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Evaluation of Aqueous and Ethanolic Extracts of Garcinia Kola on Testicular Morphology of Adult Male Wistar Rats'

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ABSTRACT

Despite its medicinal value, bitter kola is known as a prayer nut, and traditional lifestyle has brought us much food, such as "chewing of bitter kola and its resources." Thirty-five male Wistar rats weighing between 160. to 200.g were divided into seven groups based on weight similarity. For 21 days, Group A was the control and was given only rat meal and water ad libitum, Group B (LDAEG) and C (HDAEG) were given500mg/kg and 1000mg/kg of *G.* kola aqueous extract, Group D (LDEEG) and E (HDEEG) were given500mg/kg and 1000mg/kgof *G.* kola ethanolic extract, and Group F (LDAEEG) and G (HDAEEG) were co-treated with 500mg/kg and 1000mg/kg of *G.* kola aqueous and ethanolic extracts respectively. The rats were weighed and sacrificed on day 21, and their testes were harvested and fixed for histological studies. SPSS version 23 was used to analyse the data, and each group's mean and standard deviation were calculated. Our findings show that the experimental group had a considerable rise in body weight above the control group. The histological results of the testes showed normal features in the control group and mild inactive seminiferous tubules in the test groups. According to our findings, bitter kola has no negative effects on the Wistar rats' testes, especially when taken in low doses.

KEYWORDS: Garcinia kola,Testes, Seminiferous tubules,Testicular morphology.

ARTICLE DETAILS

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INTRODUCTION

Sexual health is an important aspect of a person's overall happiness and well-being. Unfortunately, it's a condition that many doctors overlook as they try to cope with the disease's life-threatening implications. Consumption of G. kola on a regular basis has long been thought to increase men's sexual performance, among other things (Tita, R. K, et al 2001).

Garcinia kola is a dicotyledonous plant from the Clusiaceae/Guttiferae family. It is commonly known in Englsh as bitter kola, male kola and false kola, Orogbo in Yoruba, Cidagoro in Hausa, Akuilu or Ugugolu in Igbo, Efiari (Efik), and Igoligo (Idoma), and it is found in the tropical forests of Sierra Leone, Angola and Nigeria (Dalziel, I.M. 2017) .

G. kola is a miracle plant that grows in the southern Nigerian rainforest and has been proven to have medicinal properties in all of its parts. Because of its bitter flavor, it is also known as "bitter kola" or "male kola" due to its sexual effects (Iwu, M et al 1999). Garcinia kola seeds are particularly high in flavonoids, which have been demonstrated to have antibacterial properties Hong – X. I and Song F. L. 2001.

Other phytochemical constituents of Garcinia kola seeds include phenols, alkaloids, tannins, and saponins, which have a variety of beneficial effects in humans and animals. They are used to prevent or treat colic in babies, as well as to treat upper respiratory tract infections, when chewed fresh. They've been used for centuries in several West African countries to treat head and chest colds, dysentery, diarrhea, and urinary infections, as well as as a poison antidote.The seeds are said to be used as a stimulant in the treatment of liver and diarrhea, diabetes, bronchitis, and throat infections Braide V.P 1991. It is a highly desired product due to its widely perceived medicinal properties and the fact that when consumed in large quantities, it does not cause indigestion.

According to Iwu et al. 1999, the plant can be used to treat jaundice, high fever, and as a purgative.

Kolaviron, the main component of Garcinia kola seeds, comprises biflavanones (GB1, GB2, and kolaflavanone),

which have been shown to protect the liver from hepatotoxicity caused by a variety of toxins Braide V.P 1993. Tannins and guttiferin are abundant in the nut of the plant. G. kola has been proven in previous research to have neuroprotective and hepatoprotective qualities as a result of its antioxidant capabilities.

In folkloric medicine in Africa, Garcinia kola seed is often used to cure diabetes and its consequences.

In STZ-induced diabetic rats, garcinia kola seed administration reduced blood glucose levels, improved the antioxidant system, inhibited lipid peroxidation, and improved the architecture of the kidney, liver, and testes (Bravo, L. 1999). Furthermore, in STZ-induced diabetic rats, G. kola seed intervention was observed to recover kidney and liver function indicators (Conley, C. L 1974).

Anti-inflammatory, antibacterial, antiviral, and antiulcer activities are also found in the seed.

Although studies on the effects of G. kola on the blood, gastrointestinal system, and neurological system have been published, there is a scarcity of research on the effects on the reproductive system. As a result, it's critical to explore the underlying morphological effect of aqueous and ethanolic extracts of Garcinia kola on Wistar rats' testicular functioning using Wistar rats as models. The current probe is justified in light of this.

MATERIALS AND METHODS

Ethical approval

The group created international standards, norms, and recommendations for the use of animals in research National Institute of Health. Laboratory Animal Welfare 1985.

The substance used

Dry kola nuts purchased from a local market in Eke-Okigwe Market, Okigwe, Imo State, Nigeria, were utilized in this investigation. The university's plant and agricultural department determined that they were good and viable for use.

PICTURES OF THE DRY *GARCINIA* KOLA PICTURES OF PEELED *GARCINIA* KOLA

Preparation of aqueous extract of *Garcinia* Kola.

The outer coatings of the G. kola nuts were removed after they had been sun-dried for a few days, and the seeds were chopped into bits and oven-dried. The dried seeds were ground into a fine powder, and the extraction was carried out in the Abia State University Uturu Biochemistry laboratory. 20 g of the extract was weighed and dissolved in 100 mL of distilled water, yielding a G. kola aqueous extract with a concentration of 200 mg/mL.

Preparation of Ethanolic Extract of *Garcinia* Kola.

To remove the testa, the dried seeds of Garcinia kola were peeled. These were then processed into a smooth powder after being chopped into smaller pieces. A known weight (200 g) of powder was extracted for 72 hours at room temperature in 1000 ml of ethanol. The extract was filtered through Whatman No. 1 filter paper (Maidstone, UK) and concentrated in a Rotary Evaporator. The mixture was then taken to a steam bath, where it was evaporated to produce the brownish-black residue necessary. The needed doses (500 and 1000 mg/kg body weight) were then reconstituted in distilled water for use in the study.

Phytochemical Screening

Preliminary phytochemical screening was carried out using the protocols outlined by Awe and Sodipo and Mainasera et al., 2012 to determine the presence of alkaloids, saponins, tannins, steroids and flavonoids, steroids.

Experimental design

35 male Wistar rats weighing between 160.3 and 210.9 grams were utilized in the study. The rats were housed in wire gauze cages with seven compartments, each containing five rats, and allowed to acclimate for two weeks in the animal house of Abia State University's Department of Anatomy before being administered. They were provided unlimited amounts of rat food and water during the trial. The rats were divided into groups A through G at random.

Group A (Control) -was given only rat meal and water ad libitum.

Group B(Low dose aqueous extract group) were orally administered 500mg/kg body weight of *G.* kola aqueous extract for 21 days.

Group C(High dose aqueous extract group) were orally administered 1000mg/kg body weight of *G.* kola aqueous extract for 21 days.

Group D (Low dose ethanolic extract group) were orally administered 500mg/kg body weight of *G.* kola ethanolic extract for 21 days.

Group E(High dose ethanolic extract group) were orally administered 1000mg/kg body weight of *G.* kola aqueous extract for 21 days.

Group F(Low dose aqueous and ethanolic extract group) were orally co-treated with 500mg/kg body weight of *G.* kola aqueous and ethanolic extract for 21 days.

Group G(High dose aqueous and ethanolic extract group) were orally co-administered with 500mg/kg body weight of *G.* kola aqueous and ethanolic extract for 21 days.

Collection and preparation of testes for analysis

The rats were anaesthetized in a chloroform filled chamber at the end of the experiment. The rats were placed on a dissecting board and dissected quickly with dissecting kits, with the organs to be studied being removed.

For histopathology, four rats from each group were employed, and they were fixed in 10% formal saline.

Histopathological studies

The tissues were treated at Ebonyi State University's Anatomy Department's Histology Laboratory for histological examinations. For histological research, the tissues went through the standard phases of tissue processing,

STATISTICAL ANALYSIS OF RESULTS

M ± SEM of triplicate measurements were used to calculate the results. All of the data was analyzed using the Statistical Package for Social Sciences (SPSS) version 23. (IBM Corp., Armonk, New York). Significant differences at the $P< 0.05$ significance level were used to test hypotheses.

RESULTS

Table 1: Preliminary phytochemical analysis

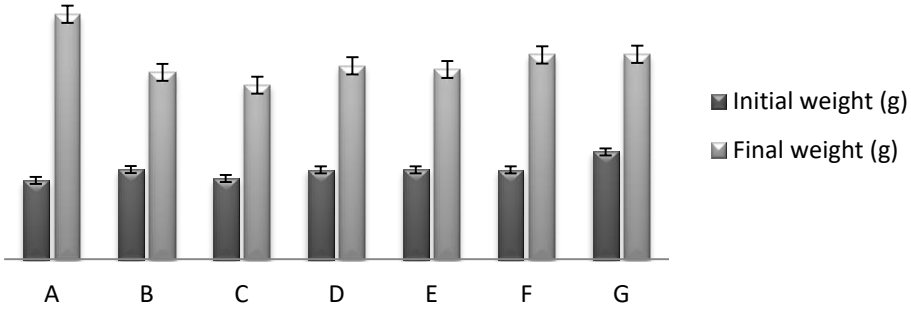
Active ingredients	Ethanol	Aqueous
Alkaloid	0.13±0.71	0.14±0.40
Saponin	1.41±0.03	0.94±0.02
Tannins	0.48±0.01	ND
Flavonoids	0.26±0.02	0.29±0.17
Cynadie	0.02±0.05	ND
Phenols	0.18±0.01	ND
Steroid	0.16±0.21	0.04±0.10

Preliminary phytochemical screening of ethanolic and aqueous extracts of *Garcinia kola* seed reveals the presence of alkaloids, saponins, tannins, steroids and flavonoids.

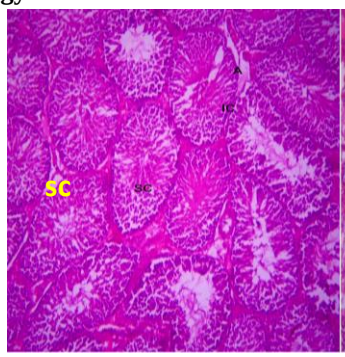
Table 2: Values of body weight instudy animals.

Groups	Initial weight (g)	Final weight (g)
A	77.60±1.97	241.40±3.92
B	88.40±2.54[a]	184.20±1.77[a,b]
C	79.60±2.38	171.60±3.54[a,b]
D	88.00±2.07[a]	190.60±5.35[a,b]
E	88.20±2.35	187.00±4.68[a,b]
F	88.00±2.07a	201.40±4.60[a,b]
G	105.80±2.27[a,b]	202.00±6.80[a,b]

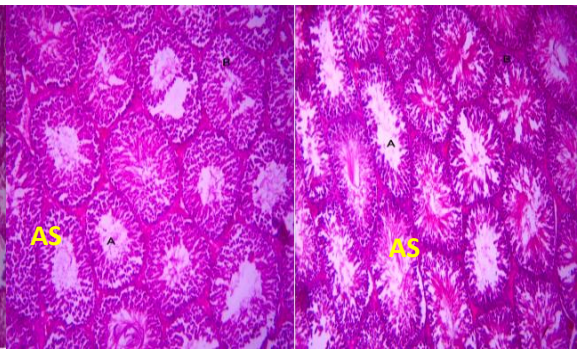
Fig 1: Values of body weight in study animals.



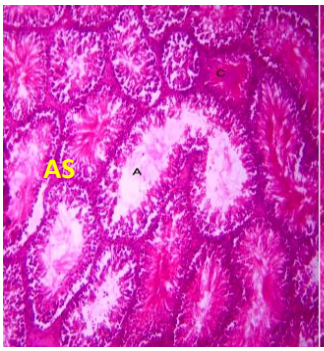
Histology of the testes



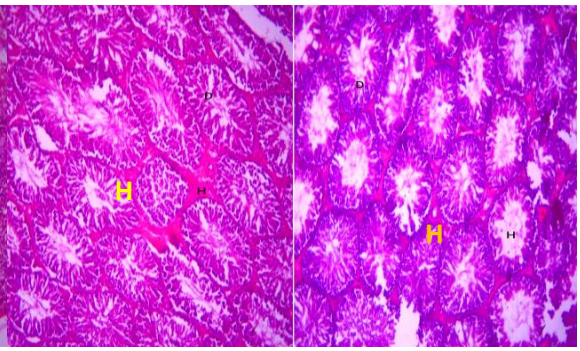
PLT A (Control) x400 mag, H/E



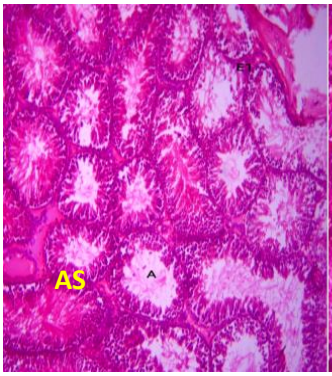
PLT B (LDAEG) x400 mag, H/E



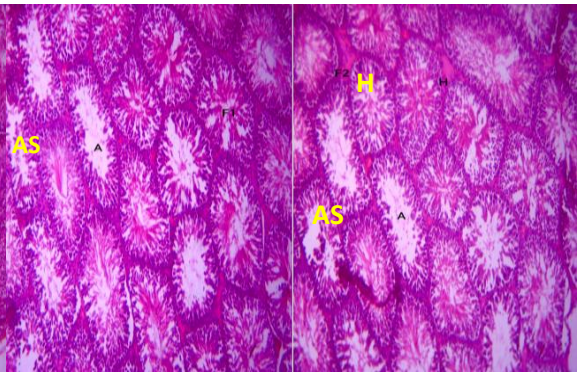
PLT C (HDAEG) x400 mag, H/E



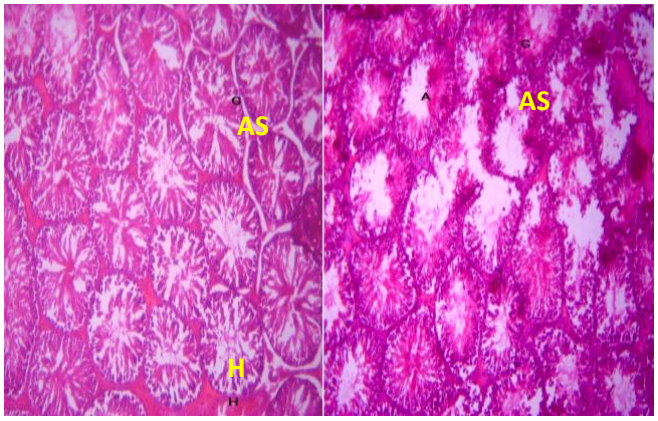
PLT D (LDEEG) x400 mag, H/E



PLT E (HDEEG) x400 mag, H/E



PLT F (LDAEEG) x400 mag, H/E



PLT G (HDAEEG) x400 mag, H/E

Group A (control) segment of the testis (x400) (H/E) photomicrograph demonstrates typical testicular architecture with active seminiferous tubules lined by interstitial cells of the leydig (ICL), sertoli cell (SC), and well improved spermatogenesis (WES). Photomicrograph of B (LDAEG) section of testes treated with a low dosage of bitter cola aqueous extract (x400) (H/E) reveals active seminiferous tubules and minor spermatogenesis arrest (AS).Photomicrograph of the C (HDAEG) section of testes after treatment with a high dosage of bitter cola aqueous extract (x400)(H/E) reveals inactive seminiferous tubules and moderate spermatogenesis stoppage (AS). Active seminiferous tubules with considerable spermatogenesis arrest (AS) in r2 and intersitial hemorrhage (H) inside the basal layer are seen in a photomicrograph of D (LDEEG) section of testes injected with low dosage ethanolic extract of bitter cola (x400)(H/E). Photomicrograph of the E (HDEEG) section of testes after treatment with a high dosage ethanoic extract of bitter cola (x400)(H/E) reveals active seminiferous tubules and a minor spermatogenesis arrest (AS).Photomicrograph of the F (LDAEEG) section of testes after treatment with low dosage ethanoic and bitter cola aqueous extract (x400)(H/E) reveals active seminiferous tubules with significant spermatogenesis arrest (AS) and slight intersitial hemorrhage (H). Photomicrograph of G (HDAEEG) segment of testes after treatment with high dose ethanoic and bitter cola aqueous extract (x400)(H/E) reveals active seminiferous tubules with considerable spermatogenesis arrest (AS) and moderate intersitial hemorrhage (H).

DISCUSSION

Our regular consumption of traditional substances has had a variety of effects on humans, animals, and other organisms. This action has caused traditional substances to be seen in various ways, but their cases and ability to make the mouth active and mobile has distinguished them as a highly classified substance.

There is always the belief that "the more you take this kola, the higher and stronger your sexual strength will be." This belief has gained widespread acceptance because, while sugar is not a helper, the perception of this as a bitter substance aids in the resolution of the problem.The phytochemical analysis of both extracts was provided in table 1, and it was obvious that the ethanol extract was superior. Was more concentrated than the aqueous extract, which could indicate that ethanol extract takes out more of the material's key element, bitter kola, which is not surprising given Aguwa, U.S., et al 2018's findings that ethanol extract brings out the chemical composition of a substance. This could be why older people mix this chemical (bitter kola) with alcohol because it is much more bitter than water.With our findings, we can see that saponins are more abundant in the extract of bitter kola in the ethanol group, and cynadie is more abundant in the aqueous group, while tannins, phenols, and steroid are absent in the aqueous group, indicating that its important productivity is not primarily obtained from aqueous extract, as one of its constituents, steroid, which aids in sexual performance, is missing.

There were observations made during the trial, such as they never looked or lost weight since they were not physically affected, which supports Abarikwu SO et al 2012 claim that bitter kola has been known to affect animal physical weight.Our weight result corroborated our physical observations, and our findings backed up other discoveries such as bitter kola having little to no influence on weight. The constant progression of the weight shows that the rats, when properly fed and cared for, as well as the fact that bitter kola has nutritional benefits, were not in any oxidative stress, as evidenced by the physical and significant weight gain. This supports the work of Singh et al 2005, who found that the rats were not in any oxidative stress.

In table 4.4, it was discovered that Group F had the highest significant weight increase compared to the other experiment groups, except for the control, which could be due to the low dose and combination of both ethanol and aqueous constituent (Lorke D (1983). It was also discovered that Group G had the lowest significant weight increase, which showed that at high doses of the same both substance, it has an effect of reduction, though not when compared to its increase rate but when compared to other ethanol and aqueous constituent.This indicates that at large dosages, it is not of interest; nonetheless, this study tends to validate Obi AU,

Nwoha PU (2014) findings that high doses of the same drug can cause inflammation and constipation in humans.

The photomicrograph of the control testes revealed typical testicular architecture, including seminiferous tubules bordered with cells and well-enhanced spermatogenesis, indicating that the animals were in good health.

In the experiment group B, the testes given the same low dose showed active seminiferous tubules and mild spermatogenesis, indicating that at low levels of aqueous bitter kola extract, the animals' reproductive systems remain healthy, indicating that there is little or no effect at this stage, which is consistent with previous findings (Tcheghebe TO et al 2016). .Due to the total absence of steroids in the aqueous extract of bitter kola, inactive seminiferous tubules with moderate arrest of spermatogenesis were found in the testes of group C, indicating that a high dose of the substance has a total effect on the testes, which can cause further impairment on the male reproductive system, resulting in low sperm count and weak erection.The testes in group D revealed active seminiferous tubules with moderate arrest, indicating that there is a recognized effect at low doses as well as large doses. This is supported by the fact that (Mosunmola BO et al 2017) believes that at large doses, there could be a variety of negative effects on human health. It reveals active seminiferous tubules with minor spermatogenesis arrest in group E. This raises the possibility that, despite the high dose, the bitter kola has no effect on the testes. It can be shown that ethanol increases the concentration of bitter kola, which is beneficial to the male reproductive system.This has led to the realization that bitter kola is best consumed with alcohol because it increases its productivity. This is in contrast to (Ahumibe AA et al 2009) who claim that alcohol increases side effects because bitter kola is served with alcohol. This is seen as the primary reason why elderly people consume it with hot drinks on a regular basis.

A photomicrograph of the group F section of the testes after treatment with a modest dose of ethanoic acid and aqueous extract of bitter kola reveals active seminiferous tubules, significant spermatogenesis arrest, and slight interstitial haemorrhage. Ovie F.O et al 2019

A photomicrograph of the group G section of the testes after treatment with a high dose of ethanoic and aqueous extract of bitter kola reveals active seminiferous tubules, significant spermatogenesis arrest, and moderate interstitial haemorrhage.

CONCLUSION

Our research revealed that there was no effect on the testes, especially when it came to the seminiferous tubules in the ethanol extract, but inactive seminiferous tubules were found in the aqueous group with high doses, indicating that there was a deterrative effect on the testes when kola was consumed with water on a high dose.

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