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INVESTIGATION OF THE EFFECTS OF ETHANOL SEED EXTRACT OF *GARCINIA KOLA* ON HEMATOLOGICAL AND HEPATORENAL INDICES IN WISTAR ALBINO RATS

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ABSTRACT: *Garcinia kola* is a known herbal plant which is widely consumed for many ethnomedicinal uses. This study was aimed at determining the effect of ethanol extract of *G. kola* seeds on levels of hematological as well as blood indices of liver (aspartate transaminase, alanine transaminase, alkaline phosphatase, total protein, and albumin) and kidney function (creatinine and urea). Extract (50, 100, 200 or 300 mg/kg) was orally administered to different groups of rats (n = 6) daily for thirty days. Control animals were given vehicle (DMSO + distilled water, 2:1). Blood levels of the parameters mentioned above were measured at the end of extract treatment. Livers and kidneys of animals were removed and weighed and organ-to-body weight ratio was obtained. Extract had no effect on serum levels of liver and kidney indices, except that it reduced ($P<0.05$) ALP level at 300 mg/kg. Organ-to-body weight ratio of liver and kidney were not affected by extract. Furthermore, the extract increased ($P<0.001$) platelet count, but had no effect on RBC and its indices (mean cell volume, mean cell haemoglobin, and mean cell haemoglobin concentration), Hb and PCV compared to control. It also caused reduction ($P<0.001$) in WBC and neutrophil counts, while lymphocyte was increased. The result suggests that *G. kola* seed may have no adverse influence on hematologic, hepatic or renal system, and may additionally have hepato-protective property, and enhance blood clotting and lymphocyte function.

INTRODUCTION: *Garcinia kola* is a species of flowering plant that belongs to the Family, Clusiaceae or Gutiferae. Its natural habitat is subtropical or tropical moist lowland forests. The plant grows to a height of about 15 m and produces reddish, yellowish or orange color fruit containing 2 to 4 seeds.

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Garcinia kola is consumed amongst Africans, either leisurely or for medicinal purposes. The fruit, Seed, nut and bark of *Garcinia kola* have been used for centuries in alternative medicine to treat a variety of ailments like cough, fever, bronchitis, asthma, throat infections, diarrhoea, and as antidote for poisons [1 - 5].

Other conditions include rheumatism, headache, constipation, abdominal colic, oral hygiene, and liver disorders [2, 4, 6]. *Garcinia kola* contains phyto-chemicals which have potent biological activities [7 - 9], and these have been attributed to most of the plant's medicinal properties. Presently, the plant is believed to be highly efficacious and therefore used

as an alternative to orthodox medicines for treatment of different illnesses by many in Nigeria and other African countries. It is growing in popularity as a natural and effective remedy for weight reduction, and particularly popular for controlling cough, diabetes and diarrheal episodes. However, there is no sufficient knowledge about the safety of prolonged consumption of the plant. A study to assess the toxicity potential of prolonged use of the plant on liver and kidney functions may therefore be useful. The liver is a vital organ which functions include, detoxification of chemical compounds, synthesis of essential molecules and metabolism [10, 11]. The kidney disposes off waste products from the body.

The objective of this study was to assess the effects of ethanol extract of *Garcinia kola* seeds on biochemical indices of liver and kidney functions in Wistar rats. Its effect on hematological indices was equally evaluated.

MATERIALS AND METHODS:

Materials:

Chemicals / Reagents: Dimethyl sulphoxide, DMSO (Zhuzhou Hansen Chemical Industry Co. Ltd., Shanghai, China), ethanol (JHD Chemicals, Guangdong, China), biochemical test kits (Randox Inc., USA)

Equipment: Analytical balance (Model CPA 3245, Sartonus AG Goghingen, Germany), spectrophotometer (Spectrum Lab., USA), oven (Model DHG - 9101 - ISA, Health Medical Equipment, England), water bath (Finlab Company, Lagos, Nigeria).

Methods:

Preparation and Extraction of Plant Material: Fresh *Garcinia kola* seeds were purchased from a Fruit Market (Oil Mill Market), Aba Road, Port Harcourt, Nigeria in 2016. It was identified by a botanist, Mr. M. Suleiman of the Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Nigeria and a specimen number (DPH048) was assigned to it and deposited in the Faculty's herbarium. The seeds were air-dried for about one week and oven-dried at 40 ºC until a constant weight was obtained. The seeds were then ground using an electric blender and 1000 g weight powder was macerated in 5000 ml of 70% ethanol in a wide mouthed extraction bottle with continuous agitation for seven days. Extraction mixture was filtered with Whatman number 51 filter paper. The filtrate was evaporated using a rotary evaporator to obtain a pasty extract which was transferred into a crucible and remaining liquid was evaporated using hot water bath. The dry extract was then transferred into a clean container and stored in a refrigerator, and percentage yield was determined (19.4 %).

Phytochemical Analysis of Ethanol Extract of *Garcinia kola* Seeds: Phytochemical analysis of the extract was done using conventional methods [12].

Animals: Thirty Wistar albino male rats (180 - 200 g body weight) were obtained from the Animal House in Department of Experimental Pharmacology and Toxicology, University of Port Harcourt, Nigeria and used for the study. They were maintained at room temperature (24 ± 3 ºC), under natural lighting condition, and fed with rodent's pellets and tap water (given *ad libitum*). Animal experiments were done according to approved guideline of my Institutional Research Ethics Committee (UPH/CHREC/APP/049/2016) and all animals were cared and handled according to stipulated standard guidelines.

Experimental Design: Animals were divided into five groups, each containing six rats, and given 0, 50, 100, 200 and 300 mg/kg of extract daily by oral gavage for 30 days. Vehicle used to dissolve extract was DMSO and distilled water (2:1). Control group was given 0.5 ml vehicle. Doses of extract administered were below the LD_{50} of 5000 mg/kg as reported previously [11]. The rats were anesthetized with diethyl ether 24 h after the last extract treatment and sacrificed by cervical dislocation. Blood was collected *via* cardiac puncture with sterile syringes and needles into EDTA and plain bottles.

Levels of hematological parameters- red blood cell (RBC), white blood cell (WBC), platelet, hemoglobin (Hb), packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) in whole blood in EDTA bottles were measured using an Hematology Auto-Analyzer (Sysmex 1-5-

1 Wakinohama-Kaigandori Chuo-ku, Kobe 651-0073, Japan). Serum was separated from blood in plain bottles and the concentrations of aspartate transaminase, alanine transaminase, alkaline phosphatase, albumin, total protein, urea and creatinine were assayed using a Clinical Chemistry Auto-Analyzer (Sylectra Pro S, Model: 13-9693, Netherland). Liver and kidney were also removed and weighed and organ-to-body weight ratio for each organ was determined.

Statistical Analysis: Data obtained are presented as Mean ± Standard Error of Mean. Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnette's Multiple Test for pair wise comparisons using GraphPad Prism 5 software. Probability values < 0.05 were considered significant.

RESULTS:

Phytochemical Analysis: Phytochemical analysis of extract showed the presence of carbohydrates, alkaloids, flavonoids, tannins, terpenoids and saponnins, while arthraquinones and cardiac glycosides were absent. Quantitatively, flavonoids were most while steroids were least present in extract **Table 1**.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF ETHANOL EXTRACT OF *GARCINA KOLA* SEEDS

Phytochemical constituent	Observation
Alkaloids	++
Terpenoids	++
Saponins	++
Tannins	++
Flavonoids	+++
Steroids	+
Anthraquinones	-
Cardiac glycosides	-
Carbohydrates	++

- Absent
+ Slightly present
++ Moderately present
+++ Abundantly present

Hepatorenal Blood Indices: Serum levels of total protein, alanine transaminase (ALT), aspartate transaminase (AST) and albumin were not affected in all treated rats compared to control, but alkaline phosphatase (ALP) was reduced (P = 0.02) in rats that received 300 mg/kg (the highest dose) of the extract **Table 2**.

In addition, serum urea and creatinine levels in extract treated rats were lower but not significant (P > 0.05) when compared to control group **Table 3**.

TABLE 2: SERUM LEVELS OF HEPATIC INDICES IN WISTAR RATS AFTER SUBACUTE ADMINISTRATION OF ETHANOL *GARCINIA KOLA* SEED EXTRACT

Dose (mg/kg)	ALT (U/L)	AST (U/L)	ALP (U/L)	Total protein (g/L)	Albumin (g/dl)
Control	110.01 ± 5.19	21.28 ± 2.90	74.79 ± 1.67	10.34 ± 6.13	20.99 ± 5.44
50	114.08 ± 6.66	26.88 ± 1.67	77.05 ± 3.67	8.22 ± 2.74	16.48 ± 5.29
100	95.90 ± 7.18	24.22 ± 3.35	68.28 ± 0.80	6.29 ± 2.17	13.82 ± 3.89
200	121.68 ± 10.56	18.97 ± 4.53	70.93 ± 3.57	6.97 ± 3.76	26.93 ± 2.97
300	130.32 ± 8.93	15.48 ± 2.45	61.80 ± 1.31*	7.37 ± 2.49	17.80 ± 4.25

Values are expressed as Mean ± Standard Error of Mean (SEM), n = 6 rats per group.
ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, * Significant at P < 0.05

TABLE 3: SUBACUTE ORAL TREATMENT OF ETHANOL *GARCINIA KOLA* SEED EXTRACT DOES NOT ALTER SERUM LEVELS OF UREA AND CREATININE IN WISTAR RATS

Dose (mg/kg)	Urea (mmol/l)	Creatinine (µmol/l)
Control	6.67 ± 1.63	100.77 ± 11.03
50	4.04 ± 0.43	77.41 ± 10.48
100	5.77 ± 1.59	70.35 ± 8.98
200	3.85 ± 0.44	76.15 ± 10.75
300	3.98 ± 0.41	79.56 ± 10.86

Values are expressed as Mean ± Standard Error of Mean (SEM), n = 6 rats per group.

Haematological Indices: There was non significant reduction (P = 0.5196) of RBC count in extract treated rats compared with control **Table 4**. There was no significant change in Hb concentration (P = 0.4111) or PCV (P = 0.5274) in all rats that were administered extract compared to control **Table 4**. Mean cell haemoglobin concentration (MCHC), mean cell volume (MCV), and mean cell haemoglobin (MCH) in treated rats were equally not altered when compared to control **Table 4**.

In addition, WBC count was reduced (P = 0.0002) dose-dependently in extract administered rats; lymphocytes were increased (P < 0.05), while neutrophils were reduced when compared with control **Table 5**. Platelet count was elevated in extract treated rats in a dose-dependent fashion, but only the count in rats that received the highest dose (300 mg/kg) showed significance (P < 0.0004) when compared with control rats **Table 5**.

TABLE 4: EFFECT OF SUBACUTE ORAL ADMINISTRATION OF ETHANOL *GARCINIA KOLA* SEED EXTRACT ON RED BLOOD CELL (RBC) COUNT AND IT'S MARKERS IN WISTAR RATS

Dose (mg/kg)	RBC (x10^{12}/L)	Hb (g/dL)	PCV (%)	MCHC (%)	MCV (%)	MCH (%)
Control	8.16 ± 0.25	16.60 ± 0.49	49.60 ± 1.63	33.62 ± 0.29	60.52 ± 0.15	20.35 ± 0.18
50	7.18 ± 0.62	14.60 ± 0.80	45.00 ± 2.70	32.53 ± 0.78	63.38 ± 2.19	20.64 ± 0.98
100	6.80 ± 0.59	14.52 ± 0.90	43.60 ± 2.69	33.44 ± 0.13	64.98 ± 2.73	21.63 ± 0.90
200	7.04 ± 0.99	14.34 ± 1.70	43.00 ± 5.10	33.34 ± 0.03	62.70 ± 3.11	20.91 ± 1.04
300	7.83 ± 0.12	16.03 ± 0.24	48.00 ± 0.71	33.38 ± 0.02	61.36 ± 0.71	20.36 ± 0.21

Values are expressed as Mean ± Standard Error of Mean (SEM), n = 6 rats per group.

TABLE 5: EFFECT OF SUBACUTE ORAL ADMINISTRATION OF ETHANOL *GARCINIA KOLA* SEED EXTRACT ON PLATELET AND WHITE BLOOD CELL (WBC) COUNTS IN WISTAR RATS

Dose (mg/kg)	Platelet (x10^9/L)	WBC (x 10^9/L)	Neutrophil (%)	Lymphocyte (%)
Control	232.00 ± 12.41	12.40 ± 1.29	34.40 ± 3.14	65.60 ± 3.14
50	240.00 ± 12.25	8.08 ± 1.19*	27.60 ± 2.50	59.00 ± 2.49
100	246.00 ± 13.64	6.60 ± 0.67**	41.00 ± 2.49	70.60 ± 2.80
200	262.00 ± 13.19	6.80 ± 0.98**	24.40 ± 2.80*	75.60 ± 2.80*
300	334.00 ± 12.08*	4.20 ± 0.51***	22.50 ± 1.04**	77.50 ± 1.04**

Values are expressed as Mean ± Standard Error of Mean (SEM), n = 6 rats per group.
* Significant value at P < 0.05; **Significant at P < 0.01; ***Significant value at P < 0.001

Liver and Kidney Weights: Relative weight of liver and kidney (organ-to-body weight ratio) of extract administered rats were not changed when compared with control weights **Table 6**.

TABLE 6: SUBACUTE ORAL TREATMENT OF ETHANOL *GARCINIA KOLA* SEED EXTRACT DOES NOT AFFECT LIVER AND KIDNEY WEIGHTS OF WISTAR RATS

Dose (mg/kg)	Liver		Kidney	
	Organ weight (g)	Organ-to-body weight ratio (x 10^2)	Organ weight (g)	Organ-to-body weight ratio (x 10^2)
Control	8.34 ± 1.19	2.81 ± 10.17	0.92 ± 0.40	0.13 ± 2.22
50	8.05 ± 0.26	2.18 ± 2.01	0.65 ± 0.10	0.22 ± 0.15
100	8.30 ± 1.03	3.22 ± 15.21	0.68 ± 0.11	0.23 ± 1.24
200	6.51 ± 1.02	3.20 ± 29.14	0.64 ± 0.12	0.33 ± 2.92
300	4.29 ± 0.13	2.41 ± 2.18	0.70 ± 0.11	0.34 ± 0.19

Values are expressed as Mean ± Standard Error of Mean (SEM), n = 6 rats per group.

DISCUSSION: Serum levels of alanine transaminase (ALT) and aspartate transaminase (AST) are commonly measured clinically as biomarkers of liver function [13 - 15]. ALT is present mostly in liver and to a lesser extent plasma and body tissues, whereas AST is found in a number of organs including liver, heart, skeletal muscle, kidneys, brain and red blood cells [16]. Elevation in serum ALT and AST levels is suggestive of hepatocellular injury [16].

From the results, extract treatment did not affect AST and ALT levels. Furthermore, the extract at the highest concentration reduced ALP level (which is also an indicator of liver function) but lower doses had no effect on the enzyme level. Similar results of reduction in ALP have been reported in rabbits but effect was produced by higher dose (500 mg/kg) of ethanol extract [17]. Albumin is a plasma protein primarily produced by liver cells and reduction in blood level of albumin is indicative of liver dysfunction [16]. It was observed that both albumin and total proteins in serum were not altered by extract treatment.

The above results of the present study thus suggest that ethanol extract of *Garcinia kola* seed is not harmful to the liver over the dose range and duration used and doses higher than 300 mg/kg may have hepatoprotective activity. Additionally,

extract did not affect liver-to-body weight ratio, and this adds more evidence that *Garcinia kola* does not affect liver function [18 - 20]. Furthermore, serum levels of urea and creatinine (standard indices of renal function) were not changed after extract treatment. Kidney-to-body weight ratio was equally not altered by extract suggesting that the plant would not be harmful to the kidney [18 - 20].

Hematopoietic system consists of blood, the constantly circulating fluid that serves the body with supply of nutrients and oxygen as well as removal of wastes. It also provides immunological functions, including circulation of white blood cells and detection and elimination of foreign materials. Changes in blood indices have been linked to exposure to harmful chemicals or alteration in certain physiological factors like cellular integrity and membrane permeability of cells [21].

In this study, *Garcinia kola* treatment had no effect on haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) count and its indices, including, mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC). This is in disagreement with the studies of Ahumibe and Braide [22] and Uko *et al*., [23]. Ahumibe and Braide [22] reported elevation of RBC, PCV and Hb after treatment with *Garcinia kola*, but at much higher doses (300 - 1200 mg/kg). Whereas, *Garcinia kola* was reported to cause reduction in Hb, PCV and erythrocyte count in young growing rats [23].

However, our finding was similar to the results of Dada and Ikuerowo [24] who demonstrated that *Garcinia kola* failed to cause change in erythrocyte count and PCV over a dose range of 250 - 2000 mg/kg. In addition, the extract in our study increased platelet count, which was consistent with the findings of Atsukwei *et al*., [25], but in disagreement with Ahumibe *et al*., [22] where platelet count was reported to be unaffected by extract treatment.

Furthermore, the extract did not cause any change in total white blood cell count after treatment, but increased lymphocyte level, while neutrophil was decreased at the high doses levels of 200 - 300 mg/kg. Increase in number of platelets by the extract from our result indicates that the plant may enhance blood clothing activity in animals.

On the other hand, lymphocytes play essential roles in immunological response and their elevation in this study suggests that the plant may enhance immune function. This justifies its use as an immune booster.

The phytochemical analysis of the extract revealed the presence of carbohydrates, alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, while cardiac glycosides and arthraquinones were absent. These phytochemicals may play vital role in the results that were obtained in this study.

CONCLUSION: Subacute treatment of ethanol *Garcinia kola* seed extract causes no effect on circulating markers of kidney and liver, and may have hepato-protective effect. In addition, the plant produces no effect on red blood cell count but increases lymphocyte and platelet levels, and may therefore possess blood clotting and immune boosting properties.

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CONFLICT OF INTEREST: Nil

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