Effects Of Crude Ethanolic Extract Of Garcinia Kola (Bitter Kola) On The Histology Of The Testis Of Male Adult Wistar Rats

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Abstract
Aim:
Garcinia kola seed generally known as bitter kola in Nigeria belong to the family of tropical plant. Gracinia kola is used in traditional medicine for various treatments like diabetes, guinea worm remedy and gonorrhea. The study investigated some effect of ethanotic extract of Garcinia kola (bitter kola) on the histology of the testes of male adult wistar rats.
Methodology:
20 adult male rats weighing between 150-200 g were used for the study. These groups were separated into control and experimental groups of five rats each. The control group A rats were given distilled water only for four weeks. Ethanolic extract of Garcinia kola at doses 75, 150 and 300 mg/kg body weight were administered orally to the three treatment groups B, C and D respectively for thirty days. The control rats received quantity of distilled water as contained in the experimental doses. The rats were sacrificed on the thirty first day of the experiment. The inguinal part of the rats were dissected free for collection of the testis and suspended in the semen diluting fluid (sodium bicarbonate-formalin diluting fluid). The rest of the testis was fixed in 10% Bouins fluid for histological preparation. The routine histological preparation at the end of administration revealed seminiferous tubules with occasion of widened interstitial spaces, distortion in the interstitial cells, distortion in the seminiferous tubules, distortion in arrangement of the cell of spermatogenic series. Seminiferous tubules with widened lumen, heavy blood congestion in the vessel and the wall of the vessel are thickened. The sperm count did not reveal any significant changes in the treated groups compared with control group but reduction in motility in the treated group in dose dependent manner compared with control ($P<0.05$) and the morphology also revealed no significant difference in the treated groups when compared with control.
Conclusion:
In conclusion, this finding indicates that ethanolic extract of Garcinia kola has some adverse effects on the testis of male adult wistar rats which is dose dependant.

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Introduction

Garcinia kola seed generally known as bitter kola in Nigeria belongs to the family of tropical plant known as Guthfera (Plow, 1972). It is commonly called orogbo in Yoruba Language. Garcinia kola seed is used in traditional medicine for various therapeutic purposes base on pharmacological effect of the active component (Flavonoid) in the seed and other plant of the plant (Braide and Vitritio, 1989). Despite the fact the physiological studies are still lacking to validate the therapeutic ability of Garcinia kola (Orie and Ekon, 1993) its use in Africa traditional medicine cannot be over emphasized (Holmes, 1960). Reported that Garcinia kola seed is used as an antidote to the effect of strophantris gratus. It also serves as a guinea worm remedy (Lewise *et al*, 1977) and employed in the treatment of diabetes (Tita *et al,* 2001). The sap from Garcinia kola used for parasitic skin disease while the latex used internally for gonorrhea.

The seeds are rich in flavonoid, which have been show to have antibiotic property (Hong-ix and Song, 2001), anti-inflammatory property (Braide, 1990), and anti microbial activity (Madubunyi, 1995). Despite it extensive as herbal medicine. Garcinia kola is found in the moist low and forest, and grows as medium size up to 12 meter in places like Niger, united State, East, Asia India and Central Africa.

The height of the plant is approximately 14cm and it produces reddish, yellowish or orange colour fruit containing 2 to 4 seeds.

The seeds when chewed have a bitter astringent taste chewing stick produced from Garcinia kola tree is very effective and reliable for cleaning teeth when used with toothpaste. The seed as well as the chewing stick an important product often seen in West Africa market. The chewing stick is used to cure halitosis among the Yoruba. Garcinia kola fruit and seeds are normally harvested annually between July to October which make it a highly seasonal produce.

The active constituent of Garcinia kola is a dimeric flavonoid molecules fused together – biflavonid- Biflavonoid are potent antioxidant. Other constituents include xanthones and benzophenoes (Ebong and Korubo, 1996; Encylopedia, 2002). Several works done on Garcinia kola have confirmed its hypolipidermic (Koshy and Vijaya-Laksshmi, 2001; Iwu, 1993; BBC News, 1999; Oluyemi *et al*, 2007) anti fungal (Mackeen *et al*, 2002) anti cancerous (Ho *et al*, 2007) anti can-cerous (Ho *et al*, 2002, pan *et al*, 2001), anti histanic (Nakatani *et al*, 2000), antimictobial (Iwu *et al*, 1996; Iwu *et al*, 1999) and anti ulcerogence effect (Machendran *et al*, 2002) though its effect on the reproductive system have been investigated by

(Akpantah *et al*, 2003 and Akintoye *et al*, 1999), their finding are however at variance. The presences of biflavonid and xanthone in Garcinia kola seeds have been confirmed. The compounds are potent antioxidant. Administration of Garcinia kola seeds extract caused an increased in testosterone production in Sprague dawley rats, due to the anti- oxidant properties of its constituent.

This study assessed the possible effects of crude ethanolic extract of Garcinia kola (bitter kola) on the testis of male wistar rats.

MATERIALS AND METHODS

20 adult male wistar rats weighing between 150-200 g were obtained from the animal house of the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. The rats were kept in a animal cages with plastic netting and maintained under standard laboratory conditions of normal temperature and the animal house was well ventilated, humidity and light for a period of two weeks before commencement of the experiment. During acclimatization the wistar rats were fed on standard diet (Grower's match) and water was given *ad libitum*.

EXPERIMENTAL DESIGN AND GROUPING

In the study, a total of 20 adult male wistar rats were used for the experiment and the rats were grouped randomly into four groups of five rats each. The control group A received feed and distilled water only for four weeks, while group B, C and D respectively received 75, 150 and 300 mg/kg body weight of the extract orally for period of four weeks. The animals from each group were sacrificed by cervical dislocation at the end of the administration of the extract for the period of four weeks.

Histological processing and statistical analysis of the parameters measured were carried out.

ADMINISTRATION OF THE EXTRACT

The administration of the extract was done orally proper concentration were administered by the use of metal oxopharygeal canula and calibrated hypodermic syringe. The administration of Garcinia kola (Bitter kola) extract was done once in a day for 7days of the week and for the period of four weeks.

TESTIS COLLECTION AND SEMEN ANALYSIS

The testes were dissected out via the inguinal region; the caudal epididymis were dissected free and suspended in the semen diluting fluid (sodium bicarbonate-formalin diluting fluid), containing 1% acetic acid, 4% $NaHCO_3$ and 35% formalin for semen analyses (Monica, 2000). The rest of the testis was fixed in Bouin's fluid.

RESULTS

HISTOLOGICAL FINDING

The control group shows normal somniferous tubules and well space interstitial space, collagen tissue, spermatogonia cells are intact substanticular cells, interstitial cells and wall of the vessel appears normal

Group B that received (75mg/kg) body weight of ethanolic extract of Garcinia kola shows somniferous tubule with occasion of widened interstitial space, however the spermatogonia cells and subtanticular cells appear normal.

Group C that received (150 mg/kg) body weight of ethanolic extract of Garcinia kola shows seminiferous tubules with widened lumen and occasion of interstitial space with their spermatogonia cells remained intact and distortions in the arrangement of the cells of spermatogenic series, and distortions in the seminiferous tubules.

Group D that received (300 mg/kg) body weight shows heavy blood congestion in the vessel and the wall of vessel are thickened, distortions in the seminiferous tubules and distortions in the arrangement of the cells of spermatogenic series.

SEMEN ANALYSES

When comparing control group with other treated group, there is no significant difference at the level of $P<0.05$.

SPERM MOTILITY GRADING,

RAPID PROGRESSIVE MOTILITY.

When comparing the control group with other treated group.

Group B that received (150mg/kg) body weight of ethanolic extract of Garcinia kola shows there is significant difference while Group C that received (150m+g/kg) body weight of ethanolic extract of Garcinia kola and Group D that received (300mg/kg) body weight of ethanolic extract of Garcinia kola has no significant difference at the level of $P<0.05$.

SLOW PROGRESSIVE MOTILITY.

When comparing the control group with other treated group. Group C that received (150mg/kg) body weight of ethanolic extract of Garcinia kola shows there is significant difference while group B that received (74mg/kg) body weight of ethanolic extract of Garcinia kola and group D that received (300mg/kg) body weight of ethanolic extract of Garcinia kola shows there is no significant difference at the level of $P<0.05$.

NON- PROGRESSIVE MOTILITY

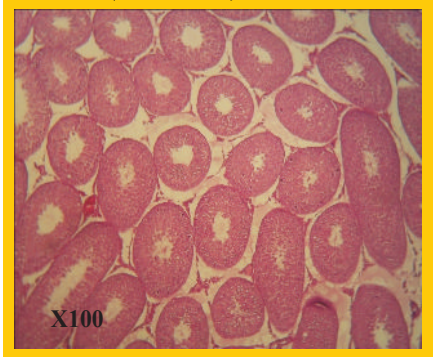
When comparing the control with other treated group. Group C that received (150mg/kg) body weight of ethanolic extract of Garcinia kola shows there is no significant difference while group B that received (75mg/kg) body weight of ethanolic extract of Garcinia kola and group D that received (300mg/kg) body weight of ethanolic extract of Garcinia kola shows there is significant different at the level of $P<0.05$.

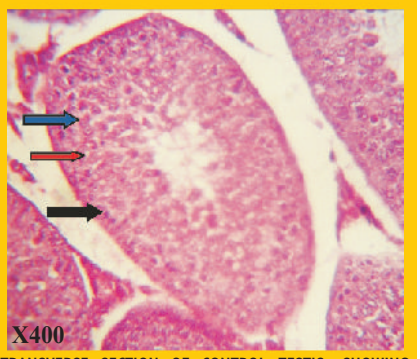
DEAD SPERM CELL.

When comparing the control with other treated group. Group C that received (150mg/kg) body weight of

HISTOLOGICAL ANALYSIS

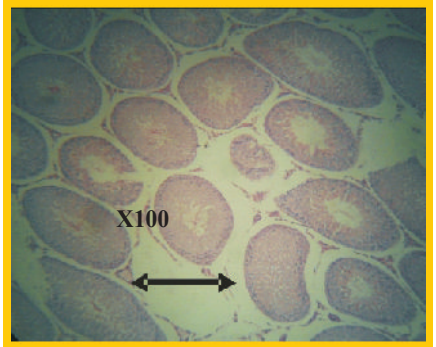
TESTIS (CONTROL)

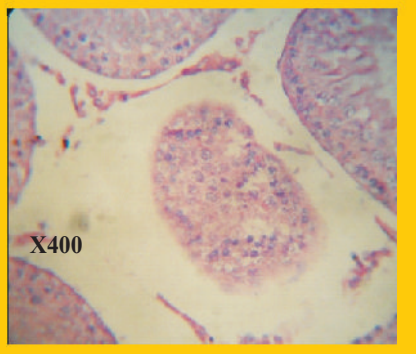




TRANSVERSE SECTION OF CONTROL TESTIS: SHOWING NORMAL SEMINIFEROUS TUBULES, COLLAGEN TISSUES (BLACK ARROW), SPERMATOGONIA CELLS (BLUE ARROW), SUBSTANTICULAR CELLS (RED ARROW) H&E STAINING

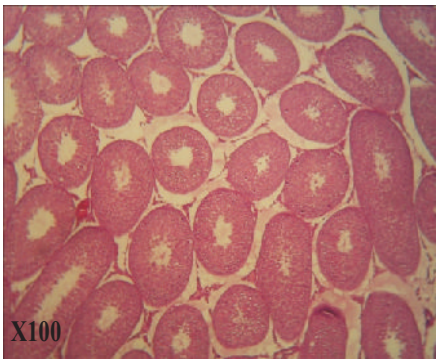
GROUP B (LOW DOSE)

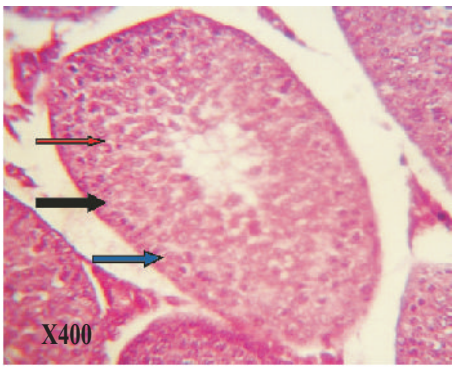




TRANSVERSE SECTION OF GROUP B TESTIS: SHOWING SEMINIFEROUS TUBULES WITH OCASSIONS OF WIDENED INTERSTITIAL SPACES AND DISTORTION IN THE INTERSTITIAL CELLS AT X100 AND ALSO SHOWING NORMAL SEMINIFEROUS TUBULES AT X400. H&E STAINING

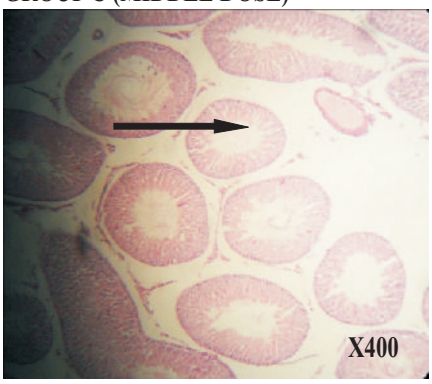
TESTIS (CONTROL)

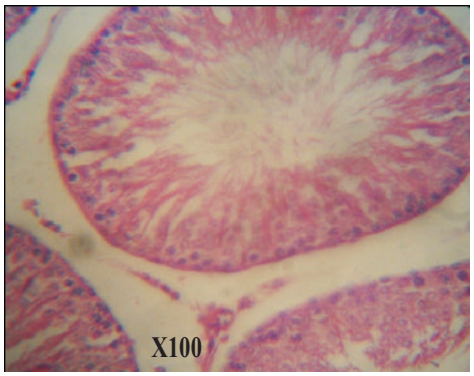




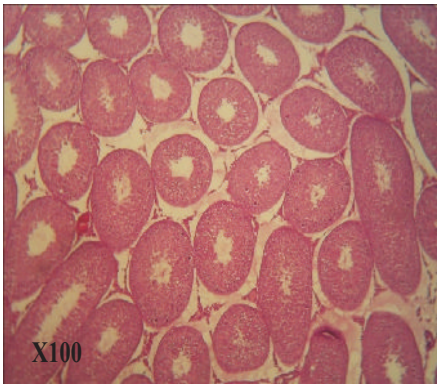
TRANSVERSE SECTION OF CONTROL TESTIS: SHOWING NORMAL SEMINIFEROUS TUBULES, COLLAGEN TISSUES (BLACK ARROW), SPERMATOGONIA CELLS (BLUE ARROW), SUBSTANTICULAR CELLS (RED ARROW) H&E STAINING

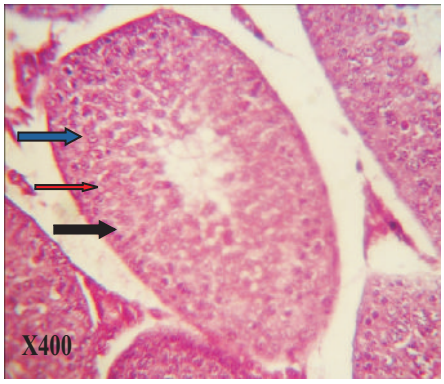
GROUP C (MIDDLE DOSE)





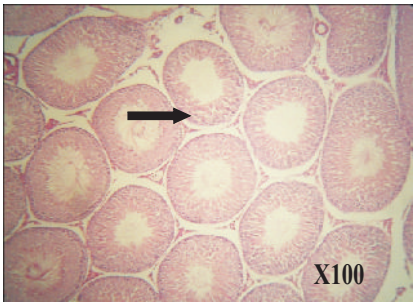
TRANSVERSE SECTION OF GROUP C TESTIS: SHOWING SEMINIFEROUS TUBULES WITH WIDENED LUMEN, DISTORTION OF SEMINIFEROUS TUBULES AND INTERSTITIAL SPACE.

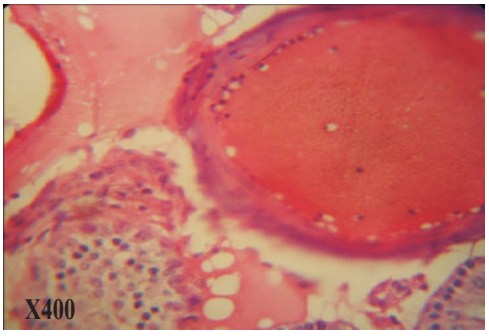




TRANSVERSE SECTION OF CONTROL TESTIS: SHOWING NORMAL SEMINIFEROUS TUBULES, COLLAGEN TISSUES (BLACK ARROW), SPERMATOGONIA CELLS (BLUE ARROW), SUBSTANTICULAR CELLS (RED ARROW) H&E STAINING

GROUP D (HIGH DOSE)





TRANSVERSE SECTION OF GROUP D TESTIS: SHOWING SEMINIFEROUS TUBULES WITH WIDENED LUMEN (BLACK ARROW) AT X100 AND X400 SHOWING HEAVY BLOOD CONGESTION IN THE VESSEL. H&E STAINING

Garcinia kola shows that there is no significant difference. While group B that received (75mg/kg) body weight and group D that received (300mg/kg) body weight of ethanolic extract of Garcinia kola shows there is significant different at the level of P<0.05.

SPERM MORPHOLOGY GRADING

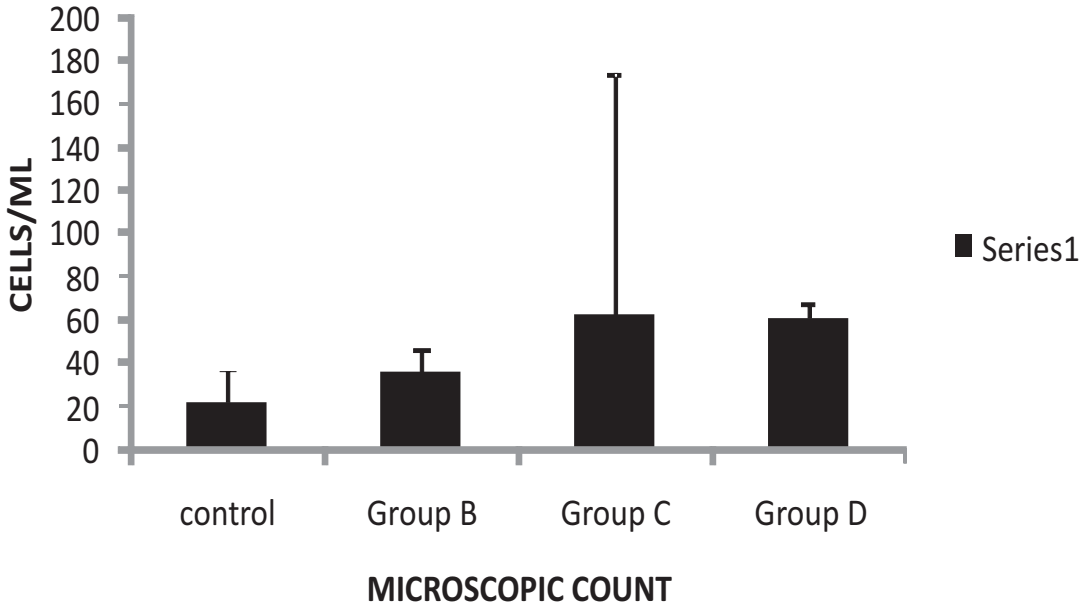
When comparing the control group with the treated groups. Normal spermatozoa, head defect, middle piece defect and tail defect the sperm morphology shows there is no statistical difference when compare at the significant level of P<0.05.

SPERM MICROSCOPIC COUNT

Table 1 shows the mean and standard error of mean of effect of Garcinia Kola on the sperm microscopic count

	CONTROL	GROUP B	GROUP C	GROUP D
Microscopic count (10^6)	22.64±14.94	35.52±10.81	62.20±11.57	60.56±6.37

FIG 1 shows the graphical representation of sperm microscopic count.



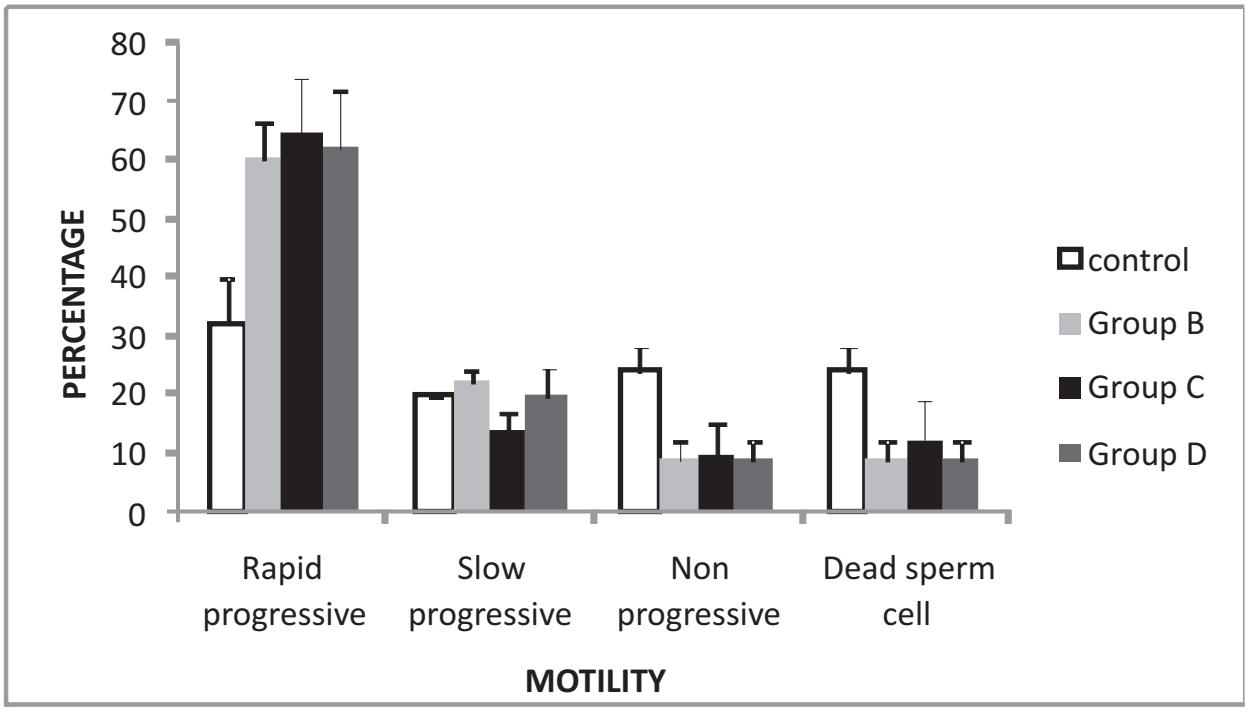
SPERM MOTILITY GRADING

Table 2 shows the mean and standard error of mean of effect of Garcinia Kola on the Sperm Motility Grading

	CONTROL	GROUP B	GROUP C	GROUP D
Rapid progressive	32.00±8.00[a]	60.00±6.33[a]	64.00±9.70	62.00±9.70
Slow progressive	20.00±0.00[a]	22.00±2.00	14.00±2.45[a]	20.00±4.47
Non progressive	24.00±4.00[ab]	9.00±2.92[a]	10.00±5.00	9.00±2.92[b]
Dead sperm cell	24.00±4.00[ab]	9.00±2.92[a]	12.00±7.00	9.00±2.92[b]

NB: Means with the same superscript on the same row are significant at the level p<0.05.

Fig 2 shows the graphical representation of sperm motility grading

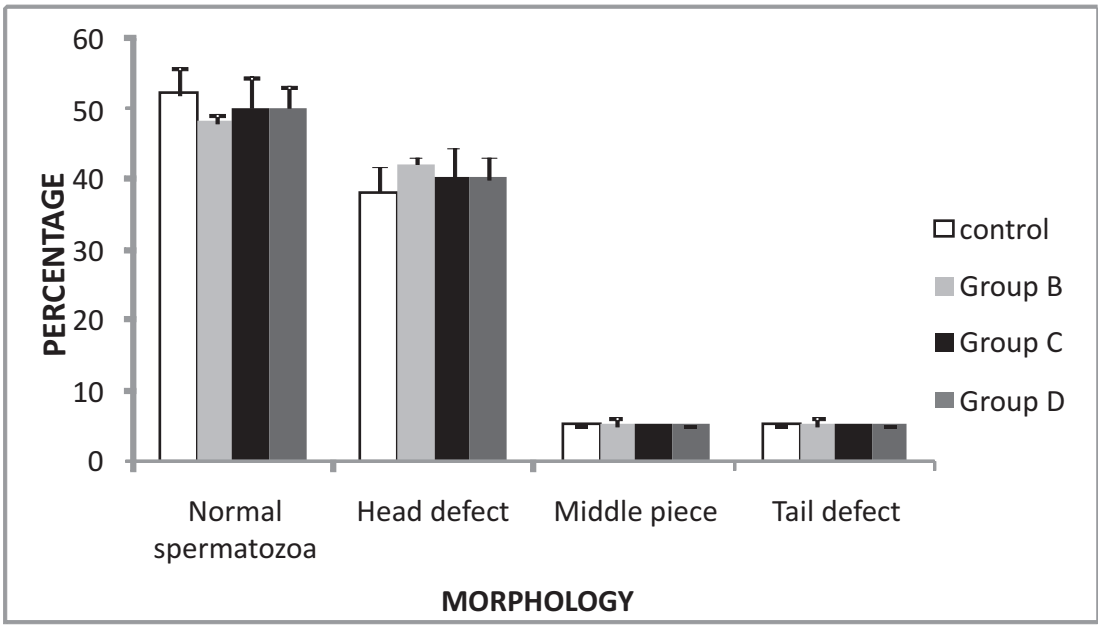


SPERM MORPHOLOGY GRADING

Table 3 shows the mean and standard error of mean of effect of Garcinia Kola on the Sperm Morphology Grading

	CONTROL	GROUP B	GROUP C	GROUP D
Normal spermatozoa	52.00±3.74	48.00±2.00	50.00±4.47	50.00±3.16
Head defect	38.00±3.742	42.00±2.00	40.00±4.47	40.00±3.16
Middle piece	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
Tail defect	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00

Fig 3 shows the graphical representation of sperm morphology grading.



Discussion

Effect of Crude Ethanolic Extract of Garcinia Kola seed on the histology and hormonal milieu of male Sprague-Dawley Rats on testicular functions. It was reported that Garcinia kola extract increase the peripheral testosterone levels in wistar rats treated with 100 mg/kg body weight of the extract. Increase spermatogenic activity in seminiferous tubules associated with the administration of Garcinia kola extract is hence due to the ability of the antioxidant compounds in the latter to increase peripheral testosterone levels (Akpantah *et al*, 2003). The Effect of Crude Ethanolic Extract of Garcinia Kola Seeds on the histology and hormonal milieu of male Sprague-Dawley Rats testes. There is significant decrease in the volume density of the lumen of the epididymis which is most likely due to the significant increase in the epithelium (pseudo stratified) of the epididymis with the corresponding increase in the volume density of the connective tissue of the epididymal wall. This is a very strong indication that Garcinia kola extract influences the epididymis in a positive direction. The ciliated epithelium is also important in the movement of the spermatozoa from the proximal to the distal portion of the tube pilor to ejaculation which under the influence of sympathetic innervations. The proximal portion of the muscular wall of epididymis exhibit slow rhythmic contractility, which gently moves spermatozoa towards the ductus deferens. Distally the smooth muscle is richly innervated by the sympathetic nervous system which produces intense contractions of the lower part of the epididymis during ejaculation (Young and Heath, 2000).

Garcinia combogia extract also causes increase in the interstitial spaces, reduction in the leydig cells population in the interstitial spaces, slight reduction in the seminiferous tubules luminal spermatozoa concentration, contraction of the seminiferous tubules, and arrangement of the cells spermatogenic of series. This finding agreed with the work of (Akinloye *et al,* 1999) but is at variance with the work of (Akpantah *et al,* 2003) who recorded no histological difference in the testes of wistar rats after administration of crude ethanolic extract of Garcinia kola. The reduction of sizes of the seminiferous tubules might be due to the contractile activities of the fibroblast of the seminiferous tubular wall. This is because testosterone causes the contraction of the fibroblasts which aid the movement of the non-motile spermatozoa into the epididymal tubule where the final maturation into spermatozoa into the epididymal tubule where the final maturation into motile spermatozoa takes place (Peter *et al*, 1999). This might be the reason for the increase in the interstitial spaces.

Oluyemi *et al,* 2007 observed increment in the diameter of seminiferous tubule of male wistar rats treated with ethanolic extracts of Garcinia kola. Akpantah *et al*, 2005 also observed increased in the peripheral testosterone levels in wistar rats treated with the extracts of Garcinia kola seeds. The result of this study showed clear effect of ethanolic extracts of Garcinia kola seeds on fecundity and egg size of C. gariepinus.

This agrees with the work of (Adesanya *et al*, 2007) who records an increased in the sperm count in wistar rats treated with ethanilic extract of Garcinia kola for 8 weeks. (Oluyemi *et al*, 2007) also found that Garcinia cambogia increase the peripheral testosterone level in wistar rats. The increased in the fecundity of C. gariepinus obtained in this study could be as a result of the presence of biflayonoid and xanthone in the plant. These compounds are potent antioxidants which are capable of increasing the production of eostrogen, the key hormone involved in the production and maturation of eggs in the ovary. Other studies in man have show that Garcinia kola helps man with infertility, with improvement in male fertility especially sperm characteristics (Adesanya *et al*, 2007).

Garcinia kola may be acting as antioxidant to either inhibit or slow down the progression of symptomatic knee osteoarthritis. It could also act as a scavenger to remove the particles that have been observed on the surfaces of human articular cartilage following trauma and osteoarthritis.

Crystal deposition in normal and diseased articular cartilage, contained calcium and phosphorus which were identified only in structurally abnormal cartilage. Bitter kola has been known to protect against the oxidation of lipoprotein, presumably through the mechanisms involving metal chelating and antioxidant activity (potential of nine edible vegetable in southwest Nigeria.

Anti-oxidant mechanism of kolaviron studies on serum lipoprotein oxidant ion, metal chelation and oxidative membrane damage in rats. The relief of pain experienced by subjects on Garcinia kola could be associated with either removal of the free radical and or revascularization of the subchondria bone through the anti-atherogenic effect. This pathway is not clear at this stage of study. It may be through activation of the cytokines selective inhibition of inducible nitric oxide synthare which has been show to reduce the progression of experimental osteoarthritis in Vivo (Pelletier *et al,* 2001). Selective inhibition of inducible nitric oxide syntheserveduce progression of experimental osteoarthritis in Vivo possible link with the reduction of chondrocyte apoptosis and caspase-3 level.

The bitter kola is believed to have aphrodisiac properties (Adaramoye *et al,* 2000). Comparative study on the antioxidant properties of flavonoid of Gracinia kola seeds. Probably related to it vasodilator effect on the genitalia smooth muscles. Reduction of intraosseous /

subchondria pressures could be the other pathway for the reduction of knee pain experienced by patient on Garcinia kola. The ability to lower intraocular pressure was earlier noted in glaucoma patient. The preliminary crude observation was confirmed scientifically in animal and human glaucoma's patient (Adefule- Ositelu *et al,* 1999). Effect of extract of Garcinia kola on the intraocular pressure and papillary diameters of laboratory animal eyes. The vaso dilatation induced could improve the subchondria blood circulation in knee osteoarthritis. The Garcinia kola extract has be show to have antithrombotic activities of falvonoid extract (Kokuiron) of Garcinia kola seeds. The effect of Garcinia kola on chondrocyte nutrition is not clearly elucidated at present. This will form the fulcrum of future studies.

Garcinia kola clinically appeared to have a significant analgesic, anti inflammatory effect in knee osteoarthritis patent. Garcinia kola is a potential osteoarthritis disease modifier. This study shows that Garcinia kola is a potential osteoarthritis disease modifier. This study shows that Garcinia kola is effective in improving locomotors function and significant pain reduction in patients with knee osteoarthritis. Further study is requiring for standardization of dosage of Garcinia kola in knee osteoarthritis.

There is degenerative changes observed in another study on the liver, kidney and small intestine which are consistent will earlier reports on the effect of prolonged ingestion of Garcinia kola (Virk and Menke *et al,* 1986). These lesions observed in the liver might account for earlier finding in which the inhibition of hepatic metabolism of drugs in rats treated with the falconoid extract of Garcinia kola seeds was reported. Also the effect of crude ethanolic extract on the testes is consistent with earlier finding on the effect of prolonged administration of aqueous extract of Garcinia kola on rabbit testes. Although Garcinia kola is traditionally used as an aphrodisiac the observed effect on histological sections of the testes of dogs in this study, as well as that reported in rabbit testes by (Akinloye *et al*, 1999) both suggested a possible anti-fertility action. (Braide *et al,* 2003) reported a marked reduction in the serum testosterone concentration of methanolic extract of Garcinia kola administered on the rats. This might have been as a result of the direct action of Garcinia kola on the testicular tissue. (Uko *et al,* 2000) had earlier reported a decrease in body mass gain in rats fed Garcinia kola and they are associated with reduced feed consumption. Although weight gain was not measured in this work, lesions found in the intestines in this work could result in poor feed consumption and ultimately a decrease in weight gain.

The histological observation of the effect ethanolic extract of Garcinia kola on the testis of male wistar rats revealed a degenerative effect with that of the low dose showing mild degenerative and those of the highest dose show high degeneration of histological architecture. Group (A) which was the control group showed the normal morphology of the testis with well spaced seminiferous tubules with a well organized series of spermatogonia and spermatocytes cells.

Group (B) which received low dose of Garcinia kola at 5mg/kg body weight showed degeneration of interstitial space and hence seminiferous tubules show occasion of widened interstitial space. However the spermatogonia cell and substanticular cells appeared normal and progression from spermatozoid to spermatocytes. This shows that ethanolic extract of garcinia kola would at low dose begin its degenerative effect by firstly damaging the interstitial space

Group C, Which received middle does of Garcinia kola at 150mg/kg body weight shows degeneration of interstitial space and occasion of widened lumen although the spermatogonia remained intact. This shows that at middle dose of Garcinia kola the degenerative effect after damaging of the interstitial space at low dose is the occasion of widened lumen which indicates reduction in spermatogenesis, although the spermatogonia cell remained intact.

Group (D) which received high dose of Garcinia kola at 300 mg/kg body weight shows heavy blood congestion with, vessels and wall of the vessel are thickened. This show that at very high dose of Garcinia kola 300 mg/kg body weight, according to this project these is corrosion of the blood supply after interstitial space has been damaged and lumen have been included at middle dose. This indicates a dose dependent degeneration of garcinia kola. This report is in agreement with report by Oluyem et al (2007) who also determine the effect of Garcinia kola extract on the diameter of the seminiferous tubules and reported a larger seminiferous tubules, prominent to group receiving high dose of Garcinia kola which was also evident when comparing to the above research work where increase in diameter of seminferousa tubules was noticed from group (A) and (C). Other reports include Young et al (2000), Akinloye et al (1999) and Peter et al (1999) who all reported that there was increase in the interstitial space, derrangement of the cell of spermatogenesis and a significant reduction in the seminiferous luminal spermatozoa on microscopic analysis. Semen analyses results in Fig:1 it was observed that Garcinia kola shows no significant change when comparing the control group with other treated group, hence different doses of Garcinia kola shows no effect on the sperm microscopic count. Fig 2: It can be observed that there is increase in sperm motility. In relation to a report by Akpantal et al (2003) that there was increase in the motility of spermatozoa. Fig 3: It can be observed that Garcinia kola shows no significant change at level of $P< 0.05$ when comparing the control group with other treated group hence different dose of Garcinia kola shows no effect on the normal head effect, middle

piece defect, tail defect of sperm microscopic analysis.

Conclusion

These findings indicate that ethanolic extract of Garcinia kola has some adverse effect on the testis of male wistar rats which is dose dependent.

Recommendation

Effect of Gracinia kola can cause infertility in male but in a dose dependant manner this implies that men should abstain from Garcinia kola.

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