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Antioxidant and antidiabetic properties, and safety profile of methanolic extract of *Garcinia kola* seed (bitter kola)

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Abstract

Background: Diabetes mellitus is growing global health concerns and conventional treatments for diabetes present limitations, prompting exploration of alternative therapeutic approaches. *Garcinia kola* seed from the family Clusiaceae is known for its medicinal properties and it is used as a traditional medicine for antidiabetic management. This study aimed to investigate the phytochemicals, antioxidant and antidiabetic activities as well as safety profile of the methanolic extract of *Garcinia kola* seeds.

Methods: Antioxidant activity was conducted using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and total antioxidant capacity (TAC) assay. Antidiabetic assay was carried out in hyperglycemic-induced diabetic rats. The safety profile of the extract was obtained by using acute toxicity model using rats in five groups (n=6).

Results: Phytochemical screening revealed the presence of tannins, saponins, terpenoids, reducing sugars, flavonoids, cardiac glycosides, and alkaloids in the extract. Antioxidant activity showed IC_{50} of 297.55 µg /mL for the extract and IC_{50} of 29.06 µg/mL for gallic acid. The total antioxidant capacity value obtained was 269.05 mg GAE/g for the extract, highlighting substantial radical scavenging ability. The antidiabetic assay indicated a significant reduction in blood glucose levels among treated diabetic rats. The methanolic extract of *Garcinia kola* seeds demonstrated significant antidiabetic activity, with the 300 mg dose and 500 mg dose achieving effects comparable to and greater than metformin respectively. The acute toxicity studies confirmed the safety of the extract up to 5000 mg/kg.

Conclusion: *Garcinia kola* seed extract can be used as both antidiabetic and antioxidant agents; the extract showed no toxicity in rats. However, further investigation is needed to identify the exact active compounds responsible for the observed activities.

Keywords: *Garcinia kola*, antidiabetic activity, antioxidant potential, acute toxicity, DPPH assay.

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Background

Diabetes mellitus is a disease that continues to pose a formidable challenge to global public health. The disease is a chronic condition marked by elevated blood sugar levels and is among the top ten (10) leading cause of death globally [1, 2]. Classified into Types 1 and 2, Type 1 accounts for 5 % to 10 % of the cases and commonly found in children and adolescents, while Type 2 accounts for around 90 % of all cases in persons older than 45 years [3, 4]. According to estimates from the International Diabetes Federation (IDF), 451 million adults worldwide have diabetes as at 2017. If no effective preventive measures are taken, this number is expected to rise to 693 million by 2045 years [2]. Additionally, children and adolescents under the age of twenty (20) years are estimated to have more than a million cases of type 1 diabetes and the prevalence of type 2 and type 1 diabetes has increased in this age group [2]. The prevalence of the disease continues to increase, most dramatically in low and middle-income nations [5]. In sub-Saharan Africa (SSA), there were about 24 million diabetics as of 2021, and by 2045, that number is expected to rise by 134 % [6]. It is also reported that over 306, 000 persons under 60 years in the SSA lost their lives to diabetes and the average direct healthcare costs in sub-Saharan Africa (SSA) per individual with diabetes was projected to be around USD 547 in 2021 alone [6]. In Ghana, the national prevalence of diabetes was reported to be between 2.80 % - 3.95 % [6]. The long-term complications of diabetes come about gradually, where the longer a person has the diabetes and the less controlled the blood sugar levels, the higher the risk of complications [7]. Over time, complications arising from diabetes can become debilitating or pose a risk to one's life. Potential complications encompass heart and blood vessel (cardiovascular diseases), nerve damage (neuropathy), kidney damage (nephropathy), eye damage (retinopathy) and foot damage [7].

Medications such as metformin (a biguanide), tolbutamide (a sulfonylurea), pioglitazone (a thiazolidinedione) and the alpha-glucosidase inhibitors are the mainstays of conventional treatment of diabetes mellitus. However, these medications can cause a number of adverse effects, including dyspepsia, hypoglycemia, lactic acidosis, metallic taste, stomach pain, anorexia, and nausea [8, 9]. Oxidative stress is closely linked to diabetes mellitus, particularly Type 2 diabetes, where it often causes micro- and macro-angiopathies. Oxidative stress has been recognized to cause damage or harm to cells, tissues and even organs by impairing or compromising important biomolecules and cells [10]. Elevated production of reactive oxygen species (ROS) results in a notable decline in antioxidant defense mechanisms, causing damage to proteins, lipids, and DNA. This damage disrupts cellular functions and can lead to cell death. However, when ROS levels are lower, they can induce subtle changes in intracellular signaling pathways [10]. A number of plants have been employed in the traditional antidiabetic medication therapy as dietary adjuvants. Hypoglycemic activity has been found in a number of plant species and alkaloids, flavonoids, terpenoids, and glycosides are among the phytochemicals that have been linked to antidiabetic benefits [9]. The *Garcinia kola* seed, commonly referred to as bitter kola, is the fruit of a tropical plant belonging to the Clusiaceae family [11]. Primarily thriving in Sub-Saharan Africa, particularly in West and Central Africa, the *Garcinia kola* plant has earned the moniker "wonder plant" due to the extensive medicinal use of nearly every part of the plant [1]. Extract of bitter kola is used in the treatment of cough, laryngitis, and ulcer. It has also been reported to be anti-inflammatory and antidiabetic [1]. The bitter kola seeds contain a wide range of useful phytochemicals, including tannins and flavonoids, proteins, lipids, carbohydrates [12]. Additionally, it contains biflavonoids and amentoflavonones [12]. Reports from many researchers indicate that the bioactivity of *Garcinia kola* seeds is linked to the presence of bioflavonoids [12]. These elements explain the antibacterial, antifungal, antiviral and antioxidant properties of the bitter kola seed extracts [12]. Therefore, it is in the light of the growing evidence of the pharmacological actions that this present study aims to investigate the methanolic extract of *Garcinia kola* seeds as an antioxidant and antidiabetic agent through *in vitro* and *in vivo* assays respectively as well as to investigate its safety profile.

Methods

Plant sample collection and identification

The *Garcinia kola* seeds were purchased from Tamale central market and authenticated by Mr. Emmanuel Adom a Lecturer at the Department of Pharmacognosy and Herbal Medicine, University for Development Studies, Tamale, Ghana. The specimen was deposited in the herbarium of the department with a voucher number UDS/PHM/NR/24/001. The seeds were cleaned and air-dried in the shade at room temperature for four weeks. The dried seeds were then milled into fine powder by using an electronic grinding machine. The powdered sample was kept for extraction.

Extraction of plant sample

An electronic grinding mill was used to powder the dry sample, and methanol was used for extraction (BDH Chemical, UK). A 0.5 kg portion of the milled material was cold macerated in 1.5 L of CH3OH for three (3) days. The residue and filtrate were separated using a Whatman filter paper after 72 hours of extraction. A water bath (Buchi, B-491) and rotavapor (Buchi R-210) at 40°C were used to concentrate the filtrate to dryness. On the extract, phytochemical screening was carried out first, before other analysis.

Phytochemical screening

A standard procedure was used to screen the methanolic extract for potential phytochemicals [13].

Test for flavonoids

A filter paper strip was used for the preliminary test. After dipping the paper into the liquid extract, it was dried and placed in a 2 M ammonia solution. When the filter paper was subjected to intense HCl fumes, a very deep yellow color was formed that eventually disappeared, indicating the presence of flavonoids.

About 0.4 g of the extract was dissolved in 2 mL of ethanol for the confirmatory test, and then 5 drops of strong HCl and magnesium turnings were added. The development of a pink hue showed the presence of flavonoids.

Test for alkaloids

The extract was first treated with ammoniacal alcohol (ammonia: 95% ethanol in a 1:9 ratio, respectively), and it was then filtered. In order to change the alkaloids into soluble salt forms, the filtrate was

evaporated and then 1% sulfuric acid was added to the residue. After filtration, the resulting solution was made alkaline with diluted ammonia and then partitioned in a separating funnel with chloroform. The solution and its content were then shaken for about three minutes. After separating the chloroform layer and evaporating the filtrate, 1% H2SO4 was added to the residue. The acidified residue was used for Dragendroff and Mayer tests.

Dragendroff's test

To the filtrate, a potassium bismuth iodide solution was added. The presence of alkaloids was indicated by the formation of red precipitate.

Mayer's test

A potassium mercuric iodide solution was used to treat the filtrate. The formation of a cream precipitate indicated the presence of alkaloids.

Test for saponins

After dissolving 2 mg of the extract in 2 mL of distilled water, the mixture was shaken. The presence of saponins was confirmed by the formation of a foam column that was at least 1 cm in height and persisted for at least 15 min.

Test for tannins

About 5 drops of iron (III) chloride (FeCl3) solution was added to 2 mL of the extract. Appearance of blue-black color indicated the presence of tannins.

Test for reducing sugars

The extract was first hydrolyzed with dilute HCl and then was treated with 20 % NaOH. The resulting solution was heated with Fehling's solutions A and B. The presence of reducing sugars was confirmed by the formation of a brick-red precipitate.

Test for triterpenes/ terpenoids (Salkowski's test)

The extract was treated with chloroform and filtered. About 4 drops of concentrated H2SO4 were added to the filtrate in a test tube. The test tube and its content were shaken for thorough mixing of the content and allowed to stand for 10 min. The formation of a brownish-red color showed the presence of triterpenes or terpenoids.

Cardiac glycosides

The extract was treated with 70 % alcohol and filtered. About 8 mL of the filtrate was added to 2 mL of anhydrous CH_3COOH with 4 drops of iron (III) chloride solution in a test tube. A dropping pipette was used to add a concentrated H2SO4 gently to the solution in the test tube. A reddish-brown colouration at the interface as a result of the presence of aglycone was observed. Hence, cardiac glycosides confirmed.

Antioxidant activity

Two main assays were employed for the antioxidant activity determination. They were 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radicals scavenging and the total antioxidant capacity assays.

DPPH radical scavenging assay

The DPPH radical scavenging activity was determined according to a standard method as previously described [14]. A solution of 20 µg/mL DPPH was prepared by dissolving 2 mg of DPPH in 100 mL of methanol. Stock solutions of 100 µg/mL of the extract and gallic acid were prepared separately in distilled water, from which various concentrations 50, 25, 12.5, and 6.25 µg/mL were made by serial dilution method. Gallic acid was used as a reference standard. The reaction mixtures of volume 200 µL were prepared by dissolving 150 µL of 20 µg/mL DPPH solution and 50 µL of various concentrations of the test solutions. The mixtures were then incubated in dark at room temperature for 30 minutes, after which the absorbance was measured at 517 nm. The experiment was independently repeated to obtain three independent sets of data for the analysis. DPPH radical scavenging (%) was calculated using the following formula:

$$Percentage\ Scanvenging\ ability = \frac{(A_{control} - A_{test\ sample})}{A_{control}} x100$$

A control denotes mean absorbance of the negative control.
A test sample denotes mean absorbance of the extracts or gallic acid (standard).

Total antioxidant capacity (TAC) assay

A modification of the methodology described by Prieto et al. was used to study the total antioxidant capacity of the methanolic extract of *Garcinia kola* seeds [15]. The method is based on the reduction of phosphomolybdic acid, Mo (VI) to phosphomolybdenum, Mo (V) blue complex by the extracts, and standard reference. Gallic acid was used as the reference standard, and distilled water was used as the blank. A stock solution of 100 µg/mL of gallic acid was prepared from which concentrations of 50, 25, 12.5, and 6.125 µg/mL were prepared through serial dilution using sterile distilled water as solvent. A test solution of concentration of 500 µg/mL of the extract in sterile distilled water was also prepared. Reaction mixtures of total volume of 10mL were prepared from 5mL of test solution mixed with 5mL phosphomolybdenum (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in series of test tubes. The mixtures were then incubated in a water bath at 95°C for 90 minutes. The absorbances of each of the solutions were then measured in triplicate, using the UV-visible spectrophotometer at 695 nm after cooling. The experiment was independently repeated to obtain three independent sets of data for the analysis. A plot of the measured absorbance of the gallic acid solutions against their concentrations was made to obtain the calibrated concentration-absorbance curve of the gallic acid. The absorbances of the extract solutions were then substituted for the dependent variable in the linear equation of the gallic acid concentration-absorbance plot to determine their corresponding independent] variables as gallic acid equivalents expressed as mGAE/g gallic acid.

Animals

The animals used were Sprague-Dawley rats (123-280 g), of both sexes and were purchased from the animal house of Centre for Plant Medicine Research, Mampong-Akwapim, Ghana. The animals were housed in the animal house facility of the Department of Pharmacology, Kwame Nkrumah University of Science and

Technology (KNUST) where both acute toxicity and antidiabetic studies were conducted. Throughout the whole experimental period, all of the animals used were treated humanely in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and the Animal Welfare Regulations. Moreover, studies on the rats were conducted with the approval of the Research and Ethics Committee, University for Development Studies Institutional Review Board (UDSIRB) with approval number UDS/ RB/ 129/24.

Antidiabetic activity

High-fat fed streptozotocin (STZ) induced type 2 diabetes model

Sprague-Dawley rats (250- 280 g) were used for this study. The animals were maintained at room temperature, 12 hours light and 12 hours dark cycles. They were accommodated in polypropylene cages and provided with high fat diet food (AGRICARE, Kumasi, Ghana) and water for 4 weeks. Afterwards, the rats were left to fast for one night, after which they received a continuous 7-day sub-diabetogenic dose of STZ (Sigma Aldrich, St. Louis, MO, USA) intraperitoneally (i. p.), 35 mg/kg body weight freshly dissolved in freshly prepared citrate buffer pH = 4.5 [16]. All STZ-injected rats with blood glucose ≥ 200 mg/dL were considered diabetic and used in the study [17]. The selected rats were put into five groups (n=6)**.** Three different doses (100, 300, 500 mg/kg) of the extracts were used for treated groups, one group for normal control and another for positive control (metformin, 200 mg/kg). Blood glucose of the rats was measured every morning for the 7 days the rats were treated.

Acute toxicity studies of the extract

The acute toxicity test on the methanolic extract of *Garcinia kola* seeds was conducted in compliance with the Organization for Economic Cooperation and Development's (OECD) 425 standard for testing chemicals [18]. The pathogen free Sprague-Dawley rats of both sexes were selected randomly and put into groups of six. The animals were allowed to acclimatize to the new laboratory environment for at least seven days. They were all kept at a room temperature. The animals were allowed to fast for 24 h with access to water prior to the test. The rats were weighed before the start of oral administration of the extract in doses of 500 mg/kg (Group II), 1000 mg/kg (Group III), 2500 mg/kg (Group IV) and 5000 mg/kg (Group V). The animals in Group I, the normal control group, received 10 mL/kg of distilled water. The experimental animals were meticulously monitored for diarrhea, decreased in movement, weight loss, lethargy, skin fur position, urination, salivation and mortality at 0 min, 30 min, 60 min, 120 min, and 200 min, as well as 24 h and 14 days after the extract was administered. Each animal was used once, after that, all the animals were euthanized at the end of the experiment.

Data analysis

The 27.0.1 version of IBM SPSS Statistics was used to analyze the data. The means ± standard errors of means was used to summarize the data. Means were compared using the analysis of variance (ANOVA), assuming statistical significance at p-value < 0.05). Differences were presented with 95 % the confidence interval.

Results

Extraction of plant material

The yield of the extract relative to the powdered plant material was calculated as a percentage. The percentage yield was determined to be 15.59 %.

Phytochemical composition

In this study, a phytochemical analysis of *Garcinia kola* was performed to identify specific compounds that may contribute to its antioxidant and antidiabetic properties. The results are presented in Table 1.

Total antioxidant capacity

The total antioxidant capacity (TAC) of the methanolic extract of bitter kola was assessed using a gallic acid calibration curve (Figure 1). The absorbance of various concentrations of gallic acid (from 0 to 500 µg/mL) was measured, and a linear relationship between absorbance (Y) and concentration (C) was established, as: Y= 0.004385*C + 0.1352 with R^2 value of 0.9964, demonstrating a high degree of predictability. Using this equation, the TAC of the bitter kola extract was calculated as 269.05 ± 0.062 mg GAE/g (milligram gallic acid equivalence per gram). This indicates a substantial antioxidant capacity in the methanolic extract.

DPPH radical scavenging capacity

The antioxidant activity of the methanolic extract of bitter kola was also evaluated using the DPPH radical scavenging assay. The half-maximal inhibitory concentration (IC_{50}) value of the extract was compared with gallic acid. The IC_{50} value for gallic acid is considerably lower than that of bitter kola as illustrated in Figure 1. The extract's IC_{50} value of 297.55 ± 9.27 µg/mL indicates that it has antioxidant activity, although it is less effective than the reference compound, gallic acid, which has an IC_{50} of 29.06 ± 4.67 µg/mL.

Antidiabetic activity

The antidiabetic activity of the methanolic extract of *Garcinia kola* seeds was evaluated using a high-fat diet and streptozotocin (STZ)-induced type 2 diabetes model. Blood glucose levels were monitored daily over seven days of treatment (Table 2). There is no significant weight-related trend observed in blood glucose responses across treatment groups as shown in Table 3. Statistical analyses further supported these findings, with independent t-tests (Table 4) indicating that all extract-treated groups significantly outperformed the normal control group (with p-value < 0.001). Moreover, the paired t-test analysis shows significant difference between the negative control group and other groups (Table 5).

Acute toxicity profile

The study assessed weight fluctuations, behavioral changes, and mortality of the experimental rats over a 14-day period. The mean weights of the rats across the various groups were determined for Day 0, Day 7, and Day 14 (Table 6). At the baseline (Day 0), there were no significant weight differences between the groups as indicated by the p-value of 0.101, confirming that they were comparable in terms of weight at the beginning of the experimental study (Table 7). The toxicity indicators regarding the physical and

behavioral reactions to the test substance have been summarized in Table 8. These indicators include death, coma, excessive salivation, abnormal movement, changes in eye color, diarrhea, urination patterns, sleep disturbances, skin and fur condition, sensitivity to touch, and defecation.

Discussion

Phytochemical screening plays a crucial role in detecting the bioactive compounds found in plant extracts, which are often linked to the pharmacological effects, whether beneficial or harmful, of the plant [19]. Phytochemical compounds in medicinal plants that are reported to have antioxidant and/or antidiabetic properties are flavonoids, alkaloids, tannins, terpenoids and saponins [20]. The findings of this study regarding phytochemical screening, is consistent with those of an earlier report [21], with the only exception that, steroids were not screened for in this study. The seed extract was found to contain tannins, which have anti-inflammatory, antidiabetic, antioxidant, anti-hypercholesterolemic and anti-cancer properties [22]. Tannins exhibit antidiabetic properties primarily by reducing glucose levels through delaying intestinal glucose absorption and mimicking insulin's effects on insulin-sensitive tissues. Additionally, they help postpone the development of insulin-dependent diabetes mellitus by maintaining the antioxidant balance in pancreatic β-cells [23]. The reduction in glucose levels can be achieved through the inhibition of α-amylase and α-glucosidase enzymes. Moreover, tannins are recognized for their ability to inhibit lipid peroxidation in vitro and to scavenge free radicals, which play a significant role in pro-oxidant conditions at the cellular level. Terpenoids were also detected in the nut extract. Terpenoids possess antioxidant, antidiabetic, antibacterial, anticancer, antimalarial properties among others [23]. Terpenoids demonstrate antidiabetic effects through a variety of mechanisms, such as enhancing glucose uptake and glycogen synthesis, boosting insulin sensitivity, inhibiting alpha-glucosidase, plasma lipase, aldose reductase, tyrosine phosphatase, and alpha-amylase, as well as preventing advanced glycation products and protein glycation [24]. Terpenoids can function as direct antioxidants by scavenging free radicals and/or as indirect antioxidants by boosting both enzymatic and non-enzymatic antioxidant defenses [25]. The extract also contains saponins which have anti-inflammatory, antioxidant, antidiabetic, anticancer activities [26, 27]. Saponins are known to produce a hypoglycemic effect through various mechanisms, such as enhancing insulin secretion from pancreatic beta cells, stimulating glycogen synthesis, suppressing gluconeogenesis, inhibiting alpha-glucosidase, and reducing the mRNA expression of glycogen phosphorylase and glucose 6-phosphate [28]. Alkaloids, the most prevalent and varied class of secondary metabolites were detected in the extract. Alkaloids possess a number of pharmacological properties including analgesic, antidiabetic, antibacterial, anticancer, and antimalarial activities [29]. They exhibit antidiabetic effects by inhibiting enzymes such as α-amylase, α-glucosidase, aldose reductase, dipeptidyl peptidase-IV, and protein tyrosine phosphatase-1B [30]. Additionally, they reduce the formation of advanced glycation products, increase insulin secretion and sensitivity, promote glucose uptake, and possess antioxidant properties [30]. In addition, flavonoids, which are also found in the extract, have numerous biological activities including antidiabetic, antioxidant, anti-inflammatory and antiviral activities [31, 32]. Flavonoids demonstrate antioxidant activity by neutralizing free radicals, donating electrons and hydrogen atoms, and binding metal cations [33]. They also exhibit antidiabetic effects by enhancing glucose absorption, boosting insulin sensitivity, inhibiting enzymes that digest carbohydrates, and reducing glucose production in the liver [32]. Additionally, flavonoids have shown protective effects against complications of diabetes mellitus, including nephropathy, neuropathy, and retinopathy [32]. Reducing sugars detected in the *Garcinia kola* seed extract suggest the presence of simple sugars, which play a crucial role in energy metabolism. Although further research is needed to clarify their involvement in antidiabetic effects, these sugars could influence the plant's bioactivity through different metabolic pathways. Also, the presence of cardiac glycosides in the extract indicates that its antidiabetic and antioxidant effects are probably related to this group of compounds. Cardiac glycosides are recognized for their use in heart therapies and some hypoglycemic properties, hence, their presence in *Garcinia kola* seed extract could be helpful in treating many hearts related conditions.

The total antioxidant potential of a plant extract is primarily influenced by its constituents and the specific test system used. Various factors can also affect the extract's activity, making it impossible to assess fully its antioxidant capacity with a single method [34]. Given the different mechanisms of antioxidant actions, the antioxidant properties of the extract were assessed through DPPH free radical scavenging and total antioxidant capacity assays. The antioxidant capacity is essential for neutralizing free radicals, and the high total antioxidant capacity (TAC) of bitter kola obtained in this study implies it contains substantial amount of phenolic compounds, that might have contributed to this effect. The TAC of bitter kola suggests it is a promising natural antioxidant source, potentially offering protection against oxidative stress and related health issues. Additional phytochemical analysis could help pinpoint the specific compounds responsible for this activity, potentially enhancing its applications in health and nutrition. The antioxidant activity of the methanolic extract of bitter kola was also evaluated using the DPPH radical scavenging assay. It was observed that the antioxidant activity of the extract was not the same as the standard drug, gallic acid. This difference is likely due to variations in the concentration and types of antioxidant compounds in the bitter kola extract compared to the pure gallic acid. Natural extracts, which consist of a blend of various compounds, typically show lower antioxidant activities compared to pure synthetic compounds like gallic acid. Nonetheless, the natural origin of bitter kola reduces the risk of potential adverse effects, making it a valuable option for nutraceutical and therapeutic applications. The DPPH assay results suggest that optimizing extraction methods or isolating the active antioxidant compounds in bitter kola could enhance its effectiveness. Combining these findings with the total antioxidant capacity results shows that although bitter kola may not match the potency of pure gallic acid in scavenging free radicals, it remains an important natural antioxidant source.

The antidiabetic activity of the same extract was also evaluated using a high-fat diet and streptozotocin (STZ)-induced type 2 diabetes model. For example, the antidiabetic effect of rats administered with 300 mg/kg of the extract and with the highest mean weight, exhibited glucose-lowering effects similar to those given 500 mg/kg of the extract. This indicates that the hypoglycemic effects were primarily influenced by the bioactive compounds in *Garcinia kola* seed rather than by differences in baseline metabolic activity due to weight. The groups treated with *Garcinia kola* seed extract displayed dose-dependent reductions in blood glucose levels, with the 100 mg dose decreasing blood glucose from 237.65± 6.34 mg/dL post induction to 187.60 ± 5.99 mg/dL (21.06 % decrease) over seven days. The 300 mg dose showed a reduction of 30.49 %, decreasing blood glucose levels

from 244.55± 6.85 mg/dL to 169.98 ± 5.35 mg/dL ($p < 0.001$). Notably, the 500 mg group, also demonstrated a significant decline in glucose levels from 264.47± 12.33 mg/dL to 155.32 ± 9.91 mg/dL representing 41.271 % reduction by the end of the experiment which was even greater than that observed in the metformin group. This affirms the efficacy of the extract even at higher glucose concentrations. The metformin-treated group (positive control) exhibited consistent reductions in blood glucose levels over the seven days period, confirming the validity of the experimental model. Statistical analyses further supported these findings, with independent t-tests indicating that all extract-treated groups significantly outperformed the normal control group (with p-value < 0.001). Moreover, the paired t-test analysis shows significant difference between the negative control group and other groups. Moreover, comparing the extract-treated groups to the normal control group revealed highly significant differences ($p < 0.001$), demonstrating the superior efficacy of the extract in controlling hyperglycemia. Comparisons between metformin and the extract further underscore the extract's therapeutic potential. While the 100 mg extract was less effective than metformin ($p < 0.001$, $t = -5.035$), the 300 mg extract showed no statistically significant difference when compared to metformin ($p = 0.119$), suggesting that at this dose, *Garcinia kola* seed extract may exhibit similar efficacy as the standard drug. Importantly, the 500 mg dose showed significant difference ($p = 0.023$, $t = 2.687$), indicating that the highest dose of the extract was more effective than the metformin in reducing the blood glucose levels in the rats. The observed antidiabetic effects can be attributed to the phytochemical constituents of the extract, such as flavonoids, alkaloids, and saponins, which are known to enhance glucose uptake, modulate insulin secretion, and protect pancreatic β-cells from oxidative stress. The extract-treated groups showed a gradual and consistent decline in glucose levels, with the 500 mg dose demonstrating the steepest decrease. This pattern suggests a sustained antidiabetic effect of *Garcinia kola* seed, likely due to cumulative action of its bioactive compounds.

The primary goal of assessing the safety of any medicinal plant is to determine the nature and importance of potential adverse effects and to identify the exposure level at which these effects become apparent. The methanolic extract of *Garcinia kola* seed was assessed for acute toxicity in Sprague-Dawley rats, in accordance with OECD 425 guidelines. The weight measurements across the groups show a consistent pattern, with the rats' body weights remaining steady over the 14-day period, although certain groups experienced some variations. The weight of the control group exhibited a consistent pattern over the 14-day period. The mean body weight of the rats in this group rose slightly from 167.68 g on Day 0 to 167.98 g by Day 7, indicating normal growth and health. By Day 14, the average weight reached 168.22 g, suggesting that natural environmental and metabolic influences, rather than experimental conditions, primarily affected the rats' development. This trend establishes a reference point for evaluating the effects of *Garcinia kola* seed extract in the treatment groups. With minimal weight changes observed in the control group, any notable variations in the treatment groups can be more reliably linked to the administered doses. The weight changes in the group that received 500 mg/kg of the extract (Group II) stayed nearly constant over the 14-day period. Starting at 161.40 g on Day 0, there were minimal increases on Day 7 (161.48 g) and Day 14 (161.63 g). This indicates that the extract, at this particular dosage, had little effect on the rats' body weight, suggesting a low level of risk for obesity. Similarly, the rats in Group III (1000 mg/kg extract) maintained consistent weights during the experiment. Beginning at 173.30 g on Day 0, their weight slightly increased to 174.10 g by Day 14. This suggests that even at a higher dose, the extract did not cause significant weight changes, indicating its safety at this dosage level. Furthermore, the rats in Group IV (2500 mg/kg) had the highest initial average body weight of 211.75 g on Day 0 and rose only slightly to 212.52 g by Day 14. The small change in weight over the period indicates that this dosage also did not cause any obesity that would significantly affect the rats' growth or health. Although, Group V (5000 mg/kg extract) received the highest dose, their weight remained consistently stable, starting at 191.52 g on Day 0 and slightly rose to 192.43 g by Day 14. This suggests that the extract, even at such a high dosage, did not adversely affect the rats' general health, as shown by their steady weight.

To compare the mean weight of the experimental rats that were administered the extracts to the normal control group, one-way ANOVA tests were performed for Days 0, 7, and 14. At the baseline (Day 0), there were no significant weight differences between the groups as indicated by the p-value of 0.101, confirming that they were comparable in terms of weight at the beginning of the experimental study. By Day 7, the p-value remains at 0.100, indicating that there were still no significant weight changes between the groups. This suggests that the intervention did not have an immediate impact on weight within the first week. On Day 14, the p-value of 0.099 similarly indicates no significant weight differences among the groups. This consistency over the two-week period suggests that the intervention did not affect weight changes during the experimental period. The absence of significant weight loss in any of the groups, even at the highest dose of 5000 mg/kg, suggests that the methanolic extract of *Garcinia kola* seed is likely non-toxic within the dosage range tested. Toxicity indicators regarding the physical and behavioral reactions to the test substance were also evaluated. These indicators include death, coma, excessive salivation, abnormal movement, changes in eye color, diarrhea, urination patterns, sleep disturbances, skin and fur condition, sensitivity to touch, and defecation.

No fatalities were observed at any dose level, suggesting that none of the administered doses had lethal effects during the study. This result is important as it indicates that the doses were well tolerated by the rats, with no signs of life-threatening toxicity and that the median lethal dose (LD_{50}) of the extract is likely higher than 5000 mg/kg. Likewise, there were no cases of coma, showing that even at the maximum dose of 5000 mg/kg, the substance did not cause significant central nervous system depression. Other parameters like salivation, movement, eye color, urination, sleep patterns, skin and fur condition, sensitivity to touch, and defecation were found to be normal across all dose groups, including the control. This consistent display of typical behavior and physical traits across all groups indicates that the substance did not trigger any significant adverse effects on these functions, even as the dosage increased. Additionally, the lack of diarrhea further supports the conclusion that the substance did not cause gastrointestinal toxicity. Overall, the absence of observable toxic effects at any of the administered dose levels points to a favorable acute toxicity profile. The absence of mortality and the steady normal physiological responses suggest that the rats could tolerate doses up to 5000 mg/kg without showing any severe or fatal toxic reactions. These findings about the safety profile of *Garcinia kola* seed extract in this study agrees with previous reports [35].

The outcome of the weight changes analysis, along with behavioral observations and mortality rates of zero across all the groups, affirms the safety profile of the extract. The stability of weight in both low and high-dose groups further supports the notion that the extract does not adversely affect the general health of the rats over the short term. Further studies could evaluate

potential long-term effects and explore different routes of administration.

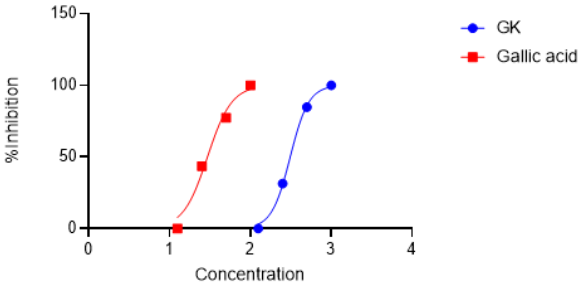

Figure 1. Comparison of the % inhibition of different concentrations of the extract and standard.
Mean IC_{50} ±SD: 29.06 ± 4.67 µg/mL for Gallic acid and 297.55 ± 9.27 µg/mL for *Garcinia kola*.

Table 1. Qualitative analysis of phytochemical constituents in the methanolic extract of *Garcinia kola* seeds.

S/N	Phytochemical constituents	Methanolic extract
1.	Tannins	+
2.	Reducing sugars	+
3.	Saponins	+
4.	Alkaloids	+
5.	Flavonoids	+
6.	Terpenoids	+
7.	Cardiac glycosides	+

Key: (+) **=** present; (-) = absent

Table 2. Mean and percentage reduction of blood glucose levels in rats

Group	Initial blood glucose level (mg/dL)	Blood glucose level after induction (mg/dL)	Blood glucose level after administration of control and test sample (mg/dL)							% Reduction in blood glucose levels
			Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
I	92.62± 1.79	-	92.55 ± 1.69	92.60 ± 1.74	92.92± 1.81	92.43± 1.91	92.05 ± 1.81	92.23± 1.84	92.40± 1.85	0.24
II	95.65± 0.78	264.10± 24.86	256.72± 25.14	249.68± 25.00	241.90± 25.25	232.57± 24.26	221.00± 21.96	199.32± 21.53	176.58± 17.77	33.14
III	95.23± 1.23	237.65± 6.34	234.73± 6.84	231.72± 7.02	227.87± 7.28	222.88± 7.55	213.68± 6.50	204.65± 6.24	187.60± 5.99	21.06
IV	95.08± 1.31	244.55± 6.85	241.20± 6.81	236.20± 6.55	231.98± 6.48	221.48± 5.43	207.72± 5.78	190.00± 6.73	169.98± 5.35	30.49
V	92.45± 3.54	264.47± 12.33	258.20± 12.15	248.05± 12.62	234.87± 11.88	221.02± 12.47	201.77± 12.49	180.42± 12.44	155.32± 9.91	41.27

Key: Data presented as Mean ± SEM; N= 6; Significance difference at $p < 0.05$. **I=** Negative control; II = Metformin 200 mg; III= Extract 100 mg; IV= Extract 300 mg; IV= Extract 500 mg

Table 3. Weights of rats used in the experiment

Group	Weights of rats in the various groups (g)						Mean weight
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	
Negative control	257.2	271.9	279.0	274.6	272.4	266.8	270.32 ± 3.08
200 mg metformin	254.5	261.3	275.3	258.9	256.7	275.5	263.70 ± 3.81
100 mg extract	278.1	269.9	252.5	279.8	266.2	254.6	266.85 ± 4.69
300 mg extract	272.7	268.1	277.8	256.7	267.9	264.9	268.02 ± 2.92
500 mg extract	263.8	257.9	268.9	256.4	268.9	257.2	262.18 ± 2.38

Table 4. Independent t-test analysis

Comparison	p- value	t-value
Negative control versus Metformin	< 0.001	11.875
Negative control versus Extract 100 mg/kg	< 0.001	43.037
Negative control versus Extract 300 mg/kg	< 0.001	38.367
Negative control versus Extract 500 mg/kg	< 0.001	33.149
Metformin versus Extract 100 mg/kg	< 0.001	-5.035
Metformin vs Extract 300 mg/kg	0.119	-1.704
Metformin vs Extract 500 mg/kg	0.023	2.687

Significance difference at $p < 0.05$

Table 5. Paired t-test analysis

Group	p-value	t-value	Cohen's d (Point Estimate)	Hedge correction (Point Estimate)
I	0.056	2.484	1.014	0.936
II	< 0.001	11.906	18.006	19.514
III	< 0.001	43.447	2.828	3.065
IV	< 0.001	38.517	4.742	5.139
V	<0.001	33.227	8.047	8.720

I= Negative control; II = Metformin 200 mg/kg; III= Extract 100 mg/kg; IV= Extract 300 mg/kg; IV= Extract 500 mg/kg**;** Significance difference at $p < 0.05$

Table 6. Mean weight (g) of rats treated with different doses of the extract

Groups	Day 0	Day 7	Day 14
Control	167.6 ± 12.13	167.98 ± 12.18	168.22 ± 12.17
500 mg/kg	161.40 ± 13.28	161.48 ± 13.35	161.63 ± 13.32
1000 mg/kg	173.30 ± 15.32	173.55 ± 15.38	174.10 ± 15.55
2500 mg/kg	211.75 ± 10.42	212.12 ± 10.37	212.52 ± 10.42
5000 mg/kg	191.52 ± 17.26	191.87 ± 17.20	192.43 ± 17.28

Key. Data presented as mean ± SEM, N = 6, Significant difference at $p < 0.05$

Table 7. ANOVA results for weight changes

Day	p-value (ANOVA)	Interpretation
0	0.101	No significant difference
7	0.100	No significant difference
14	0.099	No significant difference

*Significant difference at $p < 0.05$

Table 8. General appearance and behavioral observations of the experimental rats for tested groups (mg/kg) and the control group

Toxicity signs	Control	500 mg/kg	1000 mg/kg	2500 mg/kg	5000 mg/kg
Mortality	0	0	0	0	0
Coma	-	-	-	-	-
Salivation	NOL	NOL	NOL	NOL	NOL
Locomotion	NOL	NOL	NOL	NOL	NOL
Eye colour	NOL	NOL	NOL	NOL	NOL
Diarrhea	-	-	-	-	-
Urination	NOL	NOL	NOL	NOL	NOL
Eye colour	NOL	NOL	NOL	NOL	NOL
Sleep	NOL	NOL	NOL	NOL	NOL
Skin and fur position	NOL	NOL	NOL	NOL	NOL
Reactivity to touch	NOL	NOL	NOL	NOL	NOL
Defecation	NOL	NOL	NOL	NOL	NOL

KEY: The Table highlights physical appearance and behavioral observations of the experimental rats for acute toxicity study conducted. Number of rats per group, N = 6, 0 = no death, NOL = normal, - = absent.

Conclusion

This research examined the antioxidant and antidiabetic effects as well as safety profile of the methanolic extract of *Garcinia kola*. The results revealed that the extract contains several bioactive compounds, including tannins, flavonoids, saponins, terpenoids, cardiac glycosides, reducing sugars, and alkaloids, which likely contribute to its medicinal properties. Additionally, the extract exhibited a notable total antioxidant capacity (TAC) and a moderate free radical scavenging activity, when compared to gallic acid. The methanolic extract of *Garcinia kola* seeds demonstrated significant antidiabetic activity, with the 300 mg dose and 500 mg dose achieving effects comparable to and greater than metformin respectively. The acute toxicity assessment showed no harmful reactions, even at the maximum tested dose of 5000 mg/kg, suggesting a high safety margin. These findings validate the traditional use of *Garcinia kola* seeds in diabetes management and provide a strong foundation for further pharmacological and clinical investigations. These outcomes highlight the potential of *Garcinia kola* seeds as a natural therapeutic option for diabetes, presenting a safer alternative to standard treatments with fewer side effects.

Abbreviations

UDSIRB: University for Development Studies Institutional Review Board
TAC: Total antioxidant capacity
DPPH: 2, 2-diphenyl-1-picrylhydrazyl
GK: *Garcinia kola*
STZ: Streptozotocin

Authors' Contribution

NT and EEB carried out the study and BSM reviewed the manuscript. All authors cross-checked and approved the final version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest

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