Effects of Hydromethanol Extracts of *Garcinia Kola* on Some Biochemical Parameters of Male Wistar Rats

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Abstract: **Garcinia Kola is commonly used in traditional medicine for the treatment of diverse ailments including coronary artery diseases. Thus, this study aims to determine the effect of hydromethanol (1:4) extracts of the pulp and seed coat of Garcinia kola on serum lipid profile and its antioxidant properties. The two forms were separately dried and blended to powder. Forty male wistar rats (8 per group) were assigned into Five (5) groups. Groups were treated thus: Group one; control. Group two; 100mg/kg pulp extract. Group three; 200mg/kg pulp extract. Group four; 100mg/kg seed coat extract. Group five; 200mg/kg seed coat extract; for 30 and 60 days duration. On treatment conclusion, blood was collected for the determination of lipid profile and antioxidant properties. The higher dose of the pulp and seed coat extracts significantly (P<0.05) increased the catalase level and superoxide dismutase enzyme activity, whereas, both the higher and lower doses of the seed coat extract caused a reduction in malondialdehyde level. The serum total cholesterol was significantly elevated by the higher dose of the pulp extract while the seed coat extract caused significantly increased high density lipoprotein cholesterol level and a reduction in the low density lipoprotein level. The two extracts demonstrated marked antioxidant effects. The seed coat of Garcinia kola may possess the potential to prevent cell death due to lipid peroxidation by inhibiting the lipid peroxidation process. The seed coat extract may also be useful in preventing coronary artery disease and other atherosclerotic problems.**

Keywords: **Garcinia kola, hydromethanol, wistar rats, antioxidant**.

I. INTRODUCTION

Globally, there is increasing dependence on medicinal plants for the prevention and treatment of various illnesses [1]. Estimates, suggest that, a large number of the population of many developing countries heavily rely on medicinal plants and the services of traditional medicine practitioners to meet basic health care needs. It has been reported that 80% of the population in Africa depend on medicinal plants for primary health care [1][2][3]. The global importance attached to the use of medicinal plants is derived from its affordability, minimal side effects, and accessibility compared to modern medicines [1][2][3].

Specific compounds found in most medicinal plants are effective in the treatment and prevention of diseases. These compounds which are the active components are frequently extracted and used as raw materials in the synthesis of different drugs [5]. Also, medicinal plants are being studied to find the scientific basis of their numerously acclaimed therapeutic actions [1] [6]. *Garcinia kola* (GK) is a medicinal plant that is mainly cultivated and distributed in West and Central Africa [7]. The seeds of GK are smooth and elliptical in shape and consist of a yellow pulp and brown coat [8]. The application of the pulp in folklore remedies is a deep rooted practice in Nigeria and the African sub region and includes the use in treatment of liver disorders, hepatitis, jaundice [3] and diarrhea [11]. Although, it is difficult to find any scientific study on the physiological effects of the seed coat; studies carried out on the pulp showed that the pulp of GK has aphrodisiac properties [7][10] and also caused significant increase in the level of testosterone and sperm count [7]. There are paucity of reports on the antioxidant and lipid lowering effects of GK seed.

Antioxidants are molecules that possess the ability to delay or prevent an oxidative reaction [11], often catalyzed by free radicals. Antioxidants play important roles aimed to terminate chain reactions characterizing lipid perodixation, by removing the free radical intermediates, and inhibiting other oxidation reactions [12]. The body's antioxidant mechanisms may not sufficiently neutralize all the free radical, thereby, increasing the need for dietary intake of antioxidants to boost the antioxidant system in the maintenance of health and prevention of diseases. This study was carried out with the objective to investigate the testicular antioxidant and serum lipid effects of the pulp and seed coat of *Garcinia kola*.

II. MATERIALS AND METHODS

A. Preparation of Plant extract

The seeds of GK were procured in a local market and authenticated by the taxonomist in the Department of Plant Science and Biotechnology, University of Port Harcourt,

Nigeria. Voucher specimen was also deposited at the Herbarium of the department. The outer coat of the GK seeds was peeled off from the pulp. The seed coat and the pulp were seperately dried and blended to fine powder. The extraction was carried out with hydromethanol (1:4) as solvent at 60 – 70^{oC} using the soxhlet apparatus. The solution was filtered after 24 hours and the filtrate concentrated under reduced pressure of 60^{oC} to a semi solid form using the rotary evaporator. The net yield was weighed and the extract preserved in a refrigerator at 4^{oC}. Serial dilutions of the extracts were made to obtain concentration of 100mg/ml and 200mg/ml of solution for animal oral treatment. In addition, phytochemical screening tests were carried out on the hydromethanol extracts of the pulp and seed coat of GK in the laboratory of the Department of Pharmacognosy and Phytotherapy, Faculty of Pharmacy, University of Port Harcourt, Nigeria. The screening tests were done in accordance with established procedures Trease and Evans [13][14][15]. Intensity of colour change indicated the presence of various phytochemical constituents.

B. Animal Models

This study was approved by the ethics committee of the College of Health Sciences, University of Port Harcourt, Nigeria. Adult male rats of the wistar strain were randomly selected from the animal house of the Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria. They were kept in standard cages and allowed two weeks to acclimatize to the new environment. While they were maintained in relatively constant environmental conditions with proper ventilation and 12 hour light and 12 hour dark cycle, they were also given free access to food and water. Generally, the procedures conformed to the established principles for the care and use of laboratory animals published by the National Institute of Health, USA [16].

C. Experimental design

This study was designed to investigate the antioxidant and lipid effects of the pulp and seed coat extracts of GK. Male wistar rats were divided into five (5) groups of eight rats each. Group one (1) served as control received distilled water. Groups two (2) and three (3) were treated with 100mg/kg b.w and 200mg/kg b.w of the hydromethanol extract of the pulp respectively. Groups four (4) and five (5) were treated with 100mg/kg b.w and 200mg/kg b.w of the hydromethanol extract of the seed coat respectively. The extracts were administered orally as single daily doses throughout the period of the experiment using appropriate animal feeding tube. Administration lasted for sixty (60) days. The rats were sacrificed under chloroform anaesthesia on day 61after 24hours of last administered dose. Blood was collected by cardiac puncture into appropriate sample bottles for estimation of the serum lipid profile [Total cholesterol, High density lipoprotein-cholesterol (HDL-c), Low density lipoprotein-cholesterol (LDL-c), and Triglyceride].

The testis was excised, the superficial fatty layer was cleaned off and the organ was transferred into 0.25M sucrose solution. After blotting with tissue paper, it was cut very thinly with sterile scapel blade and again, homogenized in ice-cold 0.25M (mol/L) sucrose solution (mass-to-volume ratio of 1:5). The homogenates was centrifuged for 10 minutes at 800r/minute at 4°C to obtain the supernatant. The supernatant was then carefully aspirated with Pasteur pipette into sample bottle and stored frozen at -20°C until used for biochemical assays.

D. Determination of testicular Antioxidant activity.

Using wavelengths of 525 nm for excitation and 547nm for emission, the malondialdehyde (MDA) produced reacts with the chromogenic reagent, 2-thiobarbituric acid (TBA), to yield a pink coloured complex. The concentration of MDA was calculated using the molar extinction coefficient of the chromospheres [17].

The determination of catalase activity was based on the fact that catalase in the sample preparation split hydrogen peroxide (H_2O_2) which can then be measured spectrophotometrically at 240nm. One unit of catalase enzyme activity equals the amount of protein that converts 1µmol H_2O_2 utilized per minute [18].The measurement of superoxide enzyme activity was done spectrophotometrically.The superoxide anion reduction of nitrobluetetrazolium (NBT) to blue formazan was determined at 560nm.The ability of superoxide enzyme to cause a 50% inhibition in Nitrobluetetrazolium (NBT) reduction refers to one unit of enzyme activity [19].

The gluthathione (GSH) was measured based on its ability to react with 5,5'-dithio-bis (2-nitrobenzoic acid) (DNTB, Ellman's reagent) to produce the conjugate GS-TNB as well as the yellow TNB (5'-thio- 2- nitrobenzoic acid) detected at 412nm. The rate of TNB production was proportional to the GSH concentration in the extract [20][21].

E. Statistical analysis

Results are presented in tables and expressed as means ± standard error of mean. Significant differences were determined using the one-way ANOVA. A p value of less than 0.05 was considered

Statistically significant

III. RESULTS

A. Result of Phytochemical screening of hydro-methanol (1:4) extracts of the pulp and seed coat of Garcinia kola

Table 1 shows the result of the qualitative Phytochemical screening of hydro-methanol (1:4) extracts of the pulp and seed coat of *Garcinia kola*. Phytochemical screening indicated the presence of various bioactive constituents, such as alkaloids, triterpenoids/steroids and saponins in the seed coat but absent in the pulp. Anthraquinone and cyanogenic glycosides are absent in both

extracts. However, flavonoids, tannins, carbohydrates and cardenolides are present in both extracts.

B. Effects of hydromethanol extract of garcinia kola on some liver enzyme parameters

There was a significant ($p<0.05$) increase in the activity of superoxide dismutase (Table 2) and catalase (Table 3) enzymes for the groups treated with the higher doses (200mg/kg) of the pulp and seed coat extract respectively, compared to control. A significant ($p<0.05$) reduction in the level of Malondialdehyde (MDA) (Table 2) was observed in the groups treated with the seed coat of *Garcinia kola*, compared to control. The lower dose (100mg/kg) and higher dose (200mg/kg) of the seed coat of *Garcinia kola* caused a 78.79% and 77.27% reductions in MDA level, respectively.

C. Effects of hydromethanol extract of garcinia kola on serum lipid profile

Table 4 shows the result of garcinia kola administration on serum lipid profile. There was a significant ($p<0.05$) increase in the level of serum total cholesterol, and a marginal non-significant decrease in serum LDL-c concentration as well as a non significant increase in serum HDL-c concentration, for the group treated with higher dose of the pulp extract compared to control. There was also a significant ($p<0.05$) elevation of the serum HDL-c concentration in the groups treated with lower dose (100mg/kg) and higher dose (200mg/kg) of the seed coat extract whereas, a significant reduction in the serum LDL-c concentration was observed with the higher dose of the seed coat extract; suggesting that the seed coat of *Garcinia kola* may be useful in reducing the risk of lipid associated cardiovascular events.

IV. DISCUSSION

Phytochemical screening of hydro-methanol (1:4) extracts of the pulp and seed coat of *Garcinia kola* indicated the presence of various bioactive constituents. Some of these constituents such as alkaloids, triterpenoids/steroids and saponins are present in the seed coat but absent in the pulp. The anthraquinone and cyanogenic glycosides are absent in both extracts. The flavonoids, tannins, carbohydrates and cardenolides are present in both extracts. The bioactive constituents found in plant extracts are reportedly responsible for the biological actions of the plant. Specific compounds found in most medicinal plants which possess antioxidant properties may also be effective in the treatment and prevention of diseases. The antioxidants have positive influence on testicular function and are generally known to protect spermatogenesis especially in animals exposed to reproductive toxicants [22]. The present study showed that the pulp and seed coat extract of *Garcinia kola* possess antioxidant property. This assertion is based on the result obtained following administration of these extracts. There was a significant ($p<0.05$) increase in the activity of superoxide dismutase (Table 2) and catalase (Table 3) enzymes for the groups treated with the higher dose (200mg/kg) of the pulp and seed coat extract respectively. This can be observed in the result when the value of enzyme activity obtained in the test group is compared to control. The increase in superoxide and catalase enzyme activities implies that both extracts of *Garcinia kola* are capable of boosting the antioxidant production in the experimental animals.

A significant ($p<0.05$) reduction in the level of Malondialdehyde (MDA) (Table 2) was observed in the groups treated with the seed coat of *Garcinia kola*, when the test groups were compared to control. The lower dose (100mg/kg) and higher dose (200mg/kg) of the seed coat of *Garcinia kola* caused a 78.79% and 77.27% reductions in MDA level, respectively. MDA is a product of lipid peroxidation. Extensive lipid peroxidation would lead to a decrease in membrane fluidity and cell death. This may occur as a result of the peroxidation of unsaturated fatty acids and alteration of the ratio of polyunsaturated to other fatty acids [23].

The antioxidant effect of many plants has been attributed to the presence of certain phenolic components such as flavonoids [24][25], phenolic diterpenes and phenolic acids [26]. Phenols, flavonoids and tannins are good antioxidants useful in prevention or control of oxidative stress and related disorders [27][28]. Some of these components with antioxidant effects were discovered in the two extracts (pulp and seed coat) of *Garcinia kola* and may be responsible for the antioxidant actions of these extracts.

The findings in this study are collaborated by those reported in another study [29], which stated that the seeds of *Garcinia kola* exhibited antilipoperoxidative effect. The ability of *Garcinia kola* to inhibit lipid peroxidation may also have prevented a buildup of free radicals which has the tendency to initiate further peroxidation and damage to cell.

Lipid profile or lipid panel serves as an initial broad medical screening tool for lipid abnormalities; whose result may be useful in identifying some genetic diseases and to determine the approximate risks for cardiovascular diseases. It is usually considered in the evaluation of dyslipidemia, but emphasis is placed on LDL-c which has been referred to as “bad lipoprotein” [30]. There is overwhelming evidence showing that an elevated LDL-c concentration is atherogenic whereas, a high HDL-c is cardioprotective [31][32][33].

The lipid panel was done to investigate the effects of the pulp and seed coat extracts of *Garcinia kola* on serum lipid parameters. In this study, there was a significant ($p<0.05$) increase in the level of serum total cholesterol (Table 4), and a marginal non-significant decrease in serum LDL-c concentration as well as a non significant increase in serum HDL-c concentration, for the group treated with higher dose of the pulp extract when compared to control. There was also a significant ($p<0.05$) elevation of the serum HDL-c concentration (Table 4) in the groups treated with lower dose (100mg/kg) and higher dose (200mg/kg) of the seed coat extract whereas, a significant reduction in the serum LDL-c

concentration was observed with the higher dose of the seed coat extract (Table 4); suggesting that the seed coat of *Garcinia kola* may be useful in reducing the risk of lipid associated cardiovascular events. The triglyderide level was not significantly altered by both extracts.

It has been demonstrated in various studies that even when the conventional lipid parameters appear to be apparently normal, lipid ratios such as the Castelli's risk index-I (CRI-I), Castelli's risk index-II (CRI-II), and others may be used as an alternative diagnostic tool in predicting the risk of developing cardiovascular disease [34][35][36].

V. CONCLUSION

The pulp and seed coat extracts of *Garcinia kola* has the potential to prevent cell death due to lipid peroxidation by inhibiting the lipid peroxidation process.The seed coat extract reduced serum LDL-c and elevated serum HDL-c concentration. The phytochemical compounds present in the pulp and seed coat of *Garcinia kola* may be responsible for the pharmacological effects observed in the plant extracts.

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LEGEND TO TABLES

Table 1: shows the qualitative phytochemical screening of pulp and seed coat of *Garcinia kola.*

Table 2: shows the Mean level of Gluthathione and Superoxide dismutase activity.

Table 3: shows the Mean level of Malondialdehyde and Catalase activity

Table 4: shows the Effect of pulp and seed coat extracts on the serum lipid profile.

Table 1

	Phytochemical compounds	Pulp	Seed coat
1	Alkaloids	-	+
2	Tannins	+	+
3	Flavonoids	+	+
4	Anthraquinone	-	-
5	Triterpenoids/steroids	-	+
6	Cardenolides	+	+
7	Carbohydrates	+	+
8	Cyanogenic glycosides	-	-
9	Saponins	-	+

Table 2

Groups/ Extracts (mg/kg)	Testicular antioxidants			
	Gluthathione (µg/min/mg.protein)	% Change	SOD (Ug/mg.protein)	% Change
Control	0.14±0.02	0	0.35±0.05	0
Pulp				
100	0.16±0.02	14.29	0.38±0.05	8.57
200	0.17±0.01	21.43	0.61±0.12*	74.29
Seed coat				
100	0.16±0.02	14.29	0.51±0.08	45.71
200	0.17±0.02	21.43	0.70±0.11*	100

Values expressed as Mean ± SEM. n=8. Significant at [*(P<0.05)] when compared to control group

Table 3

Groups/ Extracts (mg/kg)	Testicular antioxidants			
	MDA (Umol/mg.protein)	% Change	Catalase (Units/mg.protein)	% Change
Control	0.66±0.24	0	22.45±0.66	0
Pulp				
100	0.47±0.19	-13.85	22.71±0.79	1.16
200	0.57±0.20	-43.08	24.51±0.65*	9.18
Seed coat				
100	0.14±0.01*	-72.31	24.43±0.89	8.82
200	0.15±0.01*	-73.85	25.30±0.55*	12.69

Values expressed as Mean ± SEM. n=8. Significant at [*(P<0.05)] when compared to control group

Table 4:

Groups/ Extracts (mg/kg)	Serum lipid profile (mg/dl)			
	Total cholesterol	Triglyceride	LDL-c	HDL-c
Control	2.31±0.12	0.67±0.05	1.01±0.13	2.05±0.06
Pulp				
100	2.39±0.11	0.64±0.06	0.84±0.17	2.16±0.08
200	2.71±0.12*	0.70±0.04	0.66±0.17	2.20±0.10
Seed coat				
100	2.38±0.14	0.67±0.03	0.69±0.16	2.35±0.14*
200	2.37±0.13	0.72±0.04	0.29±0.05*	2.34±0.15*

Values expressed as Mean ± SEM. n=8. Significant at [*(P<0.05)] when compared to control group.