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THE EFFECT OF GARCINIA KOLA (BITTER KOLA) ON TESTOSTERONE AND THE HISTOLOGY OF THE TESTES IN MALE ALBINO WISTAR RATS

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ABSTRACT

The study determined the effect of Garcina kola on testosterone and the histology of the testes in male albino wistarrats. Fifteen (15) male rats of the Wistar strain weighing 115g-220g were divided into groups of 3 (control, low dose, high dose) consisting of 5 male rats in each of the 3 groups to determine the effects of Garciniakola extract on testosterone. The control group was given only water and rat feed while the experimental groups of low dose and high dose were administerd 5g/kg and 10g/kg respectively per body weight for a period of 28 days. The rats were sacrificed and testosterone from the blood in these 3 groups, were studied. Statistical analyses of data were determined by one way analysis of variance (ANOVA) followed by Post-hoc Turkey's test. It was observed that the hormone testosterone from group 2 with values of 0.50 ±0.15 recorded a significant increase in testosterone when compared to group 1 (control)with values 0.29 ± 0.08,while group 3 with values 0.23 ± 0.08 recorded no significant changes in testosterone when compared with group 1 (control).

Keywords: Garcina Kola, Testosterone, Histology of the Testis, Wistar Rats

INTRODUCTION

Garcinia kola, also known as bitter kola, is a fruit of a nut-bearing tropical tree native to Nigeria's coastal rainforests. It was named after a man called Garcin, who lived and wrote about the plant in the 18th century. It has a bitter taste followed by slight sweetness. Despite its bitter taste, Garcinia kola nuts are commonly eaten as a snack and used for their stimulant effects, due to high caffeine content. G. kolais mostly found in the southern part of Nigeria[1].

Garcinia kola contains antibacterial properties, according to clinical data which have been demonstrated to be effective in the treatment of infectious disease while simultaneously mitigating many of the side effects that are often associated with syntheticantimicrobials[2].

They agent has been revealed to be effective, yet gentle and known to exhibit response to specific organs or systems in the body. A previous study showed that Garcina kola did not possess toxins and was therefore termed "miracle drug" in the United States[3].

Garciniakola has been shown to be a popular treatment for diarrhoea and fever especially the seed extract which is antiseptic and is active mostly against gram positive bacteria. While the leaf, is active mostly against gram negative bacteria[4]. It is also very efficacious for hepatitis. In West Africa, it is now being harnessed as a cure for the Ebola virus infections and also against flu[3]. The stem, bark and the seeds have been used for acute fever, inflammation of the respiratory tract and throat infections.

Historically, Nigerians used Garcinia kola as an aphrodisiac. The seeds were also chewed to relieve hoarseness of voice, sore throat and cough. In folk medicine the seed is used for the treatment of liver disorder. It has also been used in the treatment of dysentery and diarrhoea[5].

MATERIALS AND METHOD

The plant material used for this study was garcinia kola(bitter kola) and were identified and confirmed in the department of Botany, Delta State University, Abraka. The animals used were 15 male adult albino wistar rats. These rates were procured in the animal house of the College of Health Science, Delta State University, Abraka and were observed for a period of two weeks and one day, before the commencement of the experiment. The experiment was carried out for a period of 28 days.

METHODOLOGY

Experimental design

The male albino wistar rats for the study were of comparable age, within the age range of eight(8) to ten (10) weeks, and weight range of 110g to 185g. The fifteen rats were divided into three groups of 5 rats each. Thereafter the control were fed with normal feed (growers mash produced by Bendel feeds and flower mills, limited, Ewu, Nigeria), the other two groups were given the bitter kola extract in respect of their weight and were fed with normal feed.

These three (3) groups of five rats each were arranged as:

Group 1 Control Rats
Group 2 Low dose
Group 3 High dose

Animal Handling

Each group of rats were maintained in one cage but in different compartments. The animal's room was adequately ventilated and kept at room temperature and relative humidity of 27-31^{0}c and 40-70% respectively with a 12hour natural light dark cycle. They were fed ad libitum with water and rat chow. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from cages daily.

Extraction of Raw Materials

The fresh seeds of garcinia kola were washed and cut into smaller bits and air dried for a period of two weeks. The dried specimens were grinded using mortar and pester

Two hundred grams (200g) portion of the powdered seed were extracted in soxhlet apparatus using distilled water as solvent. Filtrate recovery from soxhlet extraction was concentrated as reduced pressure with the aid of rotary evaporator. Extract sample was further dried to constant weight in oven set at 50^{o}c.

Finally extract recovered (32.5g weight) with percentage yield of 16.25% w/w was stored in the refrigerator before use.

Percentage yield = weight of final extract / Weight of powered garcinia kola X 100/1
= 32.5g/200g X 100/1
=16.25% w/w

Preparation of stock solution

Two grams (2g) and four grams (4g) of crude extract was weighed with the aid of electronic weighing balance and were constituted in 400ml of distilled water respectively. This gave a stock solution of 2000mg/400ml (5mg/ml) for low dose (50mg/kg) and 4000mg/400ml (10mg/ml) for high dose respectively. Respective volumes for experimental animals were calculated based on their body weights (BW).

Feed Administration

Group 1 (Control) was administered normal feed (growers mash and clean drinking water for four weeks. Group 2 (Low dose) was given 5mg/ml of aqueous solution of bitter cola in respect of their weight and with normal feed. Group 3 (High dose) was given 10mg/ml of aqueous solution of bitter cola in respect of their weight and with normal feeds.

Collection of Samples

The rats were allowed to fast 12 hours preceding the sample collection. After sedating the rats with chloroform; they were dissected to expose the heart. Using syringe and needles, the blood was collected from the heart and put in a labelled non-heparinized sample tube.

Research Ethics

Ethical approval was given by the ethics committee of Delta State University, Abraka under the license covering the institution of medical and scientific research ethics.the animal care guidelines of European centre for the validation of alternative method were used[8].

Biochemical Assay for Determination of Testoserone
Product: Elisa kit.
Principle: The principle follows that of enzyme immunoassay test which is a typical competitive binding schematic.

Competition occurs between an unlabeled antigen (present in standards, control and patient samples) and anenzyme-labeled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures removed unbound materials. After the washing step, the enzyme substrate was added. The enzymatic reaction was terminated by addition of the stop solution. The absorbance was then measured on a microtiter plate reader. The intensity of the color formed was inversely proportional to the concentration of testosterone in the sample. A set of standards was used to plot a standard curve from which the amount of testosterone in patient samples and controls can be directly read.

Statistical Analysis
The result of this study were expressed as mean ± SD (Standard Deviation) of five replicates, N=5. Raw data were subjected to one way analyses of variance (ANOVA) followed by post HOC – Turkey's test for multiple comparisons using statistical package for social science (SPSS, 16). Statistical Significance was declared when $P<0.05$.

Results

Table I: Showing raw data of results of the effect of Bitter cola (*Garcinia cola*) extract on testosterone in Wistar rats.

TESTOSTERONE LEVEL (ng/ml)		
Group 1 (Control)	**Group 2 (Low dose)**	**Group 3(High Dose**
0.22, 0.2, 0.3, 0.36, 0.38	0.4, 0.34, 0.7, 0.6, 0.45	0.23, 0.3, 0.1, 0.21, 0.3

The above table shows the raw data of the results of the effects of Bitter cola (*Garcinia cola)* extract on semen parameters in Wistar rats. Each group consists of five animals.
Control: Distilled water, 10ml/kg, **Low dose**: Extract, 50mg/kg **High dose**: Extract, 100mg/kg.

Table II: Showing the results of the effect of Bitter cola (*Garcinia cola*) extract on serum testosterone in male Wistar rats.

TESTOSTERONE LEVEL (ng/ml)		
Group 1 (Control)	**Group 2 (Low dose)**	**Group 3(High Dose**
0.29 ± 0.080.50 ± 0.15[a]0.23 ± 0.08[b]		

Values are expressed as mean ± Standard Deviation, n=5.
^{a}P<0.05: Statistically significantly different from group 1.
^{b}P<0.05: Statistically significantly different from group 2.
Fig. 1 (Control):Sections of the testis showing various size tubules disposed within a loose connective tissue stroma with a few interstitial call present. The tubules are lined by germ cells at various stages of normal differentiation.

Figure 2 (Low dose): Sections of the testis showing several size tubules disposed within a loose connective tissue stroma with a few interstitial call present. The tubules are lined by germ cells at various stages of normal differentiation.

Fig. 3 (High dose): Sections of the testis show several size tubules disposed within a loose connective tissue stroma with a few interstitial call present. The tubules are lined by germ cells at various stages of normal differentiation.

The effect of Bitter cola (*Garcinia cola*) extract on histology of the testis in Wistar rats.

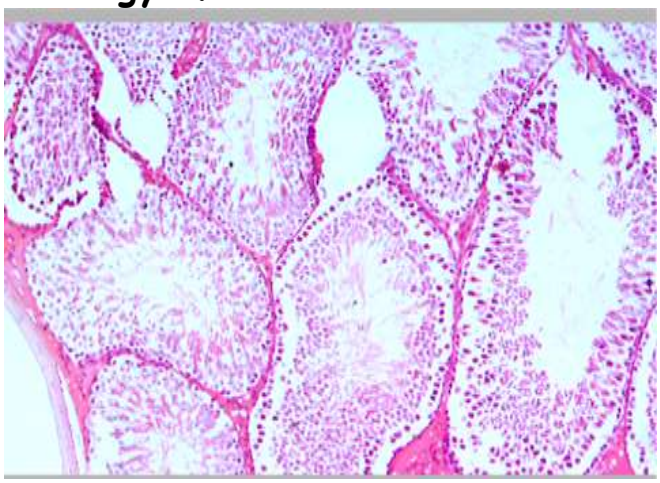

Figure 1: section of the testes show size seminiferous tubule (control) H and E*100

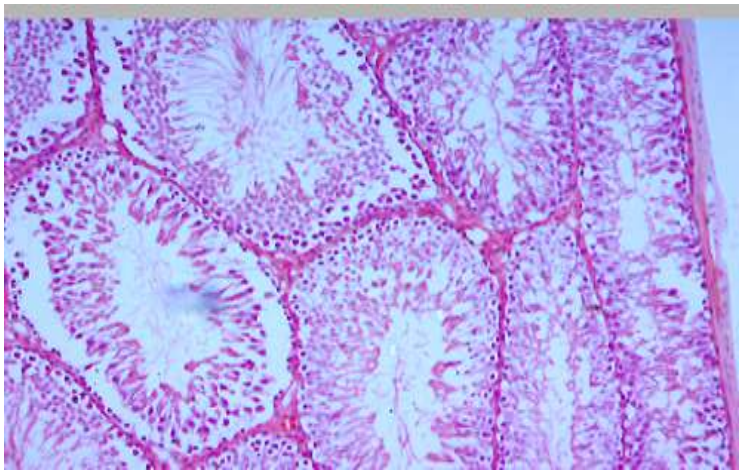

Figure 2: section of the testes show size seminiferous tubule (Low dose) H and E*100

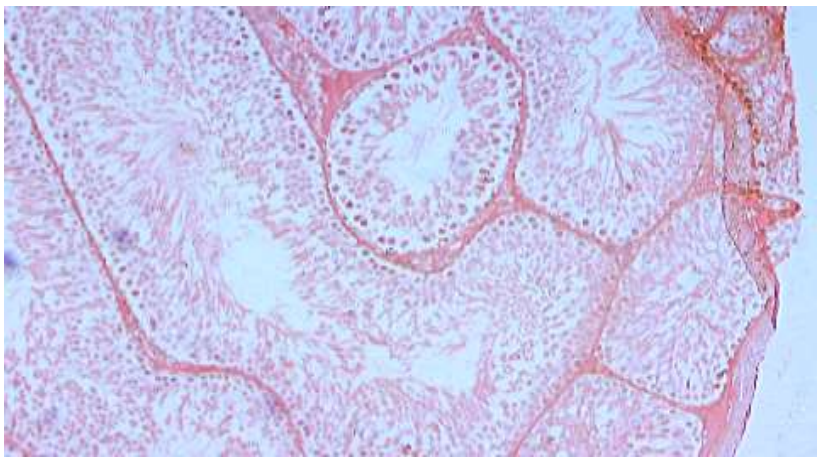

Figure 3: section of the testes show size seminiferous tubule (High dose) H and E*100

DISCUSSION

The effects of Soxhlet extract of Garcinia kola seed on the histology of the testes and the hormone testosterone of albino wistar rats was evaluated. It was reported that Garcinia kola increases spermatogenic activity through its peripheral testosterone[6]. This ability of Garcinia kola to increase peripheral testosterone and tissue enhancement has been attributed to antioxidant compounds present in them[6,7].

Serum testosterone from the analysed data in table 2, the changes in control group was 0.29 ± 0.08 ng/ml, low dose; 0.50 ± 0.15^{a} ng/ml while high dose; 0.23 ± 0.08^{b} ng/ml. Low dose extract significantly increased ($^{a}P<0.05$) testosterone level when compared with control while high dose significantly decreased ($^{b}P<0.05$) testosterone level when compared with low dose. According to Braide et al (1989)[8], plasma testosterone does not show any significant difference but the increment of testosterone level in low dose in this present research agreed with the finding of Akpantah et al (2003). Garcinia kola has been reported to increase spermatogenic activity through its tissue enhancement and ability to increase peripheral testosterone[6]. The ability of G. kola to increase

peripheral testosterone and tissue enhancement has been attributed to antioxidant compounds present in them[6,7] while the reduction of testosterone in high dose group could be traced to the presence of toxic substances (benzophenone, apigenin) in G. kola. This suggests that the effect of the toxic substances, are dose dependent.

This result showed that the extract caused no significant increase in the interstitial spaces, no significant reduction in the leydig cells population in the interstitial spaces, no significant reduction in the seminiferous tubules luminal spermatozoa concentration, no significant contraction of the seminiferous tubules, and arrangement of the cells spermatogenic series. This finding agreed with the work of Akpantah et al., (2003)[6] who recorded no histological difference in the testes of wistar rats after administration of crude ethanolic extract of Garciniakola but in variance with the work of Akinloye et al., (1999)[9].

Oluyemi et al., (2007)[7] observed increment in the diameter of seminiferous tubule of the male wistar rats treated with ethanolic extracts of Garcinia kola.

CONCLUSION

The study showed that administration of Soxhlet extract of Garcinia kola over a period of 28 days have some adverse effect on the testosterone and the testes of albino wistar rats which is dose dependent. Therefore, it is advised that the continuous usage of Garcinia kola diet should be taken with caution.

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