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Some biological activities of *Garcinia kola* in growing rats

Otor John Uko[1]*, Abdulkadir Usman[1], and Abu Mohammed Ataja[2]

[1]*Department of Veterinary Physiology and Pharmacology, Usmanu Danfodiyo University, Sokoto, Nigeria*

[2]*Department of Veterinary Public Health and Animal Production, Usmanu Danfodiyo University, Sokoto, Nigeria*

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ABSTRACT

The biological activities of water extract from *Garcinia kola* (*G. kola*) were investigated in growing Wistar rats. Three doses of *G. kola* extract (0, 10, 20 mg *G. kola*/100g body mass of rat) were administered daily by gavage to the respective groups of 15 rats for a period of 70 days. The animals were offered standard rat diet and water ad libitum. The plant extract had a depressive effect ($P<0.01$) on appetite and water intake with resultant poor ($P<0.05$) feed utilization efficiency and mass gain of rats in a dose-dependent manner. Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were elevated ($P<0.05$) but histological examinations of liver, heart and lungs of experimental rats revealed no alterations. Nevertheless, a significant ($P<0.05$) increase in leucocyte counts was adduced for possible mild degenerative changes in these organs. The extract enhanced sexual interest (libido) of the male rats but did not necessarily improve their fertility rate.

Key words: *Garcinia kola*, performance traits, organ masses, blood composition, male fertility index, rat

Introduction

Garcinia kola Heckel (*G. kola*; Guttiferae) is a dicotyledonous plant found in the rain forests of Central and West Africa. It is grown on homesteads in Southern Nigeria; a detailed description and distribution of the plant have been documented (IWU, 1993). The plant is commonly called

* Contact address:
O. J. Uko, Department of Veterinary Physiology and Pharmacology, Usmanu Danfodiyo University, P. M. B. 2346, Sokoto, Nigeria, Phone: + 060 232 285, E-mail: ojuko2002@yahoo.com

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"bitter kola" or "male kola" because of its bitter taste, or for its claimed aphrodisiac activity, respectively. *G. kola* nut is chewed extensively in Southern Nigeria as a masticatory and it is readily served to visitors, especially among the Igbo tribe in Eastern Nigeria, as a sign of peace and acceptance of visitors. It is also popular among the people of Nigeria for nervous alertness and induction of insomnia. The root of the plant is used as favourite bitter chew-sticks in West Africa. The stem bark is used as a purgative among the natives of Eastern Nigeria and the latex is externally applied to fresh wounds to prevent sepsis, thereby assisting in wound healing.

G. kola stem bark has been shown to contain a complex mixture of phenolic compounds such as biflavonoids, xanthones and benzophenone (IWU and IGBOKO, 1982) which have antimicrobial activity as kolanone (HUSSAIN et al., 1982), kolaflavanone and garciniaflavanone (IWU, 1993). The plant's nut contains a high proportion of tannins and guttiferin (ETKIN, 1981).

G. kola is reported to have a protective effect against a variety of experimental hepatotoxins (IWU, 1985; AKINTOWA and ESSIEN, 1990). However, some histological alterations in the liver, kidney and duodenum of rats fed diets containing 10% *G. kola* nut have been reported (BRAIDE and GRILL, 1990). Similarly, OLUWOLE and OBATOMI (1992) observed an increase in both basal and histamine-mediated gastric acid secretion of rats fed *G. kola* nut.

Because of the extensive consumption of *G. kola* nuts in Nigeria, this study was conducted to elucidate possible alterations of some biological systems (digestive, haematological, reproductive, etc.) of the subjects, using growing rats as a model.

Materials and methods

Plant material. Nuts of *G. kola* were obtained from Sokoto market in Nigeria and were authenticated in the Botanical Unit of Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, where a voucher specimen has been deposited in the herbarium. The nuts were chopped into pieces, air-dried and grated. About 220 g of the grated nut

was soaked in 1 litre of distilled water and allowed to stand for about 2 days on a mechanical shaker before filtering. The filtrate was evaporated at 55 °C in an oven (Gallenkamp®) for 3 days. Two distinct layers (the upper sticky and chocolate-coloured layer, and the lower powdery and dark-brown layer) of solid obtained on drying the filtrate were re-dissolved in 10 ml distilled water into a homogenous paste and was further dried in the oven to a constant weight.

Animals. Forty-five growing Wistar rats of both sexes (95-109 g), obtained from the animal house of the University of Ilorin, Nigeria, were randomly allocated to 3 treatment groups of 15 rats with similar average body mass. The rats were first generation progenies from parents whose records of reproductive performances were very good. Each rat was housed in a stainless steel cage and fed standard rat diet (Table 1) *ad libitum* with free access to drinking water. Graded doses (0, 10 and 20 mg/100 g body mass of rat/day) of *G. kola* extract were each administered in 0.5 ml of saline to the respective groups of rats for 70 days. This was done by gavage using a flexible catheter attached to a tuberculin syringe. The control group was given saline (0.5 ml saline/rat) only. Daily water intake, voluntary feed intake, mass gain and faecal output were recorded at 7-day intervals.

Male fertility test. On day 49 of the experiment, 4 sexually mature male rats were randomly selected from each of the treatment groups. Each rat was individually caged with 3, non-treated female rats known to have given birth once, but which had not been mated for approximately 35 days before the start of this test. Male and female rats were kept together for 14 days and then returned to their respective groups. Daily dosing of the males with *G. kola* extract continued as the experiment progressed. At the end of day 28 of first contact between both sexes, the female rats were sacrificed and their uteri examined for evidence of pregnancy (presence and number of embryos and foetuses). Embryos and foetuses from the test groups were expressed as percentages of the number from the control rats and were used as measure of male fertility index.

Haematology and plasma biochemistry. At termination of the experiment, blood was drawn from all the rats fasted for 12 hours, by cardiac puncture under diethyl ether anaesthesia and divided into 2 samples containing either disodium ethylenediaminetetra-acetate (EDTA) at 1.0

mg/ml, or heparin (0.2 mg/ml blood) for cell counts and plasma biochemistry, respectively. Haemoglobin concentration (Hb) was determined by the cyanomethaemoglobin method using a Beckman model spectrophotometer, erythrocytes (RBC) were counted with an improved Neubauer haemocytometer. Total leucocyte (WBC) count and packed cell volume (PVC) were measured with the QBCII Centrifugal Haematology System (Becton Dickinson Co., U. S. A.) The erythrocytic indices: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), were calculated (JAIN, 1986). Total plasma protein, albumin, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), were measured using autoanalyser (SMA 12/60 Technicon Autoanalyser, Terry-town, N. Y.).

Histopathology. The anaesthetized rats were decapitated immediately after blood sampling and eviscerated. Organs were removed, weighed fresh and fixed for 5 days in 10% neutral buffered formalin for histopathology. Their weights were later expressed as percentages of final body mass. Tissue samples from testes, kidneys, liver, heart, lungs and brain were trimmed and processed by the paraffin wax procedure, and sections of 4um thickness were cut and stained with haematoxylin and eosin for examination by light microscopy.

Statistics. All data were expressed as means ± standard errors. Statistical comparisons between groups were performed using the Student's two-tailed test for unpaired data (STEEL and TORRIE, 1980).

Results

Performance characteristics of the rats. The yield of *G. kola* extract relative to the starting material (raw *G. kola* nuts) was 11.1%. The extract induced dose-dependent reductions ($P<0.05$) of weight gain and feed utilization efficiency. Voluntary feed and water intake were markedly depressed ($P<0.01$) but faecal output was enhanced ($P<0.05$) at a dose of 20 mg extract/100 g body mass. Dry matter digestibility did not differ appreciably ($P>0.05$) between control and experimental rats. Similarly, there was no consistent trend in fertility index of the male rats placed on

Table 1. Composition of rat diet

Ingredients	(%)
Corn starch	61.8
Groundnut meal	18.9
Soya bean meal	16.0
Bone meal	1.5
Limestone	1.0
Iodized salt	0.5
Vitamin and mineral premix*	0.3
Total	100.0
Crude protein content (%)$^{+}$	15.5

* Unit–vit 15 (Roche) premix supplied the following vitamins and minerals per kg of diet: A 8,000 I.U.; D_3 1,500 I.U.; K 3.0 mg; B_2 2.5 mg; nicotinic acid 8.0 mg; D – pantothenate 3.0 mg; B_6 0.3 mg; B_{12} 8.0 mg. Minerals: Mn 10.00 mg; Fe 5.00 mg; Zn 4.50 mg; Cu 0.20 mg; I 0.15 mg; Co 0.02 mg; Se 0.01 mg.
$^{+}$ Calculated

Table 2. Performance characteristics of rats on varying doses of *G. kola* extract

Dosage of *G. kola* (mg/100 g body mass/day)				
Traits	0 (n=15)	10 (n=15)	20 (n=15)	Significance
	Mean ± SE	Mean ± SE	Mean ± SE	
Initial mass (g/rat)	109.4 ± 4.2	107.8 ± 5.3	109.3 ± 4.8	-
Mass gain (g/rat/d)	1.81 ± 0.4 ^{a}	1.2 ± 0.4 ^{b}	0.7 ± 0.4 ^{c}	0.05
Feed intake (g/rat/d)	9.8 ± 0.4 ^{a}	7.4 ± 0.3 ^{b}	4.6 ± 0.2 ^{c}	0.01
Feed: gain ratio	5.4 ± 0.5 ^{a}	6.5 ± 0.4 ^{b}	6.8 ± 0.4 ^{b}	0.05
Water intake (ml/rat/d)	26.1 ± 1.0 ^{a}	23. ± 0.9 ^{a}	11.7 ± 0.5 ^{b}	0.01
Faecal output				
(% feed intake)	14.2 ± 0.7 ^{a}	18.1 ± 0.8 ^{a}	23.6 ± 1.5 ^{b}	0.05
Dry matter				
Digestibility (%)	90.4 ± 0.7 ^{a}	84.6 ± 1.6 ^{a}	79.6 ± 1.7 ^{a}	ns
Male fertility index (%)	100 ± 3.8 ^{a}	92.3 ± 6.5 ^{a}	103.8 ± 5.2 ^{a}	ns

a, b, c = means in the same row with different superscripts are significantly different
ns = no significant difference among means in the same row
n = the number of rats per treatment

Table 3. Organ masss of given varying doses of *G. kola* extract

	Dosage of *G. kola* (mg/100 g body mass/day)		
Organs	0 (n=15)	10 (n=15)	20 (n=15)
	Mean ± SE	Mean ± SE	Mean ± SE
Testes	1.28 ± 0.02	1.15 ± 0.04	1.21 ± 0.03
Female reproduction tract	0.39 ± 0.05	0.41 ± 0.03	0.38 ± 0.04
Kidneys	0.36 ± 0.02	0.39 ± 0.02	0.39 ± 0.02
Liver	3.42 ± 0.20	3.29 ± 0.06	3.15 ± 0.05
Spleen	0.30 ± 0.03	0.36 ± 0.02	0.45 ± 0.04
Stomach	1.02 ± 0.03	1.02 ± 0.03	1.12 ± 0.05
Intestines	7.00 ± 0.19	7.36 ± 0.12	7.13 ± 0.17
Lungs	1.95 ± 0.11	1.63 ± 0.16	1.34 ± 0.08
Heart	0.65 ± 0.03	0.47 ± 0.02	0.45 ± 0.02
Brain	1.08 ± 0.12	1.25 ± 0.07	1.39 ± 0.09

Table 4. Haematological indices of rats given varying doses of *G. kola*

	Dosage of *G. kola* (mg/100 g body mass/day)		
Indices	0 (n=15)	10 (n=15)	20 (n=15)
	Mean ± SE	Mean ± SE	Mean ± SE
Haematocrit (%)	40.3 ± 1.2	38.5 ± 1.1	36.4 ± 1.4
Haemoglobin (g/100 ml)	13.9 ± 0.8	13.2 ± 0.5	12.5 ± 0.6
Erythrocytes (10^6 ml)	5.2 ± 0.3	4.8 ± 0.2	4.6 ± 0.4
MVC (μl)	77.5 ± 3.8	80.2 ± 4.1	79.1 ± 3.7
MCH (pg)	26.7 ± 1.9	27.5 ± 2.0	27.2 ± 1.8
MCHC (g/dl)	34.5 ± 3.2	34.2 ± 2.8	34.3 ± 3.3
Leucocytes (10^3 ml)	4.5 ± 0.3^a	5.5 ± 0.4^{ab}	6.1 ± 0.3^b

a, b, means with different letters in a row differed ($P<0.05$)

Table 5. Plasma biochemistry of rats on varying doses of *G. kola* extracts

Dosage of *G. kola* (mg/100 g body mass/day)			
Traits	0 (n=15)	10 (n=15)	20 (n=15)
	Mean ± SE	Mean ± SE	Mean ± SE
Total proteins (g/dl)	5.33 ± 0.16	5.22 ± 0.12	4.93 ± 0.09
Albumin (g/dl)	3.33 ± 0.06	3.21 ± 0.05	3.18 ± 0.06
Total bilirubin (μmol/L)	1.83 ± 0.04	2.00 ± 0.13	2.13 ± 0.11
Conjugated bilirubin (μmol/L)	0.80 ± 0.09	0.96 ± 0.07	1.05 ± 0.09
ALT (GPT) (μg/L)	12.25 ± 0.92 [a]	15.31 ± 0.87 [b]	16.75 ± 0.92 [b]
AST (GOT) (μg/L)	35.25 ± 2.13 [a]	39.75 ± 3.16 [b]	42.00 ± 3.28 [b]
ALP (μg/L)	24.50 ± 0.48	24.44 ± 0.68	23.65 ± 0.19

a, b, means with different letters in a row differed ($P<0.05$)

varying doses of *G. kola* extract. The number of embryos and foetuses in groups 1 (control), 2 (10 mg/100 g) and 3 (20 mg extract/100 g), respectively, were 24 and 28, 26 and 22, and 20 and 34. The treatment means for the various performance characteristics from the rats are shown in Table 2.

Organ mass and histopathology. Although none of the organs from the control and experimental rats were significantly influenced by the levels of *G. kola* extract (Table 3), there was a dose-related decrease in size of livers, lungs and hearts of rats fed the plant extract. In contrast, the extract caused mild ($P>0.05$) but consistent enlargement of spleen and brain of experimental rats compared with those of the control rats. Of the organs (testes, kidney, liver, heart, lungs and brain) examined, no microscopic alterations were observed in any of the treatment groups.

Haematology and plasma biochemistry. No significant differences were found between blood samples from the control and the experimental rats for Hb, PVC, RBC and erythrocytic indices; however, a general inverse relationship between the erythrocytic values (Hb, PVC and RBC) and increased doses of the plant extract was observed (Table 4). There was significant ($P<0.05$) proliferation of total leucocyte counts in blood samples from the experimental rats, which was dependent on doses of the extract

administered. *G. kola* extract decreased ($P<0.05$) total plasma proteins, albumin concentrations and ALP activity, but slightly ($P>0.05$) increased total and conjugated bilirubin levels. Meanwhile, ALT and AST activities in plasma samples from experimental rats were significantly ($P<0.05$) elevated above those from control rats (Table 5) and AST activities in plasma samples from the experimental rats were significantly ($P<0.05$) elevated above those from control rats (Table 5).

Discussion

The decreased body mass gain in rats fed *G. kola* extract was associated with reduced feed consumption in accordance with previous reports on some other plant materials (THOMAS et al., 1983; SUMMERS et al., 1990) although mass loss without decreased feed intake has been documented (WIGHT et al., 1987). Both food refusal and decreased feed efficiency ratios appeared to have affected weight gain negatively; however, the degree to which each of the factors contributed to the overall effect is not known. The significant reduction in water intake by experimental rats was surprising. It was expected that rats ingesting bitter kola would drink more water to 'rinse' off the material from taste buds. It would seem that some fraction(s) of *G. kola* extract may have a subtle but direct depression (toxic effect) on osmoreceptors in the digestive tract and/or higher (hypothalamic) centres. The relatively higher faecal output of the experimental rats compared to that of control rats possibly resulted from both the tannin content (ETKIN, 1981) and the sticky layer of the plant extract. This (sticky) layer is presumed to contain latex, as does the stem bark of *G. kola*, which is not readily digestible.

During the course of male fertility trial it was observed that experimental rats exhibited increased libido which was demonstrated by frequent attempts of the males to mount female rats, thus justifying the use of *G. kola* nut by the natives as an aphrodisiac (IWU, 1993). However, pregnancy rates in female rats as a measure of the male fertility index did not differ appreciably among the treatment groups. *G. kola* extract had no significant effects on the relative organ mass of the rats used in this study. Similarly, alterations in liver of rats fed diets containing 10% *G. kola* nut

have recently been reported (BRAIDE and GRILL, 1990). The absence of observable pathological lesions in organs of the experimental rats in the present study, contrary to earlier reports (BRAIDE and GRILL, 1990; VIRK and MENKE, 1986), is possibly due to non-challenge of the rats with high doses of *G. kola* extract. The rise in bilirubin values, with a corresponding decrease in the RBC values, implied mild haemolysis of RBC by the plant extract. The elevation in ALT and AST in rats fed *G. kola* extract reflected liver and heart toxicity, which may be mild but sufficient to permit leakage of the cellular enzymes through the cell membranes with no appreciable effect on cellular functions. Whereas ALT concentration is higher in liver of rats, AST occurs in a wide variety of tissues, but with high concentrations in muscular tissues and in liver (KANEKO, 1980; BUSH, 1991; DIAL, 1995). The increase in AST activity could therefore be ascribable to both myocardial and liver degeneration in the experimental rats because of a consistent decrease in relative weight of their hearts and livers.

In conclusion, the study has justified the folkloric use of *G. kola* nut to induce inappetence in the event of anticipated prolonged abstinence from food and water, and as an aphrodisiac to minimise incidence of impotence. However, ingestion of the kola nut should be restricted to adults, as the young may suffer growth retardation. Even among adults, intake of the kola nut should be as moderate as possible as chronic ingestion of high doses has the tendency to cause pathological changes in some body systems.

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SAŽETAK

Biološka aktivnost vodene iscrpine biljke *Garcinia kola* (*G. kola*) istražena je u Wistar štakora u razvoju. Nakon što su svrstani u tri skupine po 15 štakora, iscrpina je primijenjena u tri doze (0, 10 i 20 mg/100g tjelesne mase), i to peroralnim davanjem prikladnom sondom tijekom 70 dana. U tijeku pokusa štakori su hranjeni standardnom hranom i napajani *ad libitum.* U životinja u kojih je davana iscrpina biljke utvrđen je smanjen tek te slabija želja za uzimanjem vode, kao i slabija konverzija hrane ($P<0,05$) i smanjen prirast ovisan o dozi. U štakora su utvrđene visoke razine aktivnosti plazmene alanin-aminotransferaze i aspartat-aminotransferaze ($P<0,05$), što nije potvrđeno histološkom pretragom jetre, srca i pluća. Degenerativne promjene istih organa mogu se dovesti u vezu sa značajnim porastom broja leukocita ($P<0,05$). Zapaženo je da iscrpina povećava spolni nagon mužjaka iako ne povećava plodnost.

Ključne riječi: *Garcinia kola,* prirast i konverzija, masa organa, sastav krvi, plodnost mužjaka, štakor