups C, D, E compared with the Control, while group B indicated significantly higher mean when compared to the control. The mean active motility revealed no significant difference in groups C and E compared to B but showed a significant decrease in group D. The non-motile sperm showed no significant differences in groups C, and E compared to B but showed a significant increase in group D. The mean total sperm count result revealed no significance difference in group C and E, but indicated a significant decrease in group D when compared with group B.

Table 2 Effect of calcium carbide ripened *Musa sapiens* on sperm morphology and relative testicular weight following acute intake

	Normal sperm cells (%)	Abnormal sperm cells (%)	Relative testicular weight (g)
	MEAN±SD	MEAN±SD	MEAN±SD
Group A (control)	88.75±4.78	11.25±4.78	0.68±0.06
Group B (500 mg/kg of NRB)	90.00±4.35 ^a	10.00±4.35 ^a	0.58±0.07 ^a
Group C (100 mg/kg of CCRB)	86.67±2.88 ^a	13.33±2.88 ^a	0.64±0.10 ^a
Group D (200 mg/kg of CCRB)	78.33±16.07 ^a	21.67±16.07 ^a	0.55±0.17 ^a
Group E (500 mg/kg of CCRB)	87.67±6.80 ^a	12.33±6.80 ^a	0.67±0.11 ^a
F-ratio	0.98	0.98	0.86

Data was analyzed using ANOVA followed by post Hoc LSD and values were considered significant at $p < 0.05$, NRB; Normal ripened banana, CCRB; Calcium carbide ripened banana, *; significant, a; non-significant.

Data for normal sperm cells and abnormal sperm Cell indicated no significant differences in groups B, C, D and E when compared to group A. Data also indicated that the relative testicular weight indicated no significant differences in groups B, C, D, and E ($p=0.243$, $p=0.559$, $p=0.140$, $p=0.840$) compared to Control. Meanwhile the mean normal sperm cells when compared to group B, revealed no significant difference in groups C, D, and E. The abnormal sperm cells result showed no significant differences in groups C, D, and E compared to group B. The mean relative testicular weight also showed no significant difference in groups C, D, and E when compared to group B.

Table 3 Effect of calcium carbide ripened *Musa sapiens* on sperm motility and total sperm count following chronic intake

	Active motility (%)	Non-motile (%)	Total sperm count (X10 ⁶ /mls)
	MEAN±SD	MEAN±SD	MEAN±SD
Group A (control)	83.75±4.78	16.25±4.78	61.20±4.49
Group B (500 mg/kg of NRB)	90.75±7.22 ^a	9.25±7.22 ^a	65.7±3.48 ^a
Group C (100 mg/kg of CCRB)	91.75±5.37 ^a	8.25±5.33 ^a	59.02±19.24 ^a
Group D (200 mg/kg of CCRB)	84.50±11.00 ^a	15.50±11.00 ^a	60.90±6.72 ^a
Group E (500 mg/kg of CCRB)	77.50±20.68 ^a	22.50±20.68 ^a	59.80±22.59 ^a
F-ratio	1.03	1.03	0.11

Data was analyzed using ANOVA followed by post Hoc LSD and values were considered significant at $p < 0.05$, NRB; Normal ripened banana, CCRB; Calcium carbide ripened banana, *; significant, a; non-significant.

Data for Active Sperm motility and non-motile indicated no significant difference in groups B, C, D and E when compared to control group. The total sperm count indicated no significant difference in groups B, C, D, E when compared with the Control. Also, the active motility mean result showed no significant difference in groups C, D and E when compared to group B. The mean non-motile sperm also indicate no significant difference in groups C, D, and E when compared to group B. The total sperm count result indicated no significant decrease in groups C, D, and E compared to B.

Table 4 Effect of calcium carbide ripened *Musa sapiens* on sperm morphology and relative testicular weight following chronic intake

	Normal sperm cells (%)	Abnormal sperm cells (%)	Relative testicular weight (g)
	MEAN±SD	MEAN±SD	MEAN±SD
Group A (control)	88.75±4.78	11.25±4.78	0.68±0.06
Group B (500 mg/kg of NRB)	92.00±2.44 ^a	8.00±2.44 ^a	0.63±0.08 ^a
Group C (100 mg/kg of CCRB)	94.00±4.76 ^a	6.00±4.76 ^a	0.59±0.10 ^a
Group D (200 mg/kg of CCRB)	80.00±15.45 ^a	19.25±15.45 ^a	0.45±0.14 [*]
Group E (500 mg/kg of CCRB)	76.75±22.33 ^a	23.25±22.33 ^a	0.61±0.03 ^a
F-ratio	1.39	1.39	3.56

Data was analyzed using ANOVA followed by post Hoc LSD and values were considered significant at $p < 0.05$, NRB; Normal ripened banana, CCRB; Calcium carbide ripened banana, *, significant, a; non-significant

Data in the active sperm cells in groups B, C, D and E indicated no significant difference when compared to group A. Data in the abnormal sperm cells also indicated no significant difference in groups B, C, D and E when compared to group A. The relative testicular weight result showed no significant difference in group B, C, and E compared to group A; but showed a significance decrease in group D. However, the mean of the normal sperm cells also showed a no significant increase in groups C, D and E when compared to group B. The abnormal sperm cells indicated no significant difference in groups C, D and E compared to group B. The mean relative testicular weight indicates a significant decrease in group D while groups C and E had no significant difference when compared to group B.

4. Histological Results

4.1. Acute Phase of Testicular Histological report

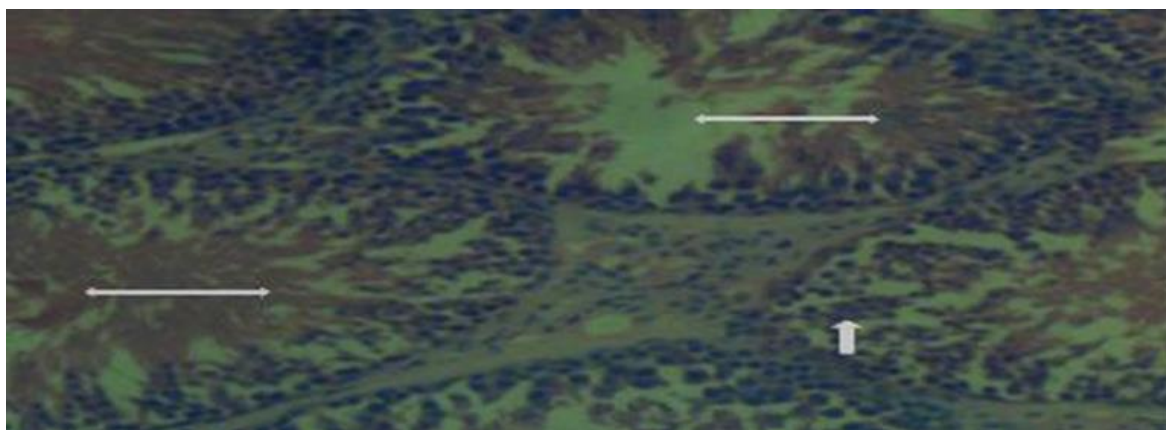


Figure 1 Group A received feed and water only: A photomicrograph section of the testes shows morphology consistent with testicular histology. The ductus epididymis (arrow), spermatozoa and the connective tissue are shown with normal architecture. The seminiferous tubules (double arrow) show active and normal spermatogonia and spermatids. (Stained with H & E; X 400)

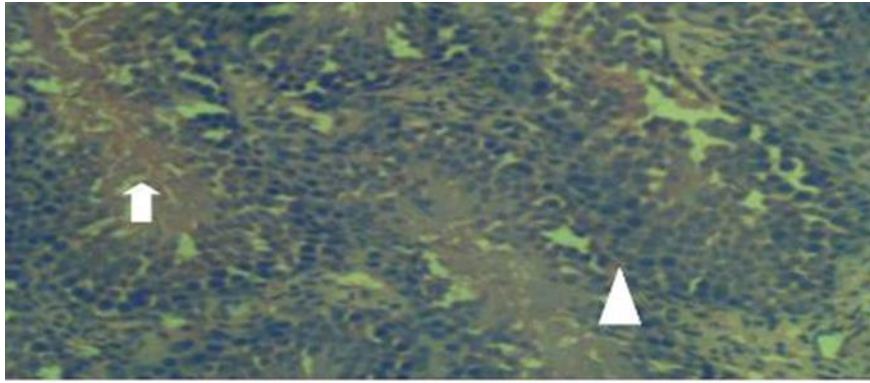


Figure 2 Group B (administered with 500 mg/kg of NRMS): Photomicrographs of testes sections show morphology consistent with testicular histology. The ductus epididymis (arrow), spermatozoa and the connective tissue are shown with normal architecture. The seminiferous tubules (arrowhead) show active and normal spermatogonia and spermatids (Stained with H & E: X 400).

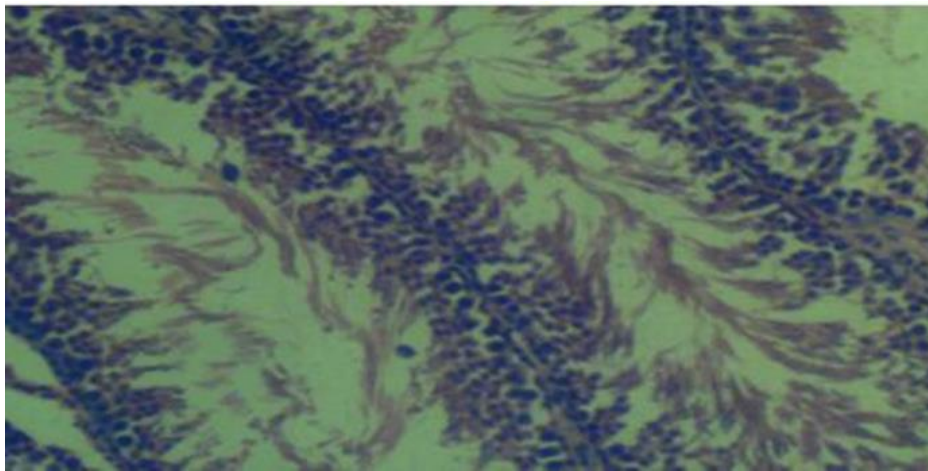


Figure 3 Group C (administered with 100 mg/kg of CCRMS): Photomicrographs of testes sections show morphology consistent with testicular histology. The ductus epididymis (arrow), spermatozoa and the connective tissue are shown with normal architecture. The seminiferous tubules (arrowhead) show active and normal spermatogonia and spermatids (Stained with H & E: X 400).

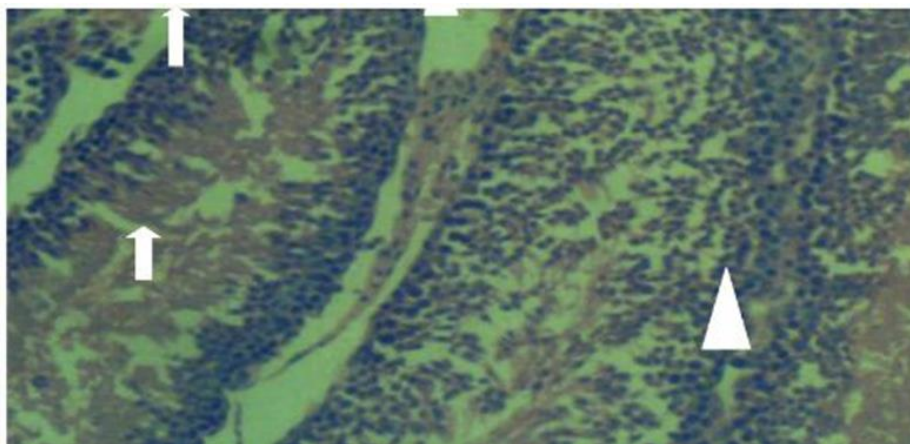


Figure 4 Group D administered with 200 mg/kg of CCRMS: Photomicrographs of testes sections show morphology consistent with testicular histology. The ductus epididymis (arrow), spermatozoa and the connective tissue are shown with normal architecture. The seminiferous tubules (arrowhead) show active and normal spermatogonia and spermatids (Stained with H & E: X 400).

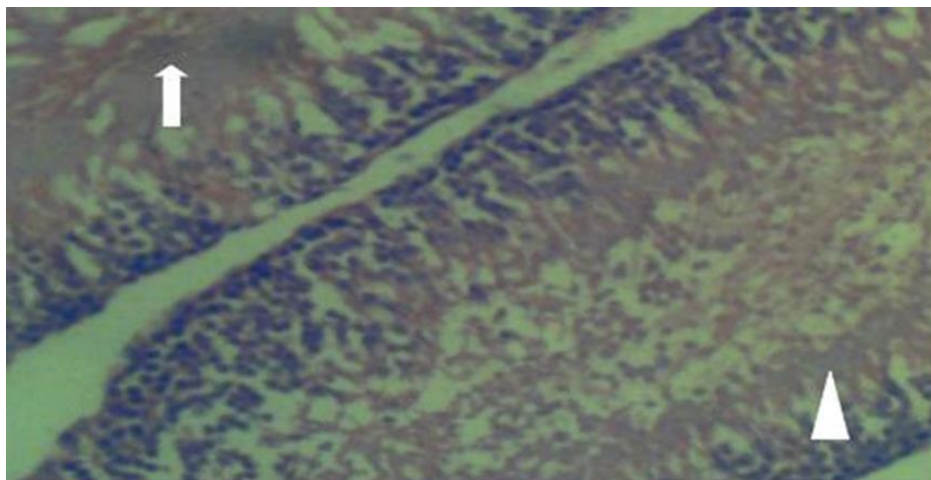


Figure 5 Group E administered with 500 mg/kg of CCRMS: Photomicrographs of testes sections show morphology consistent with testicular histology. The ductus epididymis (arrow), spermatozoa and the connective tissue are shown with normal architecture. The seminiferous tubules (arrowhead) show active and normal spermatogonia and spermatids (Stained with H & E: X 400).

4.2. Chronic phase of Testicular Histological report

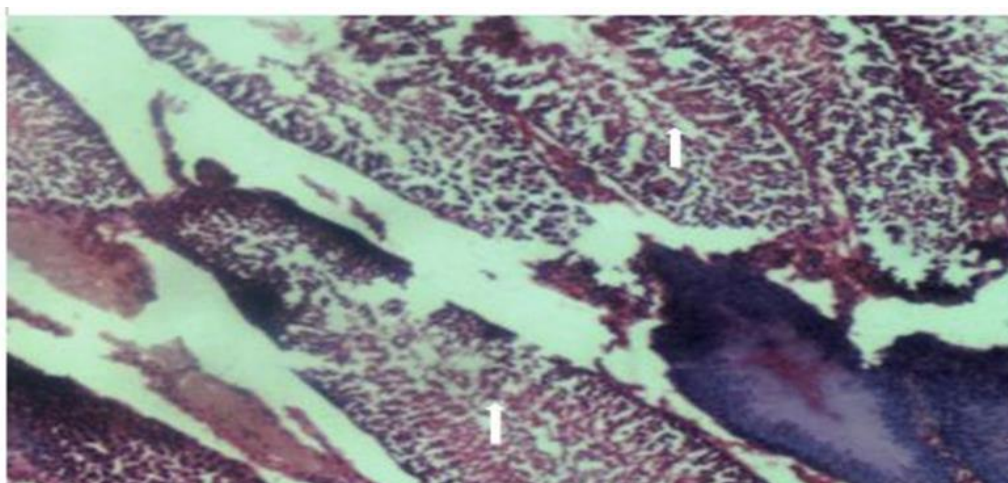


Figure 6 Group B administered with 500 mg/kg of NRMS: A photomicrograph section of the testes shows matured seminiferous tubules with intact germinal cells (arrows). Stained with H & E (X 400).

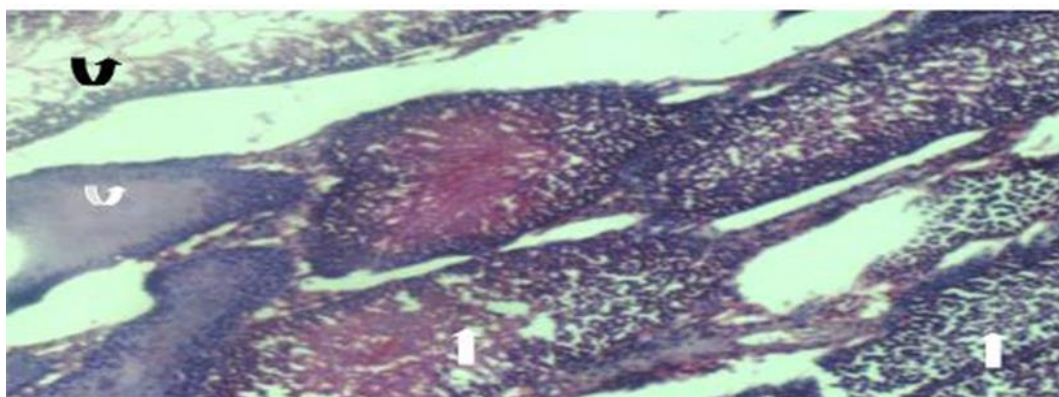


Figure 7 Group C administered with 100 mg/kg of CCRMS: A photomicrograph section of the testes shows matured seminiferous tubules with intact germinal cells (curved arrows) and either a degenerated germinal life or a reduced level of spermatogenesis at the upper left (arrows). Stained with H & E (X 400).

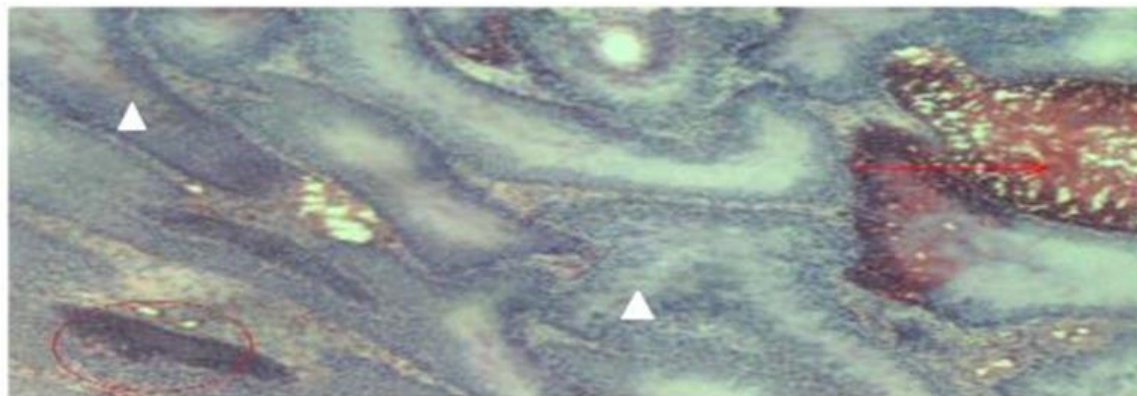


Figure 8 Group C administered with 200 mg/kg of CCRMS: A matured section of the testes showing the seminiferous tubule (arrow) and others revealing either a degenerated germinal life or a reduced level of spermatogenesis (arrow-head). Circled shows hyperplasia. Stained with H & E (X 400).

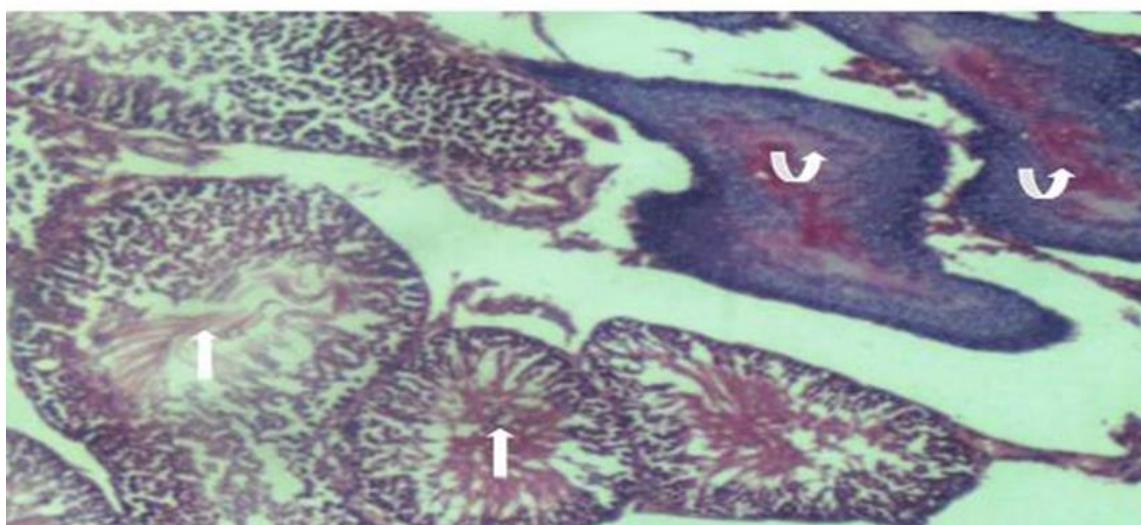


Figure 9 Group E administered with 500 mg/kg of CCRMS: A section of the testicular architecture shows matured seminiferous tubules with intact germinal cells (arrows) and either a degenerated germinal life or a reduced level of spermatogenesis at the right (curved arrows). Stained with H & E (X 400).

5. Discussion

Male infertility has stirred global concerns over the trend of its increasing prevalence and ambiguity of its etiopathogenesis [31,32]. Given that almost half of the global infertility cases involve male factors, it is essential to understand the core mechanisms of male infertility causation [33,34]. There has been an increase in the cause of some male infertility which include lifestyle or environmental factors which include heavy metals, pesticides/herbicides which can impair sperm count, motility and sexual function [35]. There is a global upsurge in terminal diseases due to unnoticed intake of toxic chemicals. One of such chemicals is calcium carbide, which has been used to ripen fruits by fruit vendors [36]. This study investigated the effect of calcium carbide ripened banana on the male reproductive function in Wistar rats.

Our findings indicated that there were no significant differences in relative testicular weight of all the test groups when compared to group A, in the acute phase. But in the chronic phase, a significant decrease in the relative testicular weight was indicated in group D, administered 200mg/kg of Calcium carbide ripened banana when compared to groups A and B. The mechanism behind the decrease in relative testicular weight of the group D rats is not very clear but may be linked to the presence of impurities in carbide, which contains traces of arsenic and phosphorous. Once dissolved in

water, these elements produce acetylene gas [37, 36], This can cause an increase in lipid peroxidation which leads to generation of free radicals' species such as reactive oxygen species (ROS), within a body system which could negatively deregulate the normal cellular function and initiate the detrimental effects on the organs [38]. A similar study by Yakubu et al., 2013 [39] reported a significant increase in the relative testicular weight of rats with aqueous extract of *Musa paradisiaca* root on testicular function parameters, thus contradicting the present findings.

The study resulted in a no significant differences in the sperm motility in the acute phase in group treated with CCRB and NRB in the acute phase compared to control. Also, the chronic phase demonstrated a non-significant increase in sperm motility in the NRB group, 100 and 200 mg/kg of CCRB, compared with the control group. However, during the acute phase, significant decrease was observed in active motility in group D treated with 200 mg/kg of CCRB compared with those treated with normal ripe banana. Dike and Etsede, 2016 [40] reported a significant reduction in the sperm motility level following intake of calcium carbide induced ripe banana fruit in wistar rats, which is in agreement with the above findings.

The study findings demonstrated a significant higher total sperm count in the NRB group compared with the control in the acute phase, while the CCRB groups showed a non-significant increase compared to control. At the chronic phase, no significant changes were found in the NRB and CCRB groups compared to control following the intake of banana fruit. The significant increase in total sperm count may be attributed to the presence of flavonoids, which has been shown to result in the regulation of the sperm count at the seminiferous tubule levels [41]. Furthermore, it was observed that during the acute phase, the group D treated with 200 mg/kg of CCRB indicated a significant decrease in total sperm count when compared with the group treated with normal ripe banana. This is in agreement with the report of Dike and Etsede, 2016 [40], in which there was a significant decline in the total sperm count following calcium carbide ripened banana intake in wistar rats.

The present findings indicated no significant changes in the sperm morphology in the NRB and CCRB treated groups in both acute and chronic phases compared to control group. The reason associated to the non-significant differences is not well understood. However, Dike and Etsede, 2016 [40], in their study, revealed a significant decrease in the sperm morphology following calcium carbide ripened banana fruits, thus disagreeing with the present findings.

The histological study reported that the intake of NRB and CCRB at the acute phase demonstrated an increased levels of active spermatogonia and spermatogenesis in testicular architecture. The increased level of spermatogenesis following the intake of NRB may be attributed to the presence of high calcium and potassium ion level [42]. Alabi et al., 2013 [21] indicated congestion of the epithelium following calcium carbide ripened banana, which disagree with the study findings. Furthermore, at the chronic stage, the CCRB treatment groups showed mild degeneration of germinal epithelium and reduced level of spermatogenesis. This may be due to a gradual elevation of intracellular ROS due to a long term CaC₂ exposure, which may result in an increase in the cellular oxidative stress and disturb the redox balance of the cell, which may gradually undergo apoptosis [38]. The degeneration of the germinal epithelium may be associated with high arsenic and phosphorous level in the calcium carbide ripened banana. The study findings align with the previous reports [21] and [43] demonstrating a congested epithelia lumen of the ovary following calcium carbide ripened banana.

6. Conclusion

The study provides evidence of the detrimental effects of calcium carbide ripened banana on male reproductive health, showing significant reduction in sperm motility, total sperm count, and testicular weight and alterations in testicular histology. These effects were more evident in rats treated with 200 mg/kg of CCRB. Though the reason why these effects were more profound in this group is not clear, however this should raise concern about the implication of consuming fruits treated with calcium carbide on the male reproductive health, thus warranting further investigations into their safety and regulation.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval was obtained from Research and Ethical committee of Basic Medical Sciences, College of Health Sciences (NAU/CHS/NC/FMBS/480).

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