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Assessment of the effect of ethanol extract of *Garcinia kola* seed on lipid profile in doxorubicin-induced cardiotoxicity in Wistar rats

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Abstract
The effect of ethanol extract of *Garcinia kola* seed on lipid profile in doxorubicin-induced cardiotoxicity in wistar rats was investigated in this study. Cardiotoxicity was induced by the administration of doxorubicin (DOX) to male Wistar rats in six equal injections intraperitoneally, over two weeks (cumulative dose, 15 mg/Kg body weight). The protective effect against doxorubicin-induced oxidative injury was studied by oral administration of *Garcinia kola* seed (GK) at 250 mg/kg body weight and 500 mg/kg b.wt respectively in 14 equal doses over four weeks (2 weeks before and 2 weeks concurrently with doxorubicin). At the end of the treatment, serum lipid profile was assayed. Results obtained from the study showed that treatment with doxorubicin significantly (p<0.05) increased serum cholesterol compared to control. There was no significant difference among rats in NC, DOX+GK 500, and *G. kola* groups, while DOX+*G. Kola* 500 mg, *G. Kola* and DOX groups showed no significant differences among them. Doxorubicin treatment resulted in the elevation of Total non-HDL-cholesterol (50.8±12.09mg/dl) which was significantly (p<0.05) higher compared to normal control (32.35±2.78mg/dl) and 27.5±3.65, 42.81±6.09, 40.38±5.62, 34.13±3.59 mg/dl in *G. kola* 500 mg, DOX + *G. kola* 250 mg, DOX + *G. kola* 500 mg and DOX + Simvastatin (SIM) treated groups respectively. Also, there was the elevation of LDL/HDL ratio to 0.62±0.29 which was significantly higher than the Normal control group (0.39±1.4). Treatment with *G. kola* 500 mg, DOX + *G. kola* 250 mg, DOX + *G. kola* 500 mg, and DOX+SIM resulted in a significant reduction of LDL/HDL ratio to 0.19±0.04, 0.36±0.13, 0.54±0.24, 0.24±0.06 respectively in the exposed animals respectively. Administration of the ethanol extract of *Garcinia kola* seed before and concurrently with doxorubicin, resulted in significant lipid-lowering properties; indicating a cardioprotective effect against doxorubicin-induced cardiotoxicity.

Keywords: Cardiovascular disease, cardiotoxicity, doxorubicin (DOX), *Garcinia kola*

Introduction
Coronary heart disease continues to represent a primary cause of mortality across the United States, Europe, and substantial regions of Asia, despite advances in lifestyle modifications and the widespread use of lipid-lowering therapies (Zhao, 2021 [31]; Wang *et al*., 2025) [30]. Developing countries are increasingly affected as well, largely due to the growing influence of Western dietary patterns and cultural practices. A well-established principle in nutritional science suggests that individuals whose diets are rich in fruits and vegetables exhibit superior health outcomes compared to those with lower consumption. (Vigar *et al*., 2019 [27]; Wallace *et al*., 2019 [29]; *Arias et al*., 2022) [5]. Consequently, considerable research efforts have been directed toward identifying the bioactive constituents within these plant-based foods that may confer such health benefits. In this context, plant-derived compounds have emerged as a significant focus of scientific investigation, owing to their diverse pharmacological and therapeutic potential. (Nasim *et al*., 2022 [22]; Chaachouay & Zidane, 2024) [12].
Medicinal plants, in particular, represent the most abundant natural source for a wide range of therapeutic agents, including those used in traditional medicine systems, contemporary pharmaceuticals, nutraceuticals, dietary supplements, folk remedies, pharmaceutical intermediates, and as precursors for the synthesis of chemical drugs (Nasim *et al*., 2022 [22];

Chaachouay & Zidane, 2024) [12]. This can be attributed to the presence of secondary metabolites which accumulate in the various parts of these plants conferring on them their pharmacological relevance; generally some of these plants especially the edible ones are eaten.

Doxorubicin (DOX) is a potent chemotherapeutic agent broadly used to treat a variety of malignacies, such as leukemias, breast and ovarian cancer, and Hodgkin and non-Hodgkin lymphoma. The major limitation of its clinical use is the development of cardiotoxicity.

The negative outcomes associated with doxorubicin are dose-dependent and range from temporary electrocardiographic changes to severe outcomes such as cardiomyopathy and congestive heart failure (Engwall *et al*., 2021 [15]; Goje *et al*., 2025) [19]. Emerging evidence indicates that the cardiotoxicity induced by doxorubicin is primarily mediated through the generation of reactive oxygen species (ROS), especially superoxide anion and hydrogen peroxide (Li *et al*., 2024; Bhutani *et al*., 2025) [9]. The main cellular damage caused by ROS includes lipid peroxidation, protein cross-linking, and DNA fragmentation (Wallace *et al*., 2020 [28]; Li *et al*., 2024; Bhutani *et al*., 2025) [9]. These effects can result in cardiac dysfunction through mechanisms such as doxorubicin-induced mitochondrial oxidative stress, impairment of mitochondrial oxidative phosphorylation, and increased mitochondrial membrane permeability. Collectively, these disturbances contribute to dysregulation of metabolic and redox homeostasis in cardiomyocytes, ultimately leading to impaired autophagy and mitophagy fluxes and elevated apoptotic activity (Wallace *et al*., 2020) [28].

Cardiovascular disease (CVD) is closely associated with prolonged unhealthy dietary patterns and lifestyle behaviours, which are major contributing factors to the onset of diabetes and obesity—both of which markedly elevate the risk of developing CVD (Sharifi-Rad *et al*., 2020) [25]. CVD may begin with the oxidation of low density lipoprotein (LDL) cholesterol, which leads to atherogenesis. If an artery becomes clogged with plaque, the result can be a heart attack or a stroke. Much of the damage that occurs in both heart attack and stroke is caused by reperfusion injury, with a burst of superoxide free radicals that flow in as the blood flow resumes. There is no particular type of therapy that can be said to possess a complete treatment for cardiac diseases.

As a result, considerable attention has been directed toward edible plants rich in antioxidants and bioactive phytochemicals for their potential as therapeutic agents in the prevention and management of various diseases. Garcinia kola, commonly referred to as “bitter kola” in Nigeria, is a member of the tropical plant family *Guttiferae*. It is also known in English as “False kola,” “Garcinia,” or “Male kola,” and is called *Agba-ilu* or *aki-ilu* by the Igbo people of southeastern Nigeria. The plant is predominantly found in tropical rainforest regions and is commonly cultivated as a multipurpose tree crop in home gardens across southern Nigeria (Nzegbule and Mbakwe, 2001) [23]. Garcinia kola grows as a medium-sized tree, typically reaching a height of 12-14 meters, and bears reddish, yellowish, or orange-colored fruits (Okwu, 2005 [24]; Adesanya *et al*., 2007) [2]. Each fruit contains two to four yellow seeds embedded in a sour-tasting pulp. When chewed, the seeds have a distinctly bitter and astringent flavor. It is highly esteemed in Nigeria for its edible nut, which are widely utilized in traditional medicine and as a dietary component. *Garcinia kola* is recognized for its wide range of pharmacological properties, including antioxidant, antibacterial, antifungal, antiviral, and anti-inflammatory activities (Adegboye *et al*., 2008) [1]. This research is designed to explore the effect of ethanol extract of *Garcinia kola* seed on lipid profile in doxorubicin-induced cardiotoxicity in albino rats.

Materials and Methods

Plant material

Plant specimens were collected from a forest location in Ihiagwa, Owerri West Local Government Area, Imo State, Nigeria. Taxonomic identification and authentication were carried out by Dr. B.N. Uwalaka of the Department of Biology, University of Agriculture and Environmental Sciences, Umuagwo. The plant was assigned voucher no: UAES/HB/0042 and a voucher specimen was deposited in the departmental herbarium for future reference. Upon collection, the samples were cleaned to eliminate foreign matter. The brown seed coats were manually removed, and the seeds were subsequently dried at ambient room temperature for one week until a constant weight was achieved. The dried material was then pulverized into a fine powder using an electric milling machine.

Fig 1: Seeds *of Garcinia kola* ()

Experimental animals

Thirty (30) healthy male albino Wistar rats (Rattus norvegicus), weighing between 80-120 g and approximately five weeks old, were utilized in this study. The animals were procured from the Animal House of the Department of Veterinary Medicine, University of Nigeria, Nsukka. They were housed in stainless steel cages under standard laboratory conditions, including a temperature of 21 ± 2 °C, relative humidity of 55 ± 5%, and a 12-hour light/dark cycle. All animals had free access to standard rat chow (Vital Finisher) and clean tap water. The rats were randomly assigned to six (6) experimental groups and kept in separate cages accordingly.

Preparation of ethanol extracts of *Garcinia kola*

The ground plant material measuring up to 400g was steeped in 2.0 litres of 80% ethanol and incubated for 4 days at room temperature. This was continuously shaken on a rotary shaker for 4 days to ensure uniform extraction. The slurry sediment was removed by coarse filtration using a sieve, and afterwards the first filtrate was re-filtered with Whatman No.1 filter paper. The final filtrate was evaporated at 49 °C under reduced pressure, using a rotary evaporator

to obtain the ethanol extracts of *Garcinia kola*. The resulting concentrated extract was subsequently stored in air tight amber container maintained in a refrigerator at 4ºC until required for analysis.

Qualitative phytochemical screening

The methods described by Trease and Evans (1996), were used to evaluate the qualitative phytochemical content of the *Garcinia kola* seed extract.

Grouping of animals

After fourteen (14) days acclimatisation period, thirty (30) healthy male albino Wistar rats were divided into six groups of five (5) animals each for the determination of cardiotoxicity studies. The animals were randomly assigned to experimental groups and treated as follows:

Group 1: Received food (vital finisher) and water only throughout the treatment period and served as normal control (NC);

Group 2: Received food and water, and doxorubicin (DOX) injected intraperitoneally in six equal doses of (2.5 mg/kg) for period of 2 weeks to achieve total cumulative dose of 15 mg/kg body weight.

Group 3: Received food, water and *Garcinia kola*. Garcinia kola was administered by intubation once every 2days at 500 mg/kg body weight over a period of 4 weeks. It served as plant control (*G. kola*).

Group 4: Received food, water, *Garcinia kola* administered by intubation and doxorubicin injected intraperitoneally in six equal doses of (2.5 mg/kg) for period of 2 weeks to achieve total cumulative dose of 15 mg/kg body weight. Garcinia kola was administered once every 2days at 250 mg/kg body weight, two weeks prior to doxorubicin administration and two weeks simultaneously with doxorubicin. It served as intoxicated test (*G. kola* 250+DOX).

Group 5: Received food, water, *Garcinia kola* by intubation and doxorubicin injected intraperitoneally in six equal doses of (2.5 mg/kg) for period of 2 weeks to achieve total cumulative dose of 15 mg/kg body weight. *G. kola* extract was administered once every 2days at 500 mg/kg body weight, two weeks prior to doxorubicin administration and two weeks simultaneously with doxorubicin. It served as intoxicated test (G.kola 500+DOX).

Group 6: Received food and water, and received Simvastatin and doxorubicin. Doxorubicin (cumulative dose 15mg/kg body weight) was injected intraperitoneally in six equal doses of (2.5 mg/kg) for period of 2 weeks to achieve total cumulative dose of 15 mg/kg body weight. Simvastatin (cumulative dose 60 mg/kg body weight) was administered orally in 12 equal doses (each treatment containing 5mg/kg) over a period of 4 weeks, 2 weeks before DOX administration and 2 weeks alternating with DOX injections. It served as Standard (SIM+DOX).

At the end of the four weeks treatment with *Garcinia kola* and doxorubicin inducement and standards for amelioration, the mortality and general condition of the animals were observed. Body weights were recorded 2 times per week during the treatment and until the end of experiment. Animals were allowed for 24hrs, lightly anaesthetized using dichloroethane and sacrificed; blood sample was collected by cardiac puncture allowed to clot for 45 minutes at room temperature. Serum was separated by centrifugation at 600×*g* for 15minutes and used for the determination of various biochemical parameters.

Biochemical Analysis

Determination of total cholesterol concentration

The enzymatic (cholesterol esterase/oxidase/peroxidase) method of Allain *et al*., (1974) [4] was used for determination of total cholesterol. The reaction generates hydrogen peroxide which is in a coupled reaction with 4-aminoantipyrine to form a coloured complex that is measured spctrophotometrically at 500nm.

Determination of triacylglycerol (TG) concentration

The glycerol phosphate oxidase/peroxidase methods as described by Bucalo and David (1973) [10] and Fossatti and Principe (1982) were used in the analysis.

Principle: Triacylglycerol in the sample originates, by means of a coupled enzymatic reaction to form hydrogen peroxide which reacts with 4-aminoantipyrine and phenol to form a coloured product that can be measured by spectrophotometry at 500nm.

Determination of low-density lipoprotein (LDL) cholesterol concentration

Low density lipoprotein cholesterol in the sample was determined according to the method of Assman *et al*., (1984).

Principle: LDL-cholesterol in the sample precipitates with polyvinyl sulphate. Their concentration is calculated from the difference between the total cholesterol and the cholesterol in the supernatant after centrifugation.

Determination of high density lipoprotein (HDL)-cholesterol concentration.

The serum HDL-cholesterol concentration was determined using the phosphotungstate/Mg-cholesterol oxidase and peroxidase method as described by Grove (1979) [20] and Burstein *et al*. (1980) [11].

Principle: Very low-density lipoprotein (VLDL) and LDL-cholesterol in the sample was precipitated with phosphotungstate and magnesium ions. The supernatant contains HDL; the HDL cholesterol was measured spectrophotometrically.

Total non-HDL-cholesterol and LDL/HDL-cholesterol was calculated as follows:

$$\text{Total non} - \text{HDL} - \text{Cholesterol} = \text{LDL (mg/dl)} + \text{VLDL (mg/dl)}$$

$$\text{LDL/HDL ratio} = \frac{\text{LDL (mg/dl)}}{\text{HDL (mg/dl)}}$$

Results and Discussion

Table 1: Phytochemical compounds present in *Garcinia kola* seed crude extract

Qualitative phytochemical screening of *G. kola* seed showed that the phytochemical compounds present are steroids, cardiac glycosides, flavonoids, tannins, saponins, and

reducing sugar while alkaloids were found to be absent in *G. kola* seed extract.

Table 1: Phytochemical compounds present in *Garcinia kola* seed crude extract.

Phytochemical compound	Result
Alkaloids	_
Steroids	+
Cardiac glycosides	+
Flavonoids	+
Tannins	+
Saponins	+
Reducing sugar	+

(+) represents presence of the phytochemical, (-) represents absence of the phytochemical,

Figure 2: Effect of ethanol extract of Garcinia kola seed administration on serum total Cholesterol, HDL-Cholesterol, LDL-Cholesterol concentration and LDL/HDL-Cholesterol ratio of male Wistar albino rats administered 2.5mg/kgbwt doxorubicin for 14 days.

The findings revealed that following doxorubicin administration the rats in DOX groups exhibited significant (p<0.05) increase in serum cholesterol compared to control (Figure 2A). Cholesterol concentration was 74.77±6.36, 97.21±10.04, 90.56±11.13, 110.53±6.60, 82.51±8.28, 112.69±3.92 mg/dl in NC, DOX group, *G. kola* 500 mg, DOX+*G. kola* 250 mg, DOX+*G. kola* 500 mg and DOX+SIM groups respectively. DOX+*G. kola* 250 mg and DOX+SIM treatment resulted in further increase of cholesterol concentration compared to the DOX group. There was no significant difference among rats in NC, DOX+ *G. kola* 500 and *G. kola* groups, while DOX+*G. kola* 500 mg, *G. Kola* and DOX groups showed no significant differences among them.

The results (Figure 2B) obtained after the exposure of the treatment groups to doxorubicin, showed that HDL concentration was 42.42±7.41, 46.41±2.54, 63.06±9.45, 67.72±9.45, 42.13±7.75 and 78.56±2.51 mg/dl in NC, DOX group, *G. kola* 500 mg, DOX + *G. kola* 250 mg, DOX+*G. kola* 500 mg and DOX+SIM respectively. This showed that doxorubicin had no significant effect on the HDL concentration when compared to the normal but *Garcinia kola* 250 mg/kg body weight caused a significant increase in the HDL level in line with the DOX+SIM group. This shows that *Garcinia kola* increased HDL cholesterol.

Results (Figure 2C) showed that LDL concentration was 16.31±2.87, 28.23±12.16, 12.29±3.82, 23.61±6.6, 21.74±5.99, 18.93±4.23 mg/dl in NC, DOX group, *G.kola* 500 mg. DOX+*G.kola* 250 mg, DOX+*G.kola* 500 mg and DOX+SIM groups respectively. Statistical analysis of the result showed that The DOX group and *G. kola* group differed significantly from the other groups.

Results showed that Doxorubicin toxicity resulted in an elevation of LDL/HDL ratio to 0.62±0.29 which was significantly higher than the Normal control group (0.39±0.14) (Figure 2D). Treatment with *G.kola* 500 mg, DOX+*G.kola* 250 mg, DOX+*G.kola* 500 mg and DOX+SIM resulted to a significant reduction of LDL/HDL ratio to 0.19±0.04, 0.36±0.13, 0.54±0.24, 0.24±0.06 respectively in the intoxicated animals respectively.

Figure 3: Effect of ethanol extract of Garcinia kola seed administration on serum triacylglycerol, VLDL-Cholesterol, and TNHDL-Cholesterol concentration of male Wistar albino rats administered 2.5mg/kgbwt doxorubicin for 14 days.

After doxorubicin exposure, the rats exhibited significant (p<0.05) increase in serum triacylglycerol compared to the control group (Figure 3A). Triacylglycerol concentration was 80.19±5.66, 112.85±9.96, 76.01±4.21, 95.98±13.29, 93.19±11.51 and 76.01±4.09 mg/dl in NC, DOX group, *G. kola* 500 mg, DOX+G. kola 250 mg, DOX+*G. kola* 500 mg and DOX+SIM groups respectively. Our results showed a further decrease in the triacylglycereol concentration in *G. kola* and DOX+SIM. There was no significant difference among animals in *G. kola*, DOX+SIM, NC, DOX+*G. kola* 500 mg and DOX+*G. kola* 250 mg groups while the animals in DOX+*G. kola* 250 mg, DOX+*G. kola* 500 mg and DOX groups also showed no significant difference.

Our results (Figure 3B) showed that there was a significant (p<0.05) increase in the VLDL concentration of rats exposed to Doxorubicin treatment above the Normal control rats. The results were as follows 16.04±1.14, 22.57±1.99, 15.2±0.84, 19.19±2.66, 18.64±2.3, 15.2±0.82 mg/dl in NC, DOX, *G.kola* 500 mg, DOX+*G.kola* 250 mg, DOX+*G.kola* 500 mg, DOX+SIM respectively. All the treatment groups aside DOX groups showed no significant difference while DOX + *G. kola* 250 mg, DOX+ *G.kola* 500 mg and DOX groups showed no significant difference.

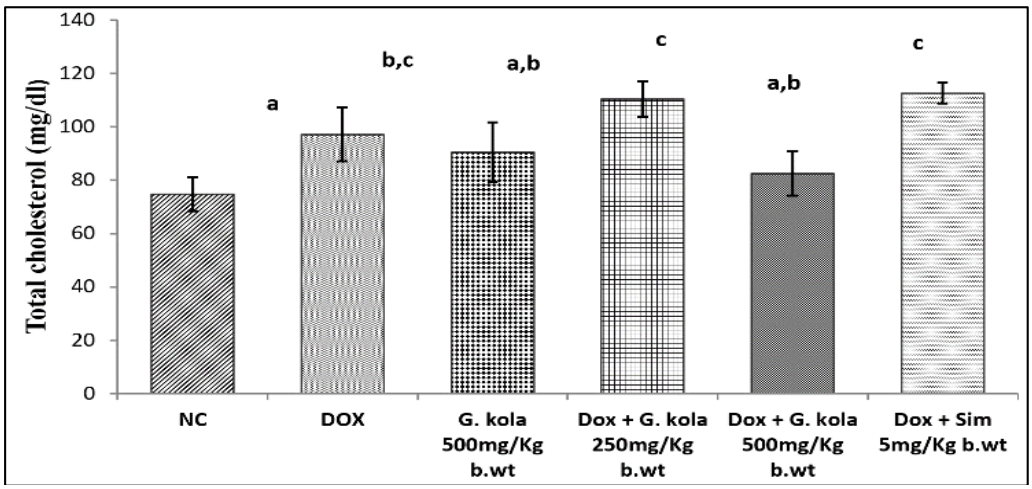

Fig 2A

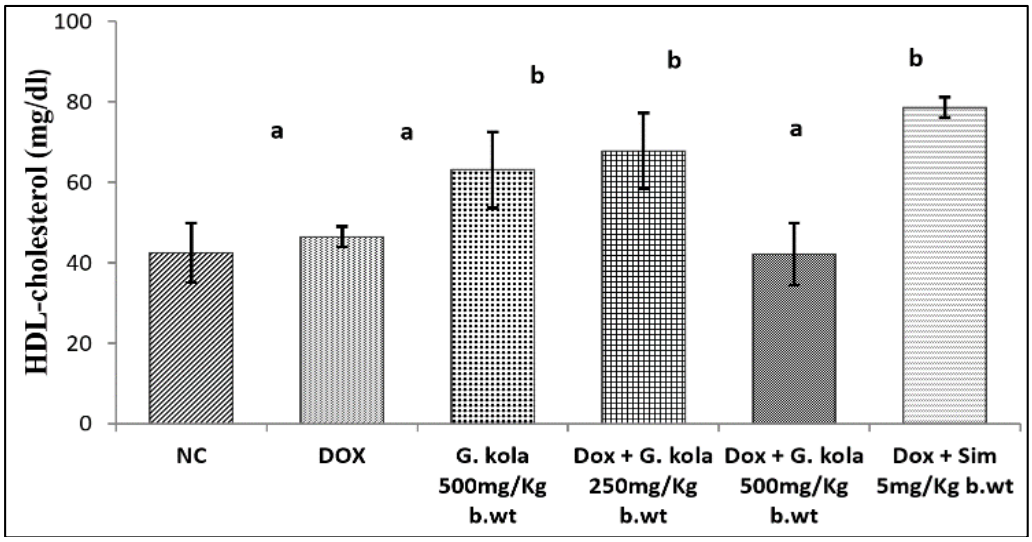

Fig 2B

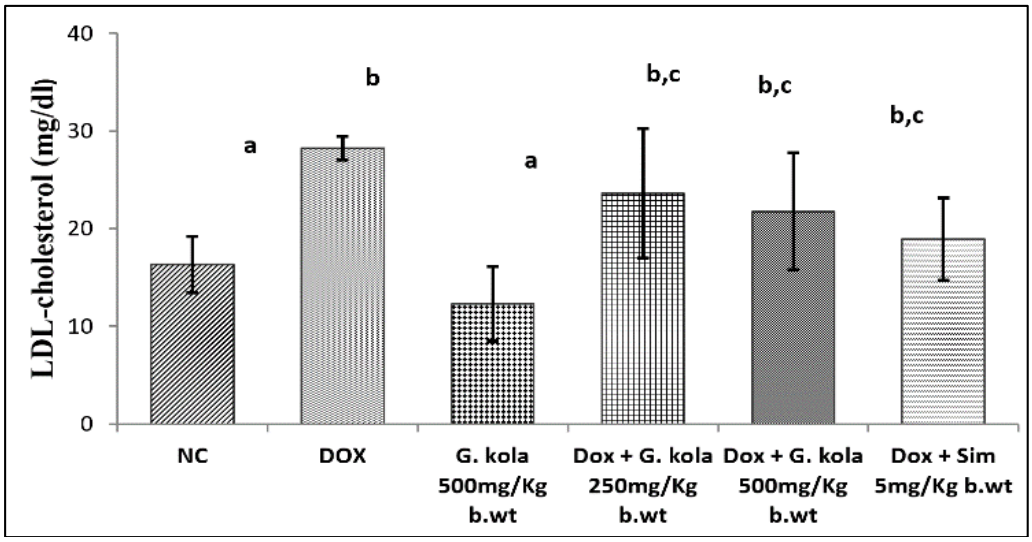

Fig 2C

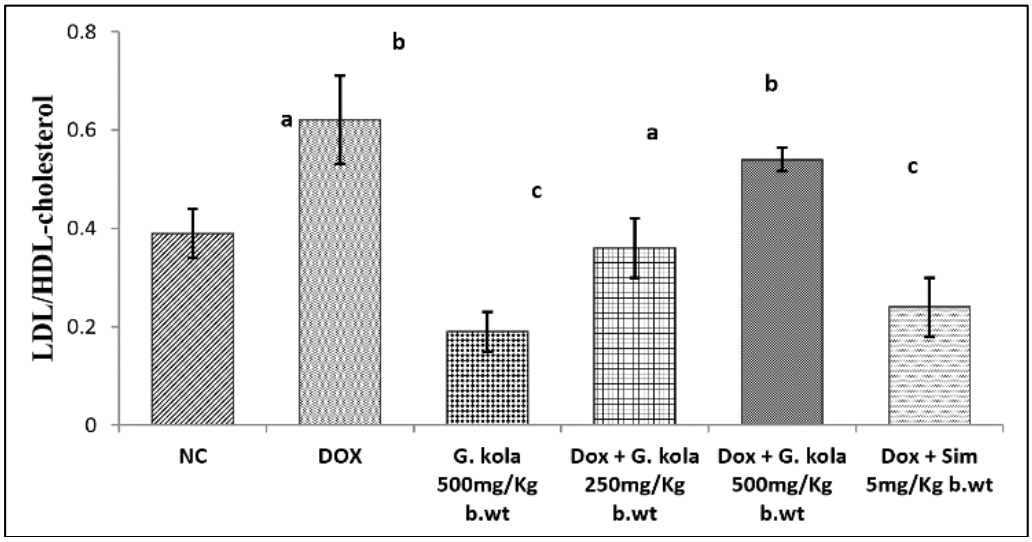

Fig 2D

Fig 2: Effect of ethanol extract of *Garcinia kola* seed administration on serum total Cholesterol, HDL-Cholesterol, LDL-Cholesterol concentration and LDL/HDL-Cholesterol ratio of male Wistar albino rats administered 2.5mg/kgbwt doxorubicin for 14 days. Results are Mean ± SD of 5 determinations and bars with different superscript are statistically significantly different (p<0.05).

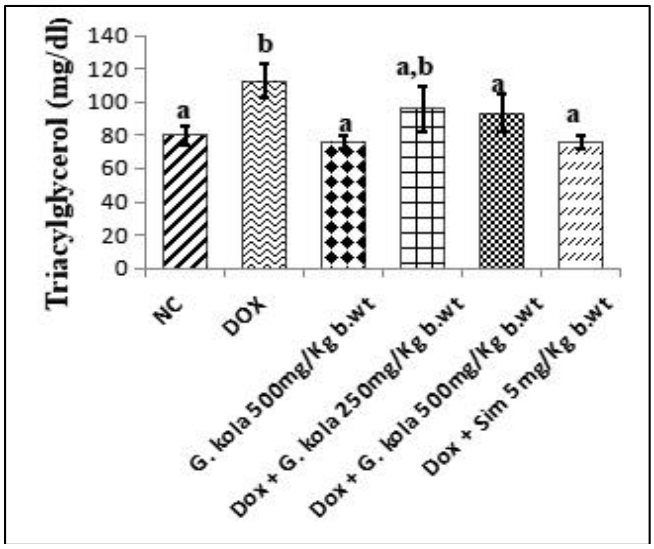

Fig 3A

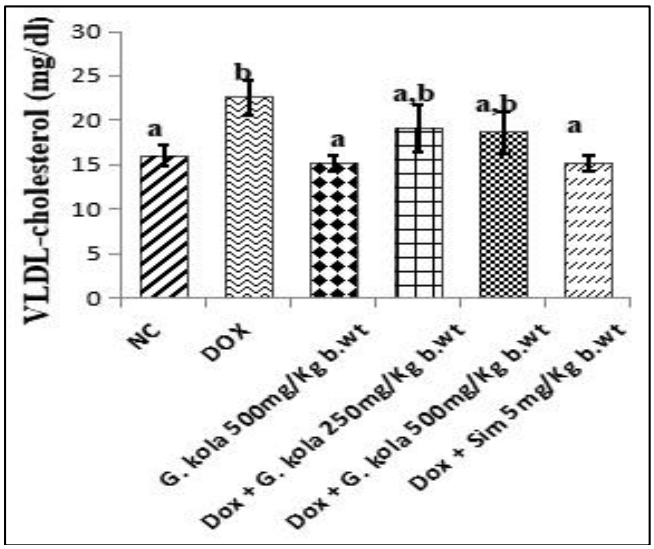

Fig 3B

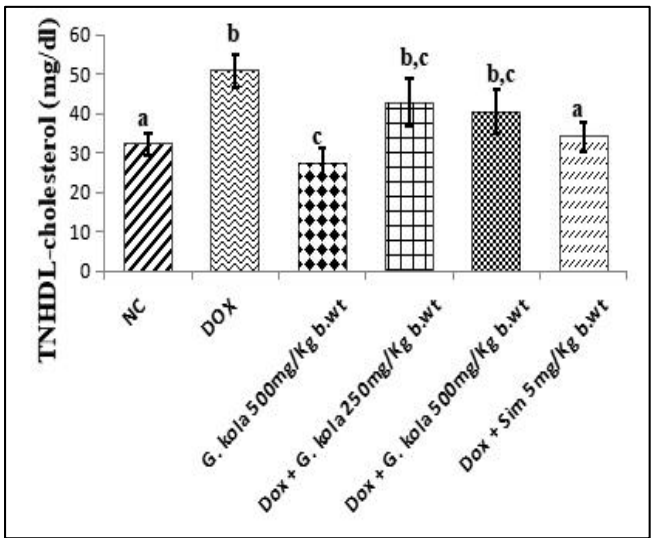

Fig 3C

Fig 3: Effect of ethanol extract of *Garcinia kola* seed administration on serum triacylglycerol, VLDL-Cholesterol, and TNHDL-Cholesterol concentration of male Wistar albino rats administered 2.5mg/kgbwt doxorubicin for 14 days. Results are Mean ± SD of 5 determinations and bars with different superscript are statistically significantly different (p<0.05).

Discussion

The present study investigated the effect of ethanol extract of *Garcinia kola* seed on lipid profile in doxorubicin-induced cardiotoxicity in albino rats. Bioactive constituents of *Garcinia kola* have been implicated in its medicinal properties. The following phytochemical compounds were identified in the crude extract of *Garcinia kola* seed; steroids, cardiac glycosides, flavonoids, tannins, saponins and reducing sugar (Table 1) while the compound alkaloids was found to be absent. The steroidal compounds found in *G. kola* seed extract are of significant interest due to their structural and functional similarity to sex hormones. *Garcinia kola* seed is also known to contain cardiac glycosides, a secondary metabolite of plants, from the result of this study (Table 1), and have been one of the major phytoconstituents reported in plants with cardioprotective functions. It is therefore speculated that the presence of this phytochemical, contributed significantly in altering the biochemical variations in the various marker enzymes assessed, the lipid profiles parameters by bringing them to near normal level after their subsequent elevation by doxorubicin. These result are corroborated by the reports of Bachheti *et al*., (2022) [7] which showed that this secondary metabolite of plants possess cardioprotective properties and this facilitates their use in the treatment of congestive heart failure.

Evaluation of the phytochemical profile of *Garcinia kola* seeds confirmed the presence of tannins (Table 1). Tannins interact with both proteins and carbohydrates, a property that has several implications for tannin-rich commodities. Tannins form complexes with proteins and carbohydrates, influencing the properties and stability of tannin-rich commodities. Their presence can lead to browning or other pigmentation issues in both fresh and processed foods, as observed in *Garcinia kola* seeds. Moreover, the occurrence of tannins in plants suggests potential astringent effects, and they may also contribute to accelerated wound and burn healing (Fraga-Corral *et al*., 2021) [17].

The bioactivity of tannins includes strong preventive effects against cancer and therapeutic benefits for ulcerated and inflamed tissues. Finally, the occurrence of tannins in numerous medicinal plants implies potential interference with iron absorption and digestive processes, similar to the effects seen in *Garcinia kola* seed (Delimont *et al*., 2017) [13]. Saponins which were found in *Garcinia kola* seeds are also useful in managing inflammation as they possess an inhibitory effect on inflamed cells. Saponins are known to precipitate and coagulate erythrocytes, and are characterized by their ability to form stable foams in aqueous solutions, exhibit hemolytic activity, and bind to cholesterol (Timilsena *et al*., 2023) [26].

Increased serum total cholesterol and triglycerides are implicated in the pathogenesis of artherosclerosis as earlier reported by Gaggini *et al*., (2022) [18]. Increase in the serum levels of triglycerides, total cholesterol, high density lipoprotein, low density lipoproteins, very low density lipoproteins as well as the HDL to LDL ratio and TNHDL in the groups treated with doxorubicin, above the level in the normal control groups, showed interference of the doxorubicin with the metabolism of these lipids (Figure 2). This increase in the levels of the serum lipid profile in the doxorubicin treated group indicated that doxorubicin may be interfering with the biosynthesis of lipids. Pre-treatment with *Garcinia kola* seed also showed some reductions in all

the serum lipid profile tests ascertained, while it was significantly evidence in some, in others it was not clearly significant. *Garcinia kola* seeds have been found to inhibit lipid peroxidation *in-vivo* according to the earlier reports of Dogara *et al*., (2022) [14]. A comparable effect has been observed with soybean isoflavones, which are known to decrease total plasma lipids, cholesterol, and triglyceride levels, aligning with the study by Baranska *et al*., (2021).

The significant reduction in the serum cholesterol concentration observed in this study following the administration of *Garcinia kola* 500 mg/kgbwt may be attributed to a reduction in cholesterol synthesis and lipogenesis. The reduced synthesis of fatty acids correspondingly reduces production of LDL particles and results in low serum cholesterol. Treatment with *Garcinia kola* seed 250 mg/kgbwt showed a marked increase in the serum HDL cholesterol amongst every other serum lipid profile results. This increase in the HDL cholesterol may be attributed to the presence of antioxidants in the *Garcinia kola* seed extract. Of the two concentrations of *Garcinia kola* seed administered, *Garcinia kola* at 500 mg/kgbwt, has been proved from the results of this study to have a better decreasing effect on the total cholesterol, triglycerides, LDL, VLDL and TNHDL cholesterol profiles when compared to *Garcinia kola* 250 mg/kgbwt.

Conclusion

The results of this present study on the assessment of the effect of ethanol extract of *Garcinia kola* seed on lipid profile in doxorubicin-induced cardiotoxicity in albino rats showed that *Garcinia kola* seed has cardioprotective effects and lipid lowering properties. It normalized the lipid profiles of rats exposed. All this properties could be rationally attributed to the phytochemical constituents; steroids, cardiac glycosides, flavonoids, tannins, saponins and reducing sugar present in the seeds of *Garcinia kola*.

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