The Effect of Ethanolic Extract of Garcinia Kola on the Sperm Parameters and Histology of the Testis of Male Wistar Rats

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Abstract

The Garcinia kola seeds have in times past been seen to possess a natural aphrodisiac effect as well as have protective and therapeutic effects amongst a host of other effects. This study however evaluated the effects of ethanolic extracts of the Garcinia kola seeds on the sperm parameters and histology of the testis in varying doses in adult male wistar rats. Fifteen adult male wistar rats within the weight range of between 140-290g were designated into three groups A, B and C of five animals each. Groups A and B received the ethanolic extracts of Garcinia kola orally as 400mg and 200mg respectively for a period of twenty-eight days while the Group C served as the control group still within this stipulated time. Twenty-four hours after the last oral administration, the animals were anaesthetized with diethyl ether and dissected for testis harvesting. The sperm parameters revealed that those of Group A had decreased sperm count and sperm motility than other groups and the Group C had the least amount of sperm morphological defects. The histological results further revealed that Group A had a slight disorientation and interstitial congestion of the cells, the Group B showed a normal interstitial space with few numbers of matured spermatozoa and regeneration of the germinal epithelium. This study therefore suggests that excessive consumption of Garcinia kola seeds could lead to an increased damaging effect on the sperm parameters as well as structure of the testis and therefore it should be advised against.

Keywords: Aphrodisiac, Garcinia kola, Sperm parameters, Testis, Wistar rats.

Introduction

Plants provide an alternative source in search for new drugs. Plant based medication has been man's ultimate therapeutic agent over the years and is still in the frontline for improving human health (Ameyaw et al 2008). There is a rich abundance of plants reputed to possess protective and therapeutic properties. These herbal plants provide the major source of ingredients in the formation and packaging of drugs. This is occasioned by their high efficacy with no or very little side effects (Ikpeme et al 2012) and Garcinia kola is one of such plants.

Garcinia kola also known as Bitter Kola, is a perennial crop found in forests distributed throughout West and Central Africa which grows as a medium sized tree, up to a height of twelve meters (Iwu 1993). In Nigeria, it is commonly called "Namijin goro", "Agbilu" and "Orogbo" in Hausa, Igbo and Yoruba lands respectively (Esomonu 2005).

The seeds of Garcinia kola are rich in flavonoids, and have been shown to have antibiotic properties (Hong Xi et al 2001). Phenols, alkaloids, tannins and saponins are other phytochemical constituents of Garcinia kola seeds and they exert various beneficial effects in humans and animals (Okwu 2005), which includes when chewed fresh, they serve as energy stimulant used to prevent or treat colic in babies and also to treat upper respiratory tract infections. They are traditionally used in several West African countries for the treatment of head and chest colds, dysentery, diarrhoea and urinary infections and as an antidote for poison (Iwu et al 2002).

Micromorphometric and stereological effect of ethanolic extracts of Garcinia kola seeds on the testis and epididymis of adult wistar rats showed that there was a significant increase in the sperm counts but reduction in motility in the treated groups in a dose dependent manner and also the volume density ratio of lumen was increased in the treated groups which receive the higher doses of extracts when compared with the control group (Adesanya et al 2006).

The effects of ethanolic extract of Garcinia kola on sexual behaviour and sperm parameters, shows that Garcinia kola possess aphrodisiac effect and as such is used traditionally in the treatment of erectile dysfunction (Ralebona et al 2012).

The effect of its crude ethanolic extract on the reproductive system of male wistar rats revealed increase in the interstitial spaces, degeneration of the Leydig cells and distortion in the arrangement of the cells of spermatogenic series revealing a significant increase in the sperm count of the experimental groups when compared statistically with the control group (Oluyemi et al 2007).

Investigation of the effects of ethanolic extracts of Garcinia kola on testicular weight, serum testosterone, luteinizing hormone and sperm count in adult male albino wistar rats after administration at different dose concentration suggested that the administration at a dose of 300 mg/kg may impair reproductive

functions (Agbai et al 2013).

A related study which investigated the effect of aqueous extract of *Garcinia kola* revealed that *Garcinia kola* seeds did not have sex enhancing potential as claimed. The study disagreed with the aged long acclaimed aphrodisiac potentials of *Garcinia kola* seeds in some parts of Western Nigeria, inferring that the acclaimed pro sexual effect of *Garcinia kola* seeds is scientifically untrue. There was a reduction in the germinative cell thickness of the seminiferous tubules in the treated groups causing them to have increased volume density when compared to the control group. There was also a significant increase in the sperm counts but reduction in motility in the treated groups in a dose dependent manner compared with control group (Yakubu *et al* 2012).

A research on the biological activities of aqueous extract of *Garcinia kola* in growing wistar rats showed the extract enhanced the libido of the growing male rats but did not necessarily improve their fertility rate (Uko *et al* 2001).

Another investigation on the effect of ethanolic extract of *Garcinia kola* on the histology of the testes of male adult wistar indicated that the ethanolic extracts had some adverse effects on the testis of male wistar rats which is dose dependent (Ajibade *et al* 2011).

The testis (the male gonad homologous with the ovary of the female produces sperms and male hormones especially testosterone) is a paired organ, with the left testis being slightly lower than the right. The testis is oval in shaped and is compressed from side to side. The external features of the testis includes: two poles or ends (upper and lower), two borders (anterior and posterior) and two surfaces (medial and lateral) (Chaurasia 2008).

The surface of each testis is covered by the visceral layer of the tunica vaginalis, except where the testis attaches to the epididymis and spermatic cord. It has a tough fibrous outer surface called the tunica albuginea, which thickens into a ridge on its internal posterior aspect as the mediastinum of the testis (Moore *et al* 2010).

The present study's objective is to further investigate the effect of *Garcinia kola* on sperm parameters as well as its effect on the histology of the testis of adult male wistar rats.

Materials and Method

Breeding of Animals and Duration of Experiment

Fifteen adult male wistar rats weighing between 140-290g were purchased from the animal house of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus and used for the experiment. They were maintained under standard conditions of temperature and illumination (12 hours dark: 12 hours light) cycle. They were fed with standard rat feed (growers mash) and provided with water *ad libitum* during the experiment. They were acclimatized for two weeks before the experimental administration which lasted for twenty-eight days bringing the entire experimental duration to six weeks.

Materials for Study

The materials used for this experiment includes the following:

- 1. Fifteen adult male wistar rats which were divided into three groups A, B and C.
- 2. Garcina kola seeds which were purchased at Nkwo market in Nnewi, Anambra state of Nigeria.
- 3. Hand grinder.

4. Growers mash; which was produced by Grand Cereal Mills, Plateau State, Nigeria was used as feed for the rats throughout the duration of acclimatization and administration.

- 5. Big mesh grid cages which were three in number.
- 6. Electronic Weighing Balance; manufactured by Napco Precision Instrument Company Limited, China.
- 7. Syringes and canula for administering the extracts.
- 8. Compound light microscope.
- 9. Slides and cover slips.
- 10. Refridgerator.
- 11. Diamond pencils.
- 12. Neubauer counting chamber; manufactured by Superior Marienfield, Germany.
- 13. Rotatory microtome.
- 14. Embedding moulds and pot.

Extract Preparation

The Garcina kola was pulverized using the hand grinder. 200g of the pulverized sample was macerated in 1000ml of ethanol and was shook at intervals for 48 hours with a mechanical shaker. The mixture was sieved using parceling cloth. It was further filtered with No. 1 Whatman filter paper. The filtrate was concentrated using rotary evaporator at reduced pressure. It was further dried using water bath at temperature of 50° C. The plant extract was stored in a refrigerator for use. This process was carried out at the Department of Pharmacognosy, Nnamdi Azikiwe University, Agulu, Nigeria.

Experimental Protocols

Fifteen apparently healthy adult male wistar rats were assigned to three different cages in a group of five each for acclimatization, for a period of two weeks, prior to the commencement of the extract administration. Group C served as the control group receiving the water and feed only while Groups A and B served as the test groups receiving 400mg/kg body weight/day of ethanolic extract of *Garcina kola* and 200mg/kg body weight/day of ethanolic extract of *Garcina kola*. These solutions were administered for four weeks respectively. Twenty-four hours after the last administration, the animals were anaesthetized using diethyl ether and then each of them was placed in the anatomical position; the testes was approached through the lower abdomen, by making a transverse incision through which the testes were harvested and semen collected by dissecting the from the epididymis.

Determination of Sperm Parameters

The sperm parameters were determined by the following protocols;

1. Sperm Motility: A drop of well-mixed liquefied semen was placed on a slide and covered with a 20×20 mm cover slip. The specimen was focused using the $10 \times$ objective lens. The condenser iris was sufficiently closed to give good contrast. The $40 \times$ objective was then used to examine several fields, assessing their motility. The percentage of motile and non-motile spermatozoa was then recorded. This method was done in duplicate for each specimen.

2. Sperm Count: A small cylinder was filled with well-mixed liquefied semen to the 1ml mark, after which sodium bicarbonate-formalin diluting fluid (Reagent No. 72) was added to reach the 20ml mark of the cylinder and mixed well. Neubauer counting chamber was then filled with well-mixed diluted semen using a Pastuer pipette. This was then viewed under the light microscope using the $10 \times$ objective with the condenser iris closed sufficiently to give good contrast and the number of spermatozoa was counted in the two large squares. This method was also done in duplicate for each specimen.

3. Sperm Morphology: A thin smear of the liquefied well mixed semen was made on a clean slide. While still wet, the smear was fixed with 95% ethanol for 5-10 minutes, and allowed to air dry. The smear was washed with sodium bicarbonate- formalin solution (Reagent No. 72) to remove any mucus which may be present. The smear was then rinsed with several changes of water. The smear was covered with dilute (1 in 20) carbon fuchsin and allowed to stain for 30 minutes. Then the stain was washed off with water. The smear was counterstained with dilute (1 in 20) Loeffler's methylene blue for 2 minutes. The stain was then washed off with water. Finally, the smear was drained and allowed to air- dry. This was viewed under the light microscope and the percentage of normal and abnormal spermatozoa using the $40 \times$ objective was determined. The $100 \times$ objective was also used to confirm abnormalities. This was done in duplicate for each specimen.

Tissue Processing

The tissue processing was done according to standard procedures for easy study under the compound light microscope, the testis tissue fixation was done in 10% formal saline for 2 hours to preserve its various cellular constituents, the tissues were then dehydrated in ascending grades of alcohol of 50%, 70%, 90% and 100% for two hours each. The tissues were then cleared in three changes of xylene for a period of one hour thirty minutes to remove the alcohol from the tissue. The xylene was removed and substituted by the molten paraffin which was maintained at a temperature of $3-5^{\circ}$ C above the melting point of the paraffin wax used, the tissues were afterwards placed into embedding mould containing molten paraffin wax. During this procedure, tissue was oriented into its proper position after which it was allowed to cool and solidify before being transferred into a bathe of ice block for easy detachment of the mould made from the embedded cassette. The mould containing the testis was then mounted and trimmed to remove excess wax, then sectioned first to 15 microns and later 4 microns with the aid of a rotatory microtome. The tissue sections were deparaffinised in 20% alcohol and hydrated in a water bath of about 45°C, the tissues were now allowed to dry on clean slides and labelled using diamond pencils, the slides were adhered to by the tissues when dried on a hot plate at 5° C. The sectioned tissue slide was stained using Erhlich's haematoxylin and eosin staining technique for general tissue structure to give its characteristic colour before being mounted using the Dibutylpthalate Polystyrene Xylene (DPX) mountant and finally covered with cover slip before viewing under the compound light microscope.

Results

Physical Observations

There were no clinical signs. However, the test groups (A and B) were noted to be very active as compared to the control group (C).

Body Weight Changes

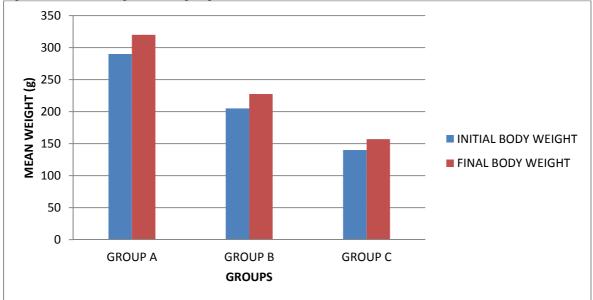
At the beginning of the experiment, the initial body weights of the animals were taken and immediately before the animals were sacrificed for tissue collection, the final body weights were taken, comparing the initial body weight mean and final body weight mean as by their groups. The changes are presented in the table and bar chart presented below;

Tuble 1. The table below shows the mean of the mitial and main body weights (g) of the three groups				
GROUP	WEIGHT	MEAN	P-VALUE	
GROUP A (400mg/kg)	INITIAL	290.00±38.29		
			P>0.05	
	FINAL	320.00±58.87		
GROUP B (200mg/kg)	INITIAL	205.00±37.85		
			P>0.05	
	FINAL	227.50±55.60		
GROUP C (control)	INITIAL	140.00±23.09		
			P>0.05	
	FINAL	157.50±26.29		

Table 1: The table below shows the mean of the initial and final body weights (g) of the three groups

(Values expressed in Mean \pm SD)

Fig 1: The bar chart below shows the comparison between the mean of the initial body weight and the final weight of each of the experimental group



Semen Analysis

The tables and bar charts below show the results of the semen analysis (sperm motility, sperm count and sperm morphology) of the respective groups used in carrying out this experiment.

Analysis of Sperm Motility

This experimental parameter showed that the rapid progressive sperm cells motility were statistically significantly decreased in Group A when compared to Group B, Group B were statistically insignificantly increased in rapid progressive sperm cells motility when compared to Group C. The analysis of the sperm motility is presented in the table and bar chart below;

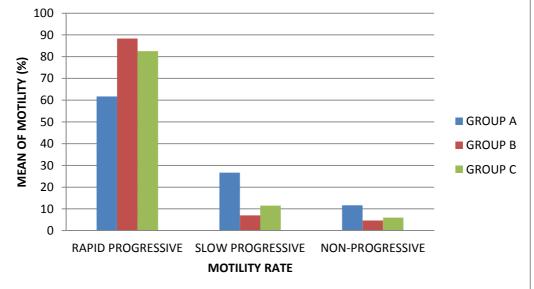
Table 2: The table below shows the percentage motility of the three groups

MOTILITY (%)	GROUP A (400mg/kg)	GROUP B (200mg/kg)	GROUP C (Control)
RAPID PROGRESSIVE	61.66±2.88**	88.33±9.42	82.50±17.67
SLOW PROGRESSIVE	26.66±2.88**	7.00±5.65	11.50±12.02
NON-PROGRESSIVE	11.66±2.88	4.66±3.77	6.00±5.65

Values expressed in Mean±SD.

**significantly different compared to Groups B and C





Analysis of Sperm Count

This parameter showed that there was a statistically insignificant increase in the sperm count of Group B compared to Group A, also there was a significant increase in the sperm count of Group A and Group B (test groups) compared to Group C (control group). The analysis of the sperm counts are presented in the table and bar chart below;

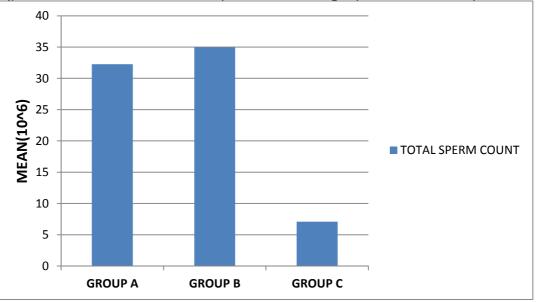
Table 3: The table below shows the mean sperm count of the three groups

	GROUP A (400mg/kg)	GROUP B (200mg/kg)	GROUP C (Control)
TOTAL SPERM COUNT (×10 ⁶)	$32.26 \pm 13.98 \times 10^{6*}$	$34.96 \pm 2.90 \times 10^{6*}$	$7.10 \pm 4.66 \times 10^{6}$

Values expressed in Mean±SD.

*significantly different compared to Group C

Fig 3: The bar chart below shows the comparison between the group and the mean total sperm count



Analysis of Sperm Morphology

This experimental parameter showed that there was a statistically significant decrease in the normal sperm percentage of Group A when compared to other groups. It also showed that there was an insignificant increase in normal sperm percentage of Group C compared to Group B. The head defect percentage was significantly higher in Group A compared to Groups B and C while the tail defect percentage was insignificantly higher in Group A compared to Group B but significantly higher compared to Group C. There was no significant difference in the

middle piece defect percentage between all experimental groups. The analyses are presented in the table and bar chart below;

Table 4: The table below shows the percentage of normal sperm, as well as head, middle piece and tail defects of all groups

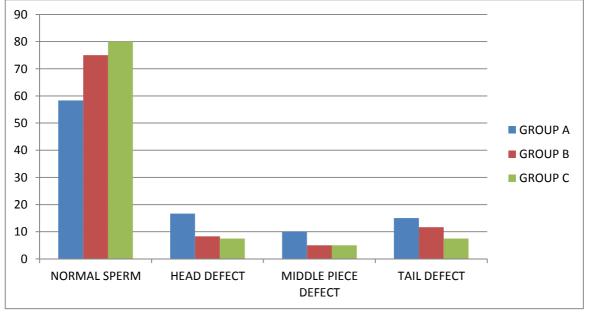
MORPHOLOGY (%)	GROUP A (400mg/kg)	GROUP B (200mg/kg)	GROUP C (Control)
NORMAL	58.33±7.63**	75.00±5.00	80.00±0.00
HEAD DEFECT	16.66±2.88**	8.33±2.88	7.50±3.53
MIDDLE PIECE DEFECT	10.00±5.00	5.00±5.00	5.00±0.00
TAIL DEFECT	15.00±0.00*	11.66±2.88	7.50±3.53

Values expressed in Mean±SD.

*significantly different compared to Group C

**significantly different compared to Groups B and C

Fig 4: The component bar chart below shows the comparison between the mean sperm morphology (%) of the three groups



Histological Findings Micrograph of Group A:

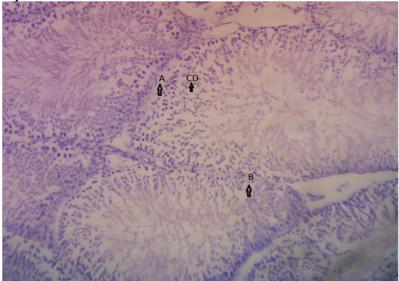


Fig A: Shows a slight disorientation of the spermatogenic cells, with a few numbers of spermatozoa in the lumen and also present, is interstitial congestion using H&E stain ($\times 100$).

A and B indicates interstitial congestion while CD indicates disorientation of the spermatogenic cells.

Micrograph of Group B:

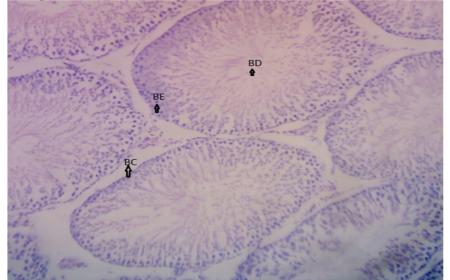


Fig B: Shows a normal interstitial space with few numbers of matured spermatozoa and regeneration of the germinal epithelium, using H&E stain (×100).

BD-lumen with matured spermatozoa; BE-regeneration of germinal epithelium; BC- Normal interstitial space.

Micrograph of Group C:

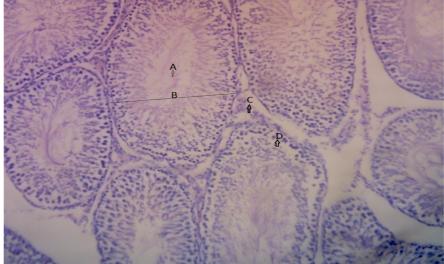


Fig C: Shows a testis with normal arrangement of the seminiferous tubules, spermatozoa and interstitial cells of Leydig, using H&E stain (×100).

A-lumen with matured spermatozoa, B-seminiferous tubule, C-Leydig cell, D-spermatogonia.

Discussion

This present study revealed the results of the administration of ethanolic extract of *Garcinia kola* resulted in an observable increase in the weight of the adult male wistar rats, although not statistically significant when comparing their initial weights to their final weights.

Other results obtained from the semen analysis in this experiment showed that there was a significant increase in the sperm count of Groups A and B as compared to the control group which is group C. This could be as a result of the presence of biflavonoid and xanthone in the plant which are potent antioxidants capable of increasing the production of testosterone (Oluyemi *et al* 2007). This revealed that at a relatively lower dose of the extract, the sperm motility and normal sperm morphology was excellent but at an increased dose there was impediment in function and reduction in the number of normal sperm cells. These disagree with that of (Ajibade *et al* 2011) where it was reported that crude ethanolic extract of *Garcinia kola* had no effect on the morphology of sperm cells and that it caused an increase in motility, though it was carried out with different doses compared to that of this study. The histological findings revealed testis with disorientation of spermatogenic cells and

interstitial congestion in Group A which received the highest dose of the extract and testis with normal interstitial space, few numbers of matured spermatozoa in the lumen and regeneration of the germinal epithelium in Group B which received the lowest dose of the extract. The disorientation of spermatogenic cells and interstitial congestion in Group A may be as a result of increased dose of the extract and these could be the reason for a recorded decrease in sperm motility and decrease in the number of normal sperm cells in Group A, because it was not recorded in Group B that received a lower dose of the extract. So in essence, the reason for the decrease in sperm motility in Group A in this study may not be due to the rapidity of development and may not be as a result of the presence of some toxic component like benzophenone.

Conclusion

The result of this present study suggests that excessive consumption of ethanolic extract of *Garcinia kola* may have an effect on sperm parameters, especially sperm motility and sperm morphology and also a significant damaging effect on the structure of the testes. Thus, the excessive consumption of *Garcinia kola* can be detrimental in males and cause male infertility.

Following the result of this study, it should be recommended that the Health/Nutritional agencies in Nigeria help regulate the various consumption doses of *Garcinia kola* seeds used for nutrition and herbal medicine and also educate people (especially in the Northern and Eastern parts of Nigeria) on the adverse effect of *Garcinia kola* at extreme consumption. Also, further researches with increased doses should be carried out so that this result can be substantiated as well as to ascertain safety with higher doses. Similar study should also be investigated in female wistar rat to determine its effect on the female reproductive system.

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