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The Effects of Ethanolic Extract of Garcinia Kola on the Testes of Male Adult Wistar Rats

Akingboye AJ[1] Ezejindu DN[2] Udemezue OO[2] Chukwujekwu IE[2] Anyabolu AE[2] Ofoego UC[2] Ogueche PN[3] Uchefuna RC[4] Obaji NN[4] Ezejindu CN[5] Ndukwe GU[6] Enemuo EH.[7]
1.Department of Microbiology, Faculty of Biological Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria
2.Department of Anatomy, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria
3.Department of Human Biochemistry, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria
4.Department of Physiology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria
5.Department of Microbiology, Faculty of Biological Sciences, Abia State University, Uturu, Abia State, Nigeria
6.Department of Anatomy, Faculty of Basic Medical Sciences, Abia State University, Uturu, Abia State, Nigeria
7.Department of Medicine, Faculty of Medicine, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria
Corresponding Authors Email:ezejindudamian@gmail.com

Abstract
This study was carried out to investigate the effects of ethanolic extract of *Garcinia kola* on the testes of adult male wistar rats. Twenty four male adult wistar rats weighing 180-270kg were used for the study. They were divided into four groups (A, B, C & D) of six animals each. Group A serve as the experimental control and were orally administered 0.2ml of distilled water; the experimental groups received 0.3ml, 0.6ml and 0.9ml of ethanolic extract of *Garcinia kola* respectively for twenty eight days. Twenty four hours after the last administration, both group A and the experimental groups were weighed, sacrificed under the influence of chloroform vapour and dissected. The testes tissues were harvested weighed and fixed in 10% formalin for histological studies. The final body weight of groups C and D decreased significantly ($P<0.001$) compare with the control group A. The mean relative organ weight of groups C and D animals increased significantly ($P<0.001$) when compare with the control group A while group B had a similar mean weight with the control group. Histological findings showed necrotic changes in the interstitial cells of the testes, loss of spermatides and multinucleated giant cells in groups C and D. The result of this study revealed that consumption of the ethanolic extract of *Garcinia kola* at high doses may cause histopathological lesions in the testicular cells.
Keywords: Testes, Organ weight, Garcinia kola, Wistar rats, Spermatide, Distilled water.

INTRODUCTION

Many herbs have shown positive results in-vitro, animal model or small-scale clinical tests (Srinivasan K 2005), while studies on some herbal treatments have found negative results (Pitter et *al.,* 2000).

In 2002, the U.S. National Center for Complementary and Alternative Medicine of the National Institutes of Health began funding clinical trials into the effectiveness of herbal medicine (National Center for Complementary and Alternative Medicine National Institute of Health). In a 2010 survey of 1000 plants, 356 had clinical trials published evaluating their "pharmacological activities and therapeutic applications" while 12% of the plants, although available in the Western market, had "no substantial studies" of their properties (Gravotto *et al*., 2010).

Gacinia kola (Heckel) and its relatives-including *G.livingstonei*, *G.gnetoides*, *G.staudtii*, *G.smeathemannii*, *G.ovalivolia, G.brevipedie llata* and *G.mannii*- are found in Nigeria as well as generally across the humid lowland plains of West Africa extending from Sierre Leone to Zaire (Vivien and Faure 1996) and Angola (Keay 1989).

Bitter kola is favoured by the three major ethnic groups in Nigeria, the Yorubas, Igbos and Hausa. Its domestic trade thus extends beyond the Southern production areas to the Northern parts of the country. Apart from being a stimulant, it has in chewing a bitter astringent and resinous taste and is often used as an aphrodisiac. It's highly valued perceived medicinal attributes, and the fact that consumption of large quantities does not cause indigestion (as cola nut do), make it a highly desired product (Dalziel 1937).

An earlier study demonstrated that long term ingestion of *Garcinia kola* seed resulted in marked spermatogenesis arrest and degeneration of spermatozoa (Udoh 1998).

Also, it has been reported that chronic consumption of *Garcinia kola* seed resulted in a marked reduction in serum testosterone in male rats (Braide *et al*., 2003).

This study is therefore aimed to investigate the effects of ethanolic extract of *Garcinia kola* on the testes of male

adult wistar rats at various doses.

MATERIALS AND METHOD

Breedings of Animals

Twenty four male adult wistar rats weighing between 180-270kg were brought from Animal House of Biochemistry Department, Nnamdi Azikiwe University, Awka, Anambra State. They were allowed for a period of seven days for acclimatization under normal temperature (27°C-30°C) before their weight were taken and recorded. They were fed with normal rat feed pallets from livestock feed Nig, Ltd. Lagos, Nigeria.

Drug Preparation

Garcinia kola when ripened, the fruits of *Garcinia kola* was collected from Okofia, Nnewi Anambra State and it was identified at the Department of Botany, Nnamdi Azikiwe University Awka. They were kept in an open cool place till the pericarp and the pulpy mesocarp become soft. After softened, the fruits are threshed to release the nuts, which are thoroughly washed to remove the sticky mucilaginous materials that sheath the nuts. The plants material was sun-dried. The dried *Garcinia kola* was milled to a powder. Extraction was done using ethanol. 250mg of this extract/kg body weight was dissolved in 10ml of distilled water and administered to the animals.

Experimental Protocols

The twenty four animals were weighed and allocated into four groups (A, B, C & D) of six animals each. Group A serve as the control and administered 0.2ml of distilled water; the experimental groups B, C & D received 0.3ml, 0.6ml & 0.9ml of ethanolic extract of *Garcinia kola* orally respectively for sixty days. After the last administration (24 hours), the animals were weighed and their weights were recorded. The animals were then anaesthetized under the influence of chloroform vapour and dissected. The testes tissues were harvested weighed and fixed in 10% formaline for about four hours for histological studies.

Tissues Processing

For easy study of sections under microscope, the tissues passed through several processes of fixation, dehydration, clearing, infiltration, embedding, sectioning and staining. Fixation was carried out in 10% formal saline for 10 hours. After fixation, the tissues were weighed in a running tap water. Dehydration of the fixed tissues was done using ascending grade of alcohol 50%, 70%, 90% and absolute. The tissues were cleared in xylene after which infiltration was done in a molten paraffin wax at 60 for two hours each in two changes. The embedding of the tissues was done in molten paraffin wax and was sectioned afterwards. Haematoxylin and eosine method was used in the staining.

RESULTS

Morphometric Analysis of Body Weight

Table 1: Comparison of the mean initial, final body weight and weight changes in all the groups (A, B, C & D) before and after the administration of the extract.

(Mean $\pm$ SEM given for each measurement)

Groups	Group A	Group B	Group C	Group D	F-Ratio	Prob. of Sig
Initial body weight	181.20$\pm$2.50	183.60$\pm$3.40	187.10$\pm$2.80	192.10$\pm$1.80	62.120	<0.001
Final body weight	201.20$\pm$4.50	198.50$\pm$4.30	171.10$\pm$4.10	169.30$\pm$2.40	41.130	<0.001
Weight change	20.00$\pm$2.00	14.90$\pm$0.90	-16.00$\pm$1.30	-22.80$\pm$0.60	7.130	<0.001

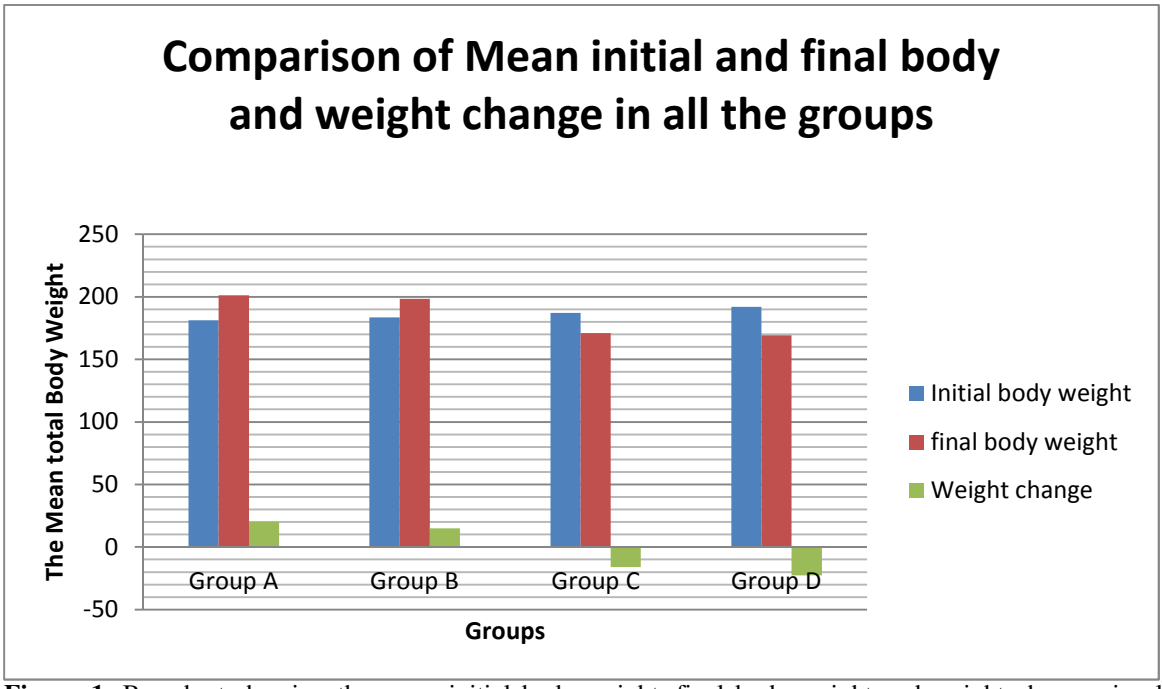

Figure 1: Bar chart showing the mean initial body weight, final body weight and weight changes in all the groups.

Morphometric Analysis of the Testes Weight

Table 2: Comparison of Mean relative testes weight of all the groups (A, B, C & D)

(Mean ± SEM given for each measurement)

Groups	Group A	Group B	Group C	Group D	F-Ratio	Prob. of Sig
Testicular weight	1.30±0.01	1.33±0.13	1.49±0.22	1.59±0.10	0.450	<0.001

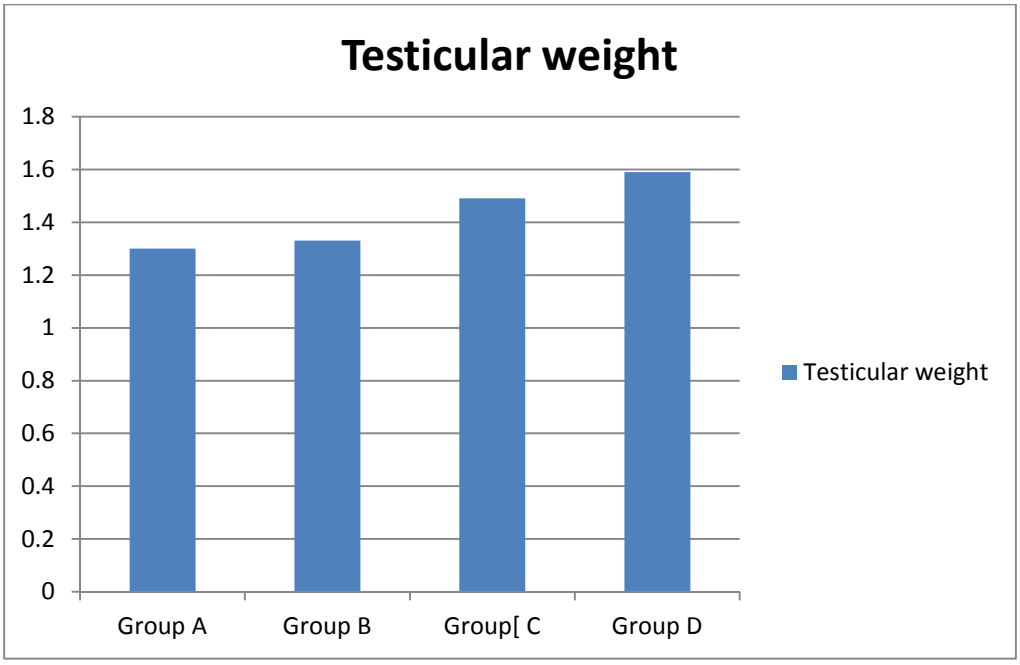

Figure 2: Bar chart showing the organ weights of all the groups

Histopatholigical Findings

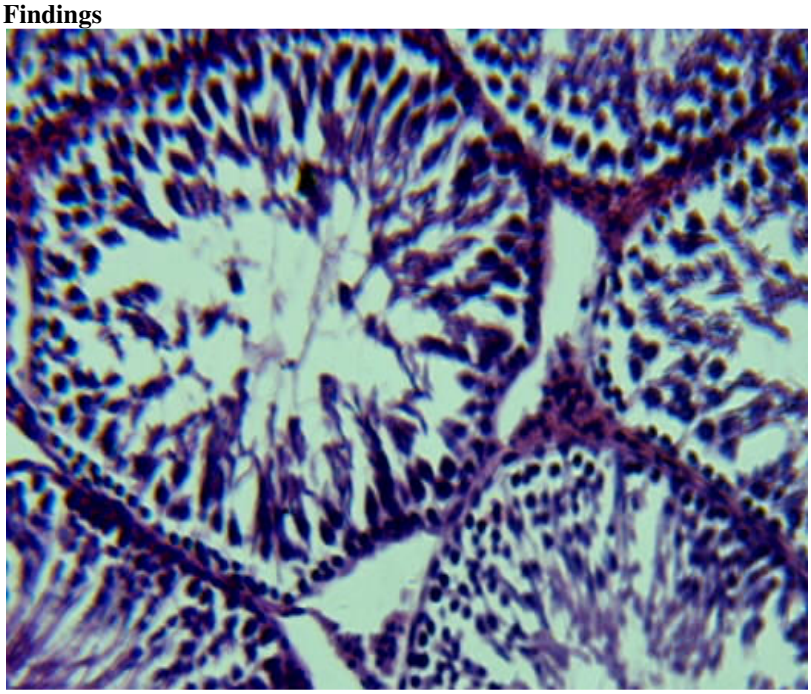

Fig 1: Micrograph 1 (control) showing normal histological structure of the testes

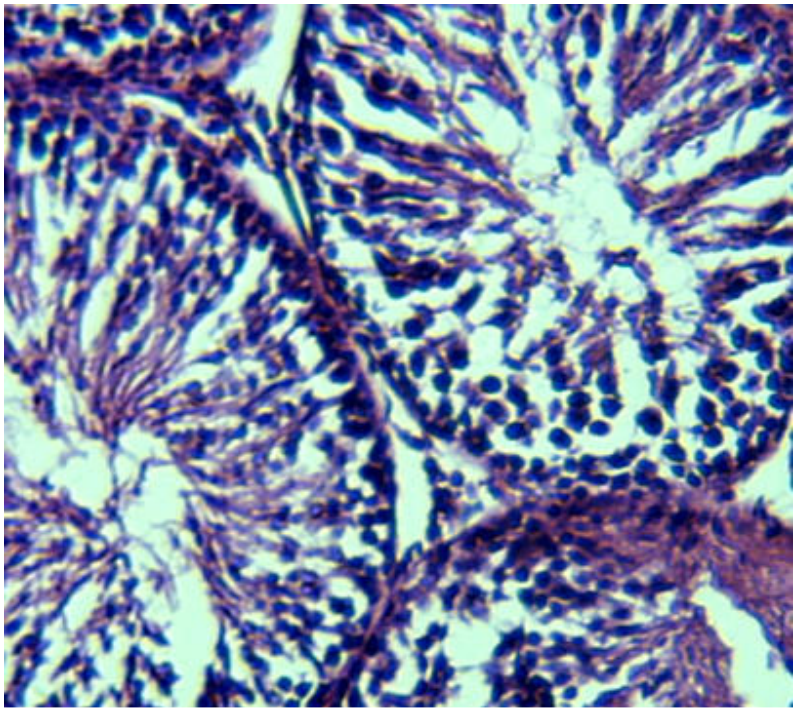

Fig 2: Micrograph 2 (Treated with 0.3ml of ethanolic extract of *Garcinia kola*) showing mild distortion of the spermatid cells.

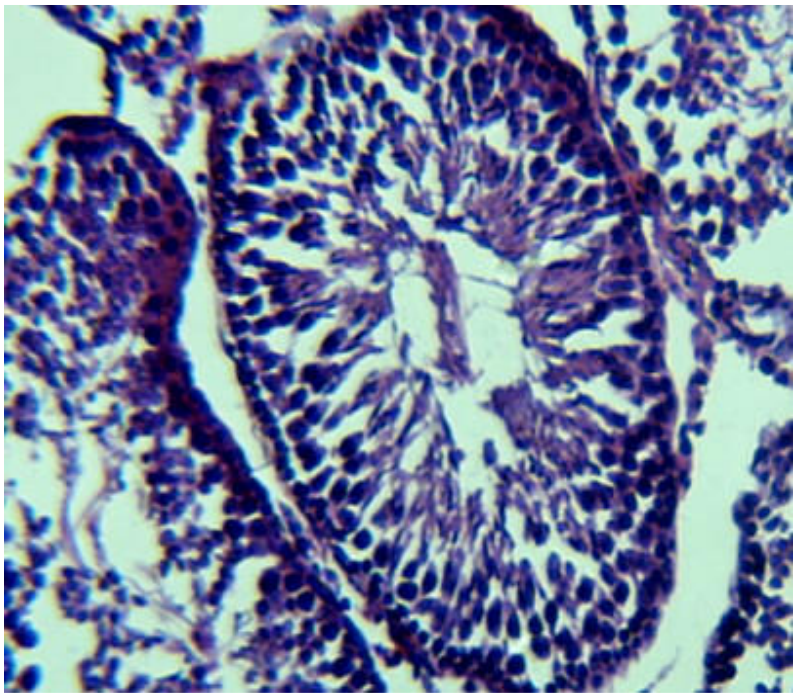

Fig 3: Micrograph 3 (Treated with 0.6ml of ethanolic extract of *Garcinia kola*) showing distortion of the spermatid cells with necrotic changes in the seminiferous tubules.

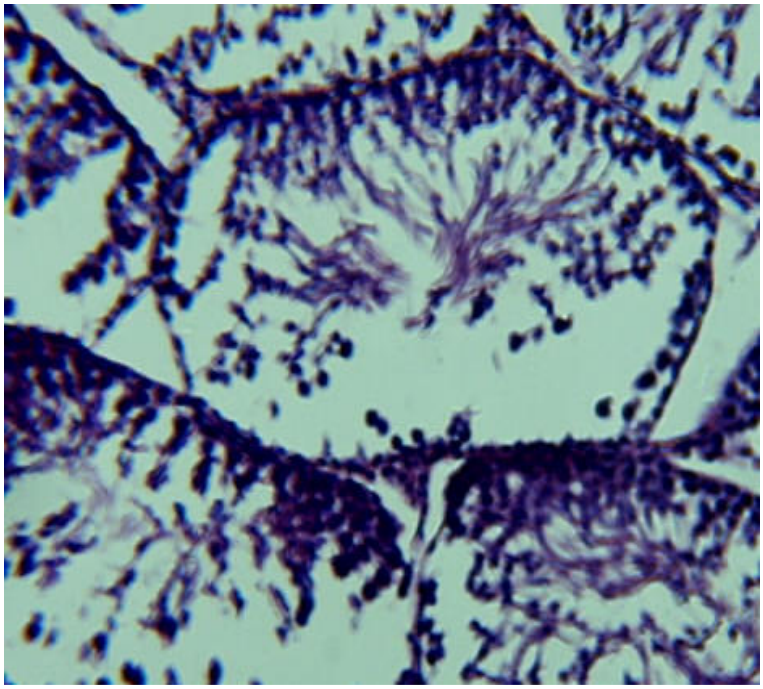

Fig 4: Micrograph 4 (Treated with 0.9ml of ethanolic extract of *Garcinia kola*) showing necrotic changes in the interstitial tissues, loss of spermatids and multinucleated gaint cells.

DISCUSSION

A number of herbs are thought to be likely to cause adverse effects (Talalay 2001). Furthermore, "adulteration", inappropriate formation, or lack of understanding of plants and drug interactions have led to adverse reactions that are sometimes life threatening or lethal (Elvin-lewis 2001). Proper double-blind clinical trials are needed to determine the safety and efficacy of each plant before they can be recommended for medical use (Vickers 2007). Although many consumers believe that herbals medicines are safe because they are "naturally" herbal medicines and synthetic drugs may interact, causing toxicity to the patient.

Herbal remedies can also be dangerously contaminated, and herbal medicines without established

efficacy, may unknowingly be used to replace medicines that do not have corroborated efficacy (Ernst 2007).

It is reported that components of *Garcinia kola* seeds exerted a toxic effect on the sertoli cells, thereby interfering with spermatogenesis (Udoh 1998). These same components may also have impact directly on the leydig cells (Naiho 2004), thereby interfering with testosterone production (Braide *et al.,* 2003).

In the present study, the final body weight for groups C and D decreased significantly ($P< 0.001$) when compared with the control. The final body weight of group B animals increased significantly with the control.

The comparison of the mean relative organ weight of groups C and D increased significantly ($P< 0.001$) when compared with the control, while group B mean relative organ weight was statistically similar with the control group A.

The histological findings indicated histological lesions in groups C and D treated with high doses of ethanolic extract of *Garcinia kola*. This result agreed with the previous study, which demonstrated that chronic consumption of *Garcinia kola* seed resulted in the disruption of the basement membrane of seminiferous tubules, near absence of sperm in the lumen and loss of interstitial cells of leydig (Naiho 2004).

CONCLUSION

From the present study, we inferred that consumption of ethanolic extract of *Garcinia kola* in high doses could cause serious damages to the testicular cells.

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