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ORIGINAL RESEARCH ARTICLE

Antioxidant Potentials and Nephroprotective Effects of Methanol Extract and Dichloromethane Fraction of Root Bark of *Garcinia kola* (Heckel) on Gentamicin-Induced Nephrotoxic Rats

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ABSTRACT

This study investigated the nephroprotective and antioxidant effects of methanol extract (ME) and dichloromethane fraction (DCMF) from the root bark of *Garcinia kola* against gentamicin-induced nephrotoxicity in rats. The root bark was collected from Iperindo, Osun State, Nigeria, and dried for eight weeks. After grinding into a powder, the root bark was extracted using 80% methanol. The extract was filtered, centrifuged, and concentrated to obtain ME. A portion of ME was fractionated using n-hexane and dichloromethane to obtain DCMF. The phenolics and flavonoid contents of ME and DMCF were analyzed. Gentamicin, ME, and DCMF were administered to forty Wistar rats for a total of fourteen days, both prior to and following therapy. The rats were split up into groups. Levels of endogenous antioxidant molecules and enzymatic antioxidant activity were measured. Phytochemical analysis revealed that the ME and DCMF contained significant amounts of phenolics (ME: 409.17 ± 16.38 mg/g TAE; DCMF: 623.98 ± 14.32 mg/g TAE) and flavonoids (ME: 377.82 ± 8.29 mg/g RE; DCMF: 192.33 ± 7.26 mg/g RE). The animal experiment results showed that both ME and DCMF significantly reduced elevated levels of non-enzymatic antioxidants caused by gentamicin. Furthermore, ME and DCMF corrected the changes in antioxidant enzyme activities caused by gentamicin exposure. These findings suggest that the ME and DCMF of G. kola contain bioactive phytoconstituents with protective effects against gentamicin-induced nephrotoxicity. The extracts were effective in restoring antioxidant balance by normalizing endogenous antioxidant levels and antioxidant enzyme activities. This highlights the therapeutic potential of G. kola in protecting kidney function against drug-induced toxicity.

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INTRODUCTION

Nephrotoxicity resulting from drugs is still a major clinical concern, especially when using aminoglycoside antibiotics like gentamicin. With data indicating that up to 30% of patients receiving gentamicin therapy experience some renal failure, gentamicin is widely known for its nephrotoxic potential. Gentamicin is frequently given to treat severe gram-negative bacterial infections (Nadeem *et al.*, 2023). The primary mechanism of gentamicin-induced nephrotoxicity is oxidative stress, in which an excess of reactive oxygen species (ROS) is produced beyond the capacity of the body's antioxidant defenses, resulting in inflammation, cellular damage, and impaired renal function (Mahi-Birjand *et al.*, 2020; Gamaan *et al.*, 2023). The search for nephroprotective drugs that can lessen this oxidative damage and maintain renal function while taking gentamicin is becoming more and more urgent.

Natural compounds with cytoprotective, anti-inflammatory, and antioxidant qualities—especially those originating from medicinal plants—have become attractive options for nephroprotection. The medium-

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sized tree endemic to Central and West Africa, *Garcinia kola* (Heckel), is one of these that has attracted a lot of interest. Often referred to as "bitter kola," *G. kola* has a wide range of medicinal applications in African traditional medicine due to its antibacterial, anti-inflammatory, and antioxidant properties (Amira *et al.*, 2020; Ogwu *et al.*, 2024). Bioactive phytoconstituents, including flavonoids, tannins, saponins, and phenolic compounds, which have shown considerable antioxidant activity in numerous studies, are especially abundant in the root-bark of *G. kola* (Kazmierczak *et al.*, 2023).

Oxidative stress is characterized by an imbalance between antioxidant defenses and the production of reactive oxygen species (ROS), and this imbalance is a key component in gentamicin-induced nephrotoxicity. Renal damage is the outcome of this imbalance, which also causes lipid peroxidation, protein oxidation, DNA damage, and apoptosis (Gao *et al.*, 2021). Antioxidants have demonstrated potential in reducing reactive oxygen species (ROS) and boosting endogenous antioxidant defenses, both of which are necessary to shield renal cells from oxidative injury (Alsawaf *et al.*, 2022). Prior research has demonstrated that flavonoids and phenolic compounds, which are essential elements of numerous plant extracts, can regulate oxidative stress through the elimination of free radicals and the activation of antioxidant enzymes (Guerreiro *et al.*, 2022). Due to *G. kola*'s high phenolic and flavonoid content, there is considerable interest in its potential to reduce oxidative stress and guard against nephrotoxicity.

Several plant extracts have demonstrated potential in repairing kidney damage caused by gentamicin in the context of nephrotoxicity. For example, in experimental models of nephrotoxicity, extracts from *Nigella sativa*, *Curcuma longa*, and *Moringa oleifera* have been shown to reduce oxidative stress and restore antioxidant equilibrium (Mahi-Birjand *et al.*, 2020). Though *G. kola*'s pharmacological qualities are widely known, less is known about the nephroprotective benefits of its root-bark extracts, especially when it comes to drug-induced kidney injury. Using a rat model that has been rendered nephrotoxic by gentamicin, the goal of this study is to examine any potential nephroprotective and antioxidant qualities of G. kola root-bark methanol extract (ME) and dichloromethane fraction (DCMF).

The primary mechanism of gentamicin's nephrotoxic effects is drug buildup in the renal proximal tubules, where it causes the generation of reactive oxygen species (ROS) and consequently causes necrosis, apoptosis, and mitochondrial dysfunction (Pakfetrat *et al.*, 2022). Key antioxidant enzymes, including glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), are disrupted by these harmful effects, and they are essential for neutralizing reactive oxygen species (ROS) and preserving cellular redox homeostasis (Stankovic *et al.*, 2020). Due to their ability to prevent or reverse oxidative damage to kidney tissues, plant-based antioxidants are assumed to possess nephroprotective qualities. This is because they can balance the production of reactive oxygen species (ROS) and the activity of antioxidant enzymes.

This study's main goal is to evaluate the *G. kola* root-bark extracts' nephroprotective efficiency in reducing gentamicin-induced kidney damage. The antioxidant ability of the root-bark's methanol extract (ME) and dichloromethane fraction (DCMF) is specifically investigated in this work by evaluating the phenolic and flavonoid content of the bark and its influence on significant indicators of oxidative stress and nephrotoxicity. This study uses a gentamicin-induced nephrotoxic rat model to investigate the potential benefits of *G. kola* for regulating antioxidant enzyme activity, restoring endogenous antioxidant levels, and preventing kidney injury.

MATERIALS AND METHODS

Materials

Plant Identification

This commercial tree's shallow root was uprooted, cleaned, and transported to the IFE Herbarium at Obafemi Awolowo University (OAU), Nigeria, in order to get the root bark of G. kola. The specimen number of the plant material was obtained (IFE 17304). The roots were peeled, air-dried for eight weeks, and then ground to powdery form.

Experimental Animals

OAU Animal House provided the forty (40) *Rattus novergicus* experimental animals used in this investigation. During their two weeks of acclimatization in the Department of Biochemistry's Animal House, the animals were provided with free access to rat feed and drink.

Ethical Clearance

Ethical clearance was obtained at the Institute of Public Health, Obafemi Awolowo University, Ile-Ife, Nigeria, with reference number HREC NO: IPHOAU/12/1424.

Methods

Preparation of the Plant Material and the Extract

A cold extraction method using 2 L of 80% (v/v) methanol was used to extract 500 g of powdered root bark over 48 hours. After the supernatant was strained, the residue was resoaked in 2 L of recently made 80% (v/v) methanol for 48 hours, and the supernatant was once more strained. The methanol extracts were pooled and filtered. With the use of a rotary evaporator, the filtrate pooled together was concentrated, labelled ME, and preserved in the refrigerator for biochemical analyses. A portion of the methanol extract (ME) obtained was fractionated using a separating funnel with n-hexane (for the removal of lipophilic substances) and dichloromethane to obtain dichloromethane fraction (DCMF).

Phytochemical Screening of ME and DMCF

Phytochemical screening of ME and DCMF was performed qualitatively utilizing thin-layer chromatography for alkaloids, diterpenes, cardiac glycoside, saponin, flavonoid, and tannins.

Estimation of Total Phenolic Content (TPC)

With a little modification, the estimation of TPC of the ME and DCMF was carried out as described by Singleton *et al.* (1999) using the technique of Folin-Ciocalteau's Phenol Reagent (FCPR) Reaction. The reaction mixture consisted of 1.5 ml of a 10% (v/v) FCPR solution, 0.8 ml of distilled water, 1.0 ml of methanol extract, and 1.0 ml of DCMF. One milliliter of 10% (w/v) $NaHCO_3$ was added after five minutes. The mixture was incubated at room temperature for ninety minutes. At 725 nm, the absorbance was measured. The standard phenolic substance that was utilized to create a standard calibration curve and interpolate the phenolic content of ME and DCMF was tannic acid.

Estimation of Flavonoid Concentrations of ME and DCMF

The quantification of the amount of flavonoid present in ME and DCMF was evaluated as explained by Zhishen *et al.* (1999) based on the principle of the aluminium chloride colorimetric reaction method. The reaction mixture was formed by adding distilled water (1.9 ml), 0.3 ml of 5% (w/v) $NaNO_2$, 0.3 ml of 10% (w/v) $AlCl_3$, and 2.0 ml of 4% (w/v) NaOH to the 1.0 ml volume of ME/DCMF (1 mg/ml) that had been added to the reaction mixture. The reaction solutions were incubated at 25 ^{O}C for 10 minutes, and then the absorbance at 500 nm was measured. The flavonoid content of ME and DCMF was interpolated using a standard calibration curve prepared using rutin as the reference flavonoid component.

Evaluation of the (DPPH)

The methanol extract and DCMF were subjected to the DPPH radical scavenging assay using Blois's (1985) method, which Bode and Oyedapo (2011) slightly modified. A 1.0 ml of 0.3 mM DPPH was mixed with 1.0 ml of ME/DCMF in 10 mM acetate buffer (pH 5.5). The reaction mixture was allowed to sit in the dark for half an hour. At 517 nm, absorbance was measured using ascorbic acid as the standard. Based on the percentage inhibition of DPPH, the following expression was used to calculate the ME/DCMF/standard's % DPPH-free radical scavenging activity:

$$\text{Percentage Scavenging Activity} = \frac{Abs_{control} - Abs_{test}}{Abs_{control}} \times 100$$

Where $Abs_{control}$ is the control absorbance, and Abs_{test} is the test absorbance at 517 nm

The IC_{50} values of ME/DCMF/standard were deduced from the plot of I_{DPPH}% against the concentrations of the ME/DCMF/standard.

Assay of Reducing Power of ME and DCMF

An assay of reducing antioxidant power was conducted using Oyaizu's (1986) methodology procedure. The compound's capacity to convert Fe^{3+} to Fe^{2+} serves as the basis for the assay. When Fe^{3+} is reduced, its reddish-brown color turns green. Higher reducing power is indicated by the reaction mixture's enhanced absorbance at 700 nm. Different amounts of methanol extract and fractions (1 ml, ranging from 0 to 250 µg/ml) were combined with 2.5 ml of 0.2 M phosphate buffer pH 6.6 and 2.5 ml of 1% (w/v) potassium ferricyanide $[K_3Fe(CN)_6]$. After 20 minutes of incubation at 50 ^{O}C, 2.5 ml of 10% (w/v) trichloroacetic acid was added to the mixture, followed by a 10-minute centrifugation at 3000 rpm. $FeCl_3$ (2.5 ml, 0.1%) and distilled water (2.5 ml) were combined with the supernatant (2.5 ml). Then, at 700 nm, the absorbance was measured in comparison to the reagent blank. As a reference, ascorbic acid (0–250 µg/ml) was utilized.

Induction of Nephrotoxicity and Treatment of Experimental Rats with ME and DMCF

Gentamicin (80 mg/kg bwt, orally) was given to induce nephrotoxicity, as established by Ataman et al. (2018). There was post-administration (administration of gentamicin 3 hours after treatment with extracts) and pre-administration (administration of gentamicin 3 hours before treatment with extracts). Forty healthy rats were used in this finding, and they were grouped into eight of 5 rats each. The methanol extract and the dichloromethane fraction were reconstituted in distilled water to give final concentrations of 250 mg/kg bwt. The suspensions were given to the rats orally for 14 days. The dose of the ME and DCMF was based on the previous study (Komolafe *et al.,* 2016)

Group I: Animals received water (Control)

Group II: Animals received 80 mg/kg bwt Gentamicin

Group III: Animals received 250 mg/kg bwt ME

Group IV: Animals received 250 mg/kg bwt DCMF

Group V: Animals received 250 mg/kg bwt ME 3 h before 80 mg/kg bwt Gentamicin

Group VI: Animals received 250 mg/kg bwt DCMF 3 h before 80 mg/kg bwt Gentamicin

Group VII: Animals received 80 mg/kg bwt Gentamicin 3 h before 250 mg/kg bwt ME

Group VIII: Animals received 80 mg/kg bwt Gentamicin 3 h before 250 mg/kg bwt DCMF

Animal Sacrifice

After an overnight fast under light ether anesthesia, the animals were sacrificed on day 15. The blood samples,

through a cardiac puncture, were carefully collected into the heparinized bottles, and kidneys were carefully removed and cleaned with physiological saline. A section of the kidney was kept for histological evaluation in 10% buffered formalin.

Blood Samples Preparation

Samples of collected blood were spun at room temperature for ten minutes at 3000 rpm. Supernatant, or plasma, was carefully removed with a sterile pipette, kept in a deep freezer, and used to measure biochemical markers.

Kidney Homogenates

The post-mitochondrial fraction of kidney homogenates (10%, w/v) was obtained. One gram of kidney was cut into pieces and thoroughly homogenized with 10 milliliters of 100 mM phosphate buffer (pH 6.8). The homogenates were put into centrifuge tubes and spun for 10 minutes at room temperature and 3000 rpm, following the previously described procedure. After the homogenate was centrifuged, the supernatant was carefully transferred into a vial, frozen, and stored for biochemical analyses.

Biochemical Analyses

Assay of Non-enzymatic Antioxidant Potentials

Reduced Glutathione (GSH) Concentrations

The kidney's GSH level was estimated, as discussed by Moron *et al.* (1979). Kidney homogenate (1 ml) was put into a sterile test tube together with 4 ml of 5% (w/v) trichloroacetic acid (TCA), mixed, and centrifuged for 10 minutes at 4000 rpm. Supernatant that had been gathered was employed. Two milliliters of freshly made 5,5'-dithiobios-2-nitrobenzoic acid (DTNB) (0.6 mM in 0.2 M phosphate buffer) were added to 0.9 ml of 0.2 M sodium phosphate buffer, pH 8.0, and 0.1 ml of supernatant were collected into a test tube. In comparison to the blank, the intensity of the yellow color that had emerged after 10 minutes was assessed at 412 nm. Expressed in µgGSH/ml sample, the GSH level was calculated by interpolating the glutathione standard calibration curve.

Estimation of Malondialdehyde Concentration

The estimation of lipid peroxidation levels was examined with thiobarbituric acid (TBA) reactive species principles as described in the method of Buege and Aust (1978) with a slight modification.

MDA (product of lipid peroxidation) + TBA form MDA-TBA adduct. The assay combination was composed of 500 µl of kidney homogenates, 500 µl of phosphate buffer (pH 7.4 + 1.15% (w/v) KCl), and 2 ml of 24% (w/v) trichloroacetic acid (TCA) in a test tube. The tube was then incubated at 50 ^{O}C for 10 minutes, spun for 10 minutes at 4000 rpm, and the top layer was collected. After collecting 1.0 ml of the upper layer, 2 ml of TBA (0.16% w/v) dissolved in 20% v/v acetic acid was added to the test tube. After 10 minutes of 95 ^{O}C incubation, the mixture was allowed to cool. In comparison to the reagent blank, the absorbance was measured at 532 nm. The MDA levels were calculated from the expression;

$$\text{MDA level} = \frac{\Delta Abs \; x \; Tv}{\varepsilon \; x \; Sv}$$

where, ΔAbs = (change in absorbance); Tv = (total assay volume); Sv = (sample volume); and ε = extinction coefficient of MDA (1.56 x10^5 $M^{-1}cm^{-1}$).

Assay of Antioxidant Enzymes

(i) Assay of Superoxide Dismutase (SOD) Activities

SOD activities were accessed using the Misra and Fridovich (1972) methodology. To create a 1: 5 dilution, 200 µl of kidney homogenate was combined with 800 µl of distilled water. The homogenate (200 µl) that had been diluted was combined with 2.5 ml of freshly made 0.05 M carbonate buffer (pH 10.2), and 0.3 ml of freshly made epinephrine (0.3 mM) was added to the cuvettes. The mixture was then inverted. For 2.5 minutes, the rise in absorbance was measured at 480 nm at 30-second intervals in comparison to the blank, which was made up of buffer rather than homogenate.

In terms of percentage inhibition of epinephrine oxidation, the SOD activity was reported. Change in absorbance of substrate per min = $\frac{A_5 - A_1}{2.5 \; min}$

Where A_5 = absorbance at 150 sec. A_1 = absorbance at 30 sec.

$$\text{Percentage Inhibition} = \frac{\Delta AS}{\Delta AB} \; x \; 100$$

ΔAS = Change in absorbance of the substrate, ΔAB = Change in absorbance of blank

One unit SOD is defined as the quantity of enzyme required to initiate 50% inhibition of the oxidation of epinephrine.

(ii) Peroxidase Activity

The peroxidase activity was accessed according to the method reported by Reddy *et al.* (1995). In the cuvette, 0.05 M pyrogallol solution (3 ml) and liver homogenate (0.02 ml) were combined. Next, 0.5 ml of 1% (v/v) H_2O_2 was added and thoroughly mixed. For three minutes, the absorbance was measured at 430 nm every 30 seconds.

The peroxidase activity was calculated as:

$$\text{Peroxidase Activity} = \frac{\Delta Abs/\min \; x \; TV \; x \; df}{\varepsilon \; x \; Ve}$$

Where extinction coefficient (ε) was 11.3, Ve = volume of enzyme

TV = total assay volume, df = dilution factor

(iii) Catalase Activity

The kidney homogenate Catalase activities were examined using Sinha's (1972) methodology. Two milliliters of 0.2 M hydrogen peroxide and 2.5 ml of 0.01 M phosphate buffer (pH 7.0) were added to separate test tubes containing kidney homogenate (200 µl, 1:5) dilution. The reaction mixture (1.0 ml) was rapidly pipetted into a second test tube along with 2 ml of potassium dichromate (5 % w/v) prepared with glacial acetic acid at a ratio of 1:3. For three minutes; this was carried out once every sixty seconds. The combination was heated for 10 minutes in a boiling water bath and then allowed to cool before absorbance was measured at 570 nm. A standard calibration curve for the measurement of catalase activity was created using hydrogen peroxide concentration ranges of 1, 2, 4, 6, 8, and 10 µM. Catalase Activity (µmol/min/mg protein) = $\frac{H_2O_2 \text{ consumed}}{mg\ protein}$

Statistical Analysis

Mean ± SEM, n = 5, was the data's expression. One-way ANOVA was used with GraphPad Prism 3 to ascertain differences between the control and treated groups, for p is less than 0.05.

RESULTS AND DISCUSSION

Although phytochemicals are not recognized nutrients, they are physiologically active substances that are present in minute amounts in plants and greatly aid in protecting them from plant predators as well as degenerative human diseases (Rabizadeh *et al.*, 2022). The flavonoids, tannins, cardiac glycosides, saponins, alkaloids, and terpenoids found in the root bark of *G. kola* belong to this group of phytoconstituents. Research has indicated that flavonoids possess biological actions such as anti-inflammatory, antioxidant, antiviral, and anti-carcinogenic qualities (Ullah *et al.*, 2020). According to Ullah *et al.* (2020), they are typically present in a wide range of foods, including oranges, berries, apples, and onions. Similar to *G. kola* seeds, the plant's root bark may also be a valuable source of flavonoids and their byproducts. Like the seeds of *G. kola*, the root bark of the plant could be another good source of flavonoids and their derivatives. Further investigation of the phytochemicals in the root-bark of G. kola revealed that the total flavonoid contents of DCMF and ME were found to be 377.82 ± 8.29 mg/gRE and 192.33 ± 7.26 mg/gRE, respectively, while the total phenolic contents were determined to be 409.17 ± 16.38 mg/g TAE and 623.98 ± 14.32 mg/g TAE (Table 1).

A stable free radical called 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) is used to screen extracts and phytochemicals that have antioxidant qualities. For the investigation of natural antioxidants, DPPH is quite popular (Baliyan et al., 2022). The yellow-colored diphenyl-picrylhydrazine was produced when the stable radical DPPH was reduced by the ME and DCMF. Thus, a free radical scavenging activity was evoked by the DCMF and ME of G. Kola's root bark (Figure 1).

One potential measure of the plant extract's antioxidant effectiveness could be its reducing capacity (Vera *et al.*, 2023). A higher ferric-reducing power was shown by a higher absorbance (Figure 2). According to earlier research, antioxidant activity and the ability of bioactive substances to donate electrons are related (Munteanu and Apetrei, 2021). Figure 2 illustrates DCMF and ME's reducing power relative to ascorbic acid. It was discovered that the reducing power varied depending on the concentration, with the DCMF exhibiting a higher reducing power than the ME. However, the most significant reducing power was generated by ascorbic acid. In terms of reducing capacity, compounds with higher reducing powers tend to have higher total phenolic and flavonoid content, and their reducing power indicates their potential as antioxidants (Ali *et al.*, 2021).

The reducing power of DCMF and ME in comparison to ascorbic acid is shown in Figure 2. It was found that the reduction power was concentration-dependent, with the DCMF showing a larger reducing power than the ME. Ascorbic acid, however, produced the greatest reducing power. In terms of reducing capacity, compounds with higher reducing powers tend to have higher total phenolic and flavonoid content, and their reducing power indicates their potential as antioxidants (Ali *et al.*, 2021). According to Munteanu and Apetrei (2021), substances with reducing power function as primary and secondary antioxidants by acting as electron donors.

Research has demonstrated that the existence of reductones, which have been demonstrated to exhibit antioxidant action by disrupting the free radical chain by donating a hydrogen atom, is typically linked to the reducing characteristics (Liu *et al.*, 2022). Therefore, of the *G. kola* root-bark extracts, DCMF may have the largest concentrations of reductones and polyphenolics. Gentamicin-induced changes in oxidative stress parameters are shown (Tables 2 and 3). Lipid peroxidation is a multifaceted process that results in lipid hydroperoxides (LOOH) when polyunsaturated fatty acids (PUFAs) in the phospholipids of cellular membranes react with oxygen (Valgimigli, 2023). Under conditions of oxidative stress, cellular biomembranes are damaged by free radicals, which results in lipid peroxidation and the transformation of unsaturated lipids into polar lipid hydroperoxides. A range of oxidized products, including reactive electrophiles like epoxides and aldehydes that can alter DNA, proteins, and other macromolecules, are also produced as a result of lipid peroxidation (Valgimigli, 2023). Isoprostanes, 2-propenal (acrolein), 4-hydroxy-2-nonenal (HNE), and malondialdehyde (MDA) are examined as indirect markers of oxidative stress.

In comparison to the control group, rats given an oral dose of gentamicin at a rate of 80 mg/kg bwt showed a 32.77% increase in MDA in kidney homogenates (Table 2). That could be because gentamicin induces lipid peroxidation through oxidative stress. The outcome is consistent with the observation made by Tomşa *et al.* (2021) that gentamicin treatment raised the MDA level in

nephrotoxic rats. Nonetheless, renal homogenates' MDA levels significantly decreased both before and after ME and DCMF treatments.

The tripeptide glutathione (GSH) is made up of cysteine, glycine, and γ-glutamic acid. It takes part in reactions that detoxify peroxides and free radicals, as well as those that involve the creation of proteins and nucleic acids (Georgiou-Siafis *et al.*, 2023). In addition to shielding cells from the damaging effects of pollution, glutathione strengthens the body's defenses against illness. Glutathione plays a critical role in antioxidant defense by providing electrons to antioxidant enzymes such as glutaredoxins and peroxidases. According to this study, the structural integrity and functionality of the cell and organelle membrane are jeopardized when the GSH content is reduced because of the generation of reactive oxygen species (ROS) and oxidative stress. After 14 days of therapy, the renal homogenates of the rats in this study showed a considerable drop in GSH levels (302.34%) following 80 mg/kg bwt gentamicin, compared to normal animals. Before and after the ME treatments, the GSH levels in kidney homogenates increased by 171.93% and 237.43%, respectively, in comparison to the group that received gentamicin alone. GSH levels rose by 172.51% and 219.88%, respectively, between the DCMF pre- and post-treatment groups and the gentamicin-only group (Table 2).

Cells are susceptible to damage from reactive oxygen species (ROS), yet they possess defensive mechanisms that capture and neutralize free radicals, thereby preventing or minimizing intracellular damage. These comprise antioxidant enzymes including thioredoxins, superoxide dismutase (SOD), catalase, and glutathione peroxidase, as well as low molecular weight antioxidants such as ascorbic acid, vitamin E, and glutathione (Kumar *et al.*, 2022).

Table 1: Levels of Total Flavonoid and Total Phenolic Content

	ME	DCMF
Flavonoid Content (mg/gRE)	377.82 ± 8.29	192.33 ± 7.26
Phenolic Content (mg/gTAE)	(409.17 ± 16.38)	623.98 ± 14.32

Mean ± SEM, n=3 readings, P ≤ 0.05
ME = Methanol Extract DCMF = Dichloromethane Fraction

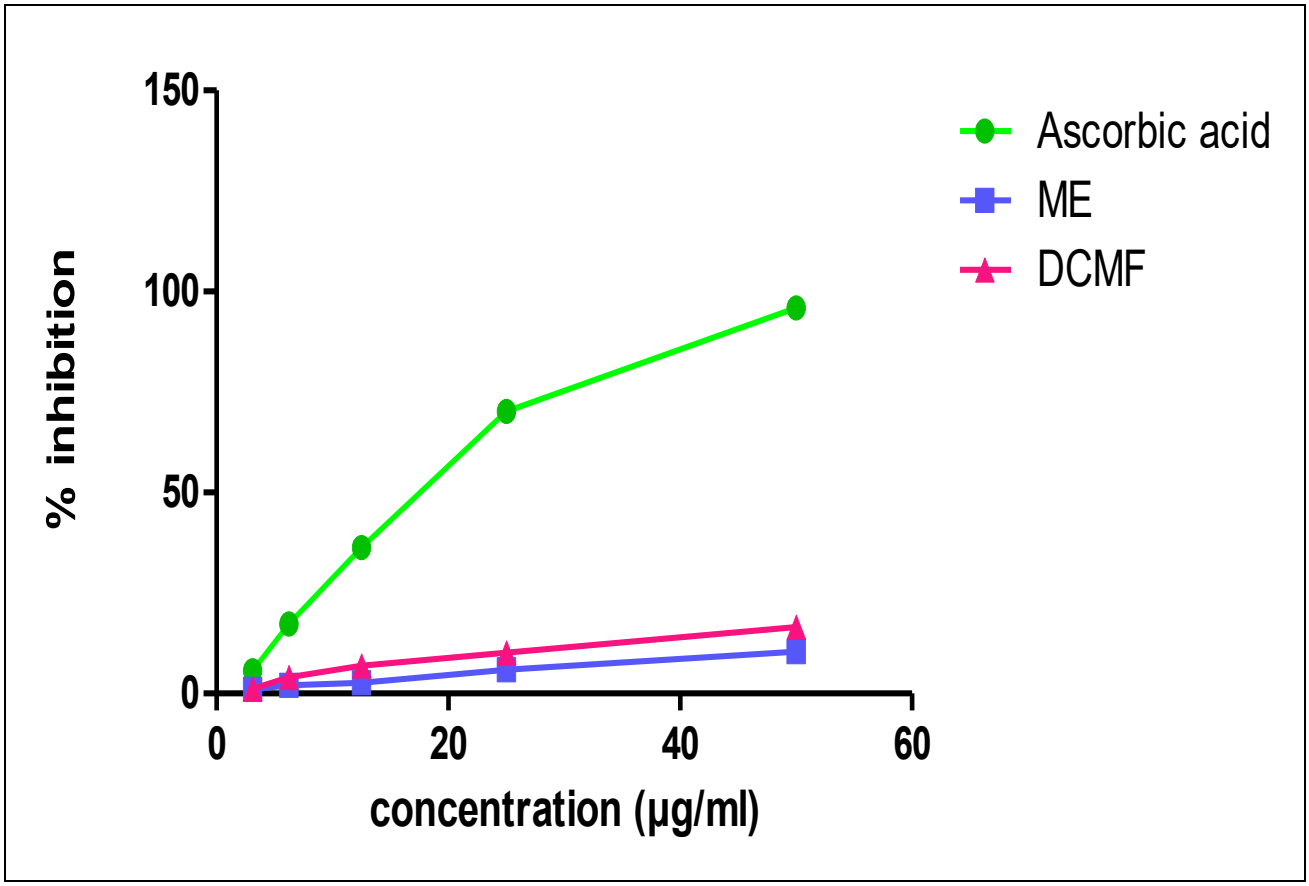

Figure 1: Representation of Percentage Inhibition of DPPH
Mean ± SEM, n=3 readings, P ≤ 0.05
ME = Methanol Extract DCMF = Dichloromethane Fraction

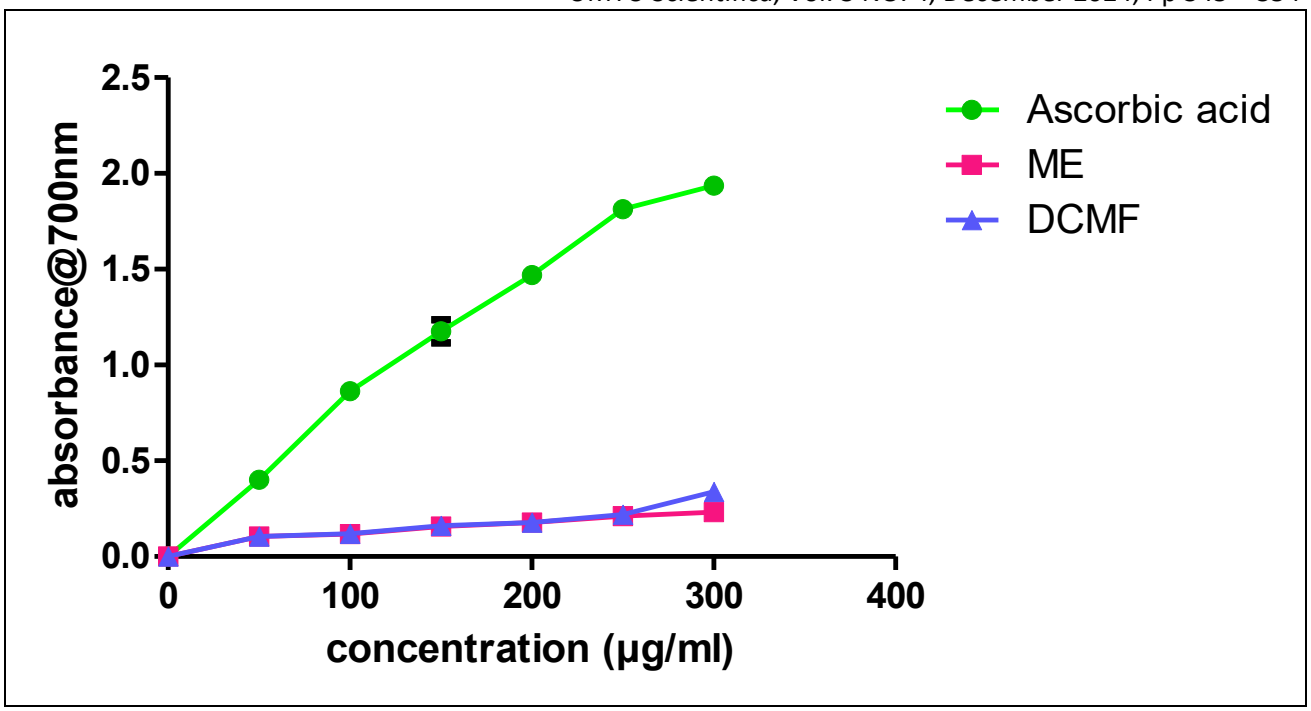

Figure 2: Reducing Antioxidant Power
Mean ± SEM of n = 3.
ME = Methanol Extract
DCMF = Dichloromethane Fraction

Table 2: Summary of the Concentrations of Non-antioxidants Enzymes in the Kidney of Experimental Animals

Group	GSH level (µg/ml)	MAL MDA(µmole)
I Animals rec. water (Control)	6.88 ± 0.63	4.41 ± 0.95
II Animals rec. 80 mg/kg bwt Gent.	1.71 ± 0.60[a]	6.56 ± 0.83
III Animals rec. 250 mg/kg bwt ME	5.06 ± 1.08[c]	5.53 ± 0.70[c]
IV Animals rec. 250 mg/kg bwt DCMF	7.02 ± 1.15[b]	5.62 ± 0.76[c]
V Animals rec. 250 mg/kg bwt ME 3 h before 80 mg/kg bwt Gent.	4.65 ± 0.48[c]	5.99 ± 0.59[c]
VI Animals rec. 250 mg/kg bwt DCMF 3 h before 80 mg/kg bwt Gent	4.66 ± 1.09[c]	5.76 ± 1.31[c]
VII Animals rec. 80 mg/kg bwt Gent. 3 h before 250 mg/kg bwt ME	5.77 ± 1.22[b]	5.87 ± 0.86[c]
VIII Animals rec. 80 mg/kg bwt Gent. 3 h before 250 mg/kg bwt DCMF	5.47 ± 0.43[c]	5.99 ± 1.27[c]

Mean ± SEM, n=5 readings, P ≤ 0.
a = significantly different from I
b = significantly different from II
c = not significantly different from II.
ME = Methanol Extract
DCMF = Dichloromethane Fraction
MAL= Malondialdehyde
rec. = received
Gent. = Gentamicin

A broad family of enzymes known as peroxidases is normally in charge of shielding cells from harm caused by free radicals such as hydrogen and lipid peroxides. Hydrogen peroxide is the best substrate for a lot of these enzymes, while some are more active with organic hydroperoxides, like lipid peroxides (Abdolahinia *et al.*, 2022). In this investigation, gentamicin administered orally dramatically raised peroxidase activity levels by 70%. This could be because gentamicin increased the creation of free radicals, which in turn caused the kidney to overproduce or overexpress this enzyme to eliminate excess peroxides and reactive oxygen species. When kidney homogenates were treated with ME and DCMF before and after, the peroxidase activity was much lower than in the group that received gentamicin alone. The outcomes demonstrated the ability of *G. kola* root bark ME and DCMF to scavenge reactive oxygen species (ROS), which ended peroxidation chain reactions and enhanced the stability and quality of biomembranes. Proteins called superoxide dismutases (SODs) that contain metals catalyze the breakdown of superoxide, producing hydrogen peroxide as a result. In contrast to the group that received gentamicin alone, the kidney homogenates' SOD activity decreased both before and after the ME and DCMF treatments.

The enzyme catalase (CAT) protects cells against internal hydrogen peroxide production. As a component of their adaptive response, cells must learn to tolerate oxidative stress (Anwar et al., 2024). In the present study, rats given gentamicin had higher levels of CAT activity than rats given control. Gentamicin may be the source of this, as it generates free radicals that lead to the kidney overproducing or overexpressing this enzyme. The CAT activity of the kidney homogenates was lower following the pre- and post-treatments with ME and DCMF compared to the group that received gentamicin alone.

Table 3: Activities of Antioxidant Enzymes in the Kidney of Experimental Animals

Group	Peroxidase unit/min/mg protein	SOD U/min/mg protein	Catalase U/min/mg protein
I	0.09 ± 0.02	12.31 ± 0.91	8.40 ± 0.78
II	0.30 ± 0.04[a]	12.57 ± 1.29	9.67 ± 0.77
III	0.11 ± 0.02[b]	8.10 ± 1.56[c]	9.01 ± 1.01[c]
IV	0.18 ± 0.03[c]	13.07 ± 3.43[c]	8.47 ± 1.94[c]
V	0.18 ± 0.03[c]	11.28 ± 1.40[c]	8.56 ± 1.08[c]
VI	0.14 ± 0.02[b]	10.42 ± 1.25[c]	9.17 ± 0.66[c]
VII	0.13 ± 0.01[b]	12.24 ± 1.35[c]	8.90 ± 0.94[c]
VIII	0.15 ± 0.06[b]	12.31 ± 5.43[c]	11.84 ± 4.23[c]

I = Animals received water (Control)
II = Animals received 80 mg/kg bwt Gentamicin
III = Animals received 250 mg/kg bwt ME
IV = Animals received 250 mg/kg bwt DCMF
V = Animals received 250 mg/kg bwt ME 3 h before 80 mg/kg bwt Gentamicin
VI = Animals received 250 mg/kg bwt DCMF 3 h before 80 mg/kg bwt Gentamicin
VII = Animals received 80 mg/kg bwt Gentamicin 3 h before 250 mg/kg bwt ME
VIII = Animals received 80 mg/kg bwt Gentamicin 3 h before 250 mg/kg bwt DCMF
Mean ± SEM, n=5 readings, P ≤ 0.
a = significantly different from I
b = significantly different from II
c = not significantly different from II.
ME = Methanol Extract DCMF = Dichloromethane Fraction

CONCLUSION

In conclusion, the kidneys were gravely harmed by the administration of gentamicin, which had detrimental nephrotoxic effects. The root-bark fractions of *Garcinia kola*, when extracted with dichloromethane or methanol, effectively recovered the altered values of biochemical parameters. These results were probably influenced by several biochemical and molecular processes that were regulated by polyphenolics. The bioactive components of the dichloromethane fraction (DCMF) and methanol extract (ME) of *G. kola* root-bark decreased the oxidative damage brought on by gentamicin and improved the kidneys' capacity for self-healing and regeneration.

To sum up, the root bark of *Garcinia kola*, with its diverse phytochemical composition, presents a possible natural remedy for preventing drug-induced nephrotoxicity. Gaining knowledge of its nephroprotective mechanisms may help create alternative treatments that use the body's natural antioxidants to reduce kidney damage and oxidative stress. This work adds to the increasing amount of data that supports the use of medicinal plants to preserve kidney function and emphasizes the potential use of *G. kola* as a treatment for nephrotoxicity brought on by commonly used medications like gentamicin.

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