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Protective effects of extract and fraction of root- bark of *Garcinia kola* (Heckel) on the renal biochemical parameters of gentamicin-induced nephrotoxic rats

Komolafe, Ige Joseph*, Akinlalu, Akintunde Oluseto, Ogunsusi, Mayowa and Oyedapo, Oluboade Oluokun

Department of Biochemistry, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria.

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The study evaluated the nephroprotective potentials of methanolic extract (ME) and dichloromethane fraction (DCMF) of root-bark of *Garcinia kola* on gentamicin-induced nephrotoxicity in rats with a view to utilize the extracts of the root of *G. Kola* in the amelioration of antibiotic-induced kidney damage. The study involved preparation of the extract, fractionation, phytochemical screening and evaluation of phenolics and flavonoid contents. Moreover, both the acute and sub-acute toxicities of ME and DCMF were determined in mice and albino rats with concentrations ranging from 10 to 5000 mg/kg body weight (bwt) respectively. Forty albino rats were divided into 8 groups of five animals each and induced with gentamicin (80 mg/kg bwt). The renal biochemical metabolites and enzymes were quantified and assayed respectively. The histopathological examinations of kidney were carried out. Phytochemical screening of the ME and DCMF of the root-bark of *G. kola* revealed the presence of flavonoids, tannins, cardiac glycosides, saponins, alkaloids and terpenoids. The extract and DCMF did not elicit any adverse effect and a dose of 250 mg/kg bwt of ME and DCMF was selected for further studies. The administration of gentamicin (80 mg/kg bwt) caused elevated levels of plasma renal biomarkers, reduction in AciPase activities. Moreover, administration of gentamicin (80 mg/kg bwt) resulted in damage to kidney structures. The treatments (pre and post) of the rats with 250 mg/kg extract and fraction reversed alterations of biochemical parameters which were supported by low levels of tubular and glomerular injuries induced by gentamicin treatment. The study concluded that, both the extract and fraction of *G. kola* root-bark elicited antioxidant and appreciable nephroprotective potentials.

Key words: Antioxidant potentials, *Garcinia kola*, Methanolic extract, Nephrotoxicity, Renal biomarkers.

INTRODUCTION

Nephrotoxicity is one of the most common kidney problems and occurs when body is exposed to a drug or

toxin (Hozayen et al., 2011). A number of therapeutic agents can adversely affect the kidney resulting in acute

*Corresponding author. Email: igekomolafe@yahoo.com

renal failure, chronic interstitial nephritis and nephrotic syndrome because there is, an increasing number of potent therapeutic drugs like aminoglycosides antibiotics, Nonsteroidal Antiinflammatory Drugs (NSAIDs) etc. Chemotherapeutic agents have been added to the therapeutic arsenal in recent years (Boudonck et al., 2009). Exposure to chemical reagents like ethylene glycol, carbon tetrachloride, sodium oxalate and heavy metals such as lead, mercury, cadmium and arsenic also induce nephrotoxicity. Prompt recognition of the disease and cessation of responsible drugs are usually the only therapy (Hozayen et al., 2011). The kidney is one of the primary sites of drug metabolism/toxicity. It is especially vulnerable to toxic insults by drugs because of the high renal blood flow. It transports, metabolizes, and concentrates chemicals present in the tubular fluid. Drugs that are known to damage the kidney glomerulus include puromycin, adriamycin, NSAIDs as well as aminoglycosides, cyclosporine, and cisplatin (Boudonck et al., 2009).

Aminoglycosides are narrow spectrum antibiotics which act mainly on aerobic gram negative bacteria including many multi-drug resistant ones. They are recommended for use in children including neonates for several serious infections like septicaemia, caused by these gram negative bacteria (Rybak and Ramkumar, 2007). Despite their beneficial effects, aminoglycosides have considerable nephrotoxic and ototoxic side effects. It has been reported that gentamicin induced free radical generation which are implicated in a variety of pathological processes (Rybak and Whitworth, 2005; Hanaa and Nora, 2012). It has been reported that at cellular level, aminoglycosides interfere with protein synthesis, especially by inhibition of translocation step (Abolfazl et al., 2011). Evidence has also suggested aminoglycoside induced inhibition of protein synthesis as one of the mechanisms of its nephrotoxicity. It has been demonstrated that elevated concentrations of aminoglycosides causes mistranslation or block incorporation of aminoacyl tRNAs into ribosomes *in vitro* (Hozayen et al., 2011). Nephrotoxicity induced by gentamicin (GM) is a complex phenomenon characterized by an increase in plasma creatinine and urea levels and proximal renal tubular necrosis, followed by deterioration of renal function and renal failure. The toxicity of aminoglycosides, including GM, is believed to be related to the generation of reactive oxygen species (ROS) in the kidney (Abolfazl et al., 2011).

Garcinia kola (Heckel) is a medium sized tree found in moist forest and widely distributed throughout West and Central Africa. The plant also known as bitter kola plant is found in countries across West and Central Africa and it is distributed by man around the towns and villages of such countries as; Nigeria, Ghana, Cameroon, Sierra Leone, Togo, Congo Democratic Republic, Angola, Liberia, Gambia etc. (Adesuyi et al., 2012). *G. kola* is regarded as a wonder plant because every part of the

plant (bark, leaves, root and seeds) has been found to be of medicinal importance. The medicinal importance of bitter kola is based mainly on its phytochemicals. From its roots to its leaves, the stem-bark, root-bark and seeds contain several phytochemicals that are noted for their medicinal importance (Adefule-Ositelu et al., 2004). The extracts of various parts of the plants are employed in the treatment of cough and as purgative (Okunji et al., 2007). The extracts of the seeds are used in the treatment of diarrhoea (Adesuyi et al., 2012), bronchitis and throat infections, liver disorders. Studies have revealed that the extracts, fractions and bioactive molecules of seeds of *G. kola* possess anti-hepatotoxic, antioxidant (Olatunde et al., 2004), hypoglycaemic, aphrodisiac anti-cancerous, anti histamic, and antimicrobial properties (Ajibade et al., 2011). Some of the chemicals that have been isolated from *G. kola* seeds included tannins, saponins, alkaloids, cardiac glycosides and flavonoid such as kolaflavone, and 2 hydroxyflavonoids (Terashima et al., 2002).

Literature is replete with scientific information on the biological activities of extracts, fractions, and compounds of *G. kola* seeds. However, there is dearth of scientific information on the activities of the extract, fractions and compounds of the root-bark of *G. kola*. This study was undertaken to provide baseline information on the antioxidant properties and nephroprotective potentials of the root-bark of *G. kola* on gentamicin-induced kidney damage.

MATERIALS AND METHODS

Collection and identification of plant material

Fresh roots of *G. kola* were collected at Babajakan Village, Ayedaade Local Government Area, Osun State, Nigeria. The plant was identified and authenticated at IFE Herbarium, Obafemi Awolowo University, Ile-Ife where the specimen copy was deposited and specimen number collected (IFE 17304).

Reagents and chemicals

All the reagents used in the study were of analytical grade. Methanol, dichloromethane, glacial acetic acid, sodium chloride, sodium hydrogen carbonate, hexane, trisodium citrate were procured from Sigma-Aldrich USA. Randox Diagnostic Kits for the assays of uric acid, creatinine, urea, protein concentrations, gamma glutamyltransferase (GGT) and alkaline phosphatase activities were procured from Randox Laboratory Limited United Kingdom.

Experimental animals

Healthy white albino rats of either sex (40) were purchased from the Animal House, Faculty of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria. Rats were acclimatized for two weeks, having free access to standard rat chow (Ladokun Feeds Limited, Ibadan) and were provided clean water.

Preparation and fractionation of methanolic extract of *G. kola* root-bark

Fresh roots were washed under running water, peeled, cut into

bits, air-dried at room temperature and then pulverized into a fine powder. Powdered root-bark (500 g) was extracted with 2 L methanol (70% v/v) for 48 h (cold extraction). The extract was collected, the residue re-soaked and re-extracted with the same solvent for additional 48 h. The extraction was carried out 5 more times until enough extracts were obtained. The methanolic extracts were combined, filtered through Whatman Paper No. 1, and followed by centrifugation at 3000 rpm for 15 min on Bench Centrifuge (Model 800D, Microfield Instrument, Essex, England) and was concentrated to dryness *in vacuo* at 40°C. The methanolic extract (15 g) was dissolved in hot distilled water (200 ml) in a separating funnel, followed by partitioning with n-hexane (150 ml x 5) to remove the fat soluble molecules from the extract. The aqueous portion was further partitioned with dichloromethane (150 ml x 5) to obtain the dichloromethane fractions (DCMF). The fractions were pooled and subjected to evaporation to dryness under reduced pressure. The fractionation was carried out five times to afford enough extracts. Both methanolic extract (ME) and DCMF were kept in the desiccator until required for further analyses and biological assays.

Estimation of total phenolic and flavonoid contents

The total phenolic contents of the ME and DCMF were determined using the Folin-Ciocalteu's Phenol reagent reaction method of Singleton et al. (1999). The determination of the total flavonoids content of the ME and DCMF was based on the aluminium chloride colorimetric reaction method according to the procedure of Zhishen et al. (1999).

Evaluation of acute toxicity of the extract and fraction

The acute toxicity study was carried out according to the method of Lorke (1983) using mice of 20-25 g bwt. The mice were divided into six groups of three animals (n = 3) each and were orally administered with a single dose of methanolic extract at increasing dose levels of 10, 100, 1000, 1600, 2900 and 5000 mg/kg bwt respectively. The animals were observed continuously for 2 h for gross behavioural changes and then intermittently once every 2 h and finally at 24 and 72 h. This was also carried out with DCMF.

Preparation and administration of extracts

The appropriate weight of ME/DCMF was dissolved in 2% (v/v) (DMSO) and diluted to a final concentration of 250 mg. The extracts were given orally to the experimental animals for a period of 14 days. The animals were also treated with gentamicin (80 mg/kg bwt) orally. Forty healthy wistar rats of either sex were divided into eight (8) groups of five (n=5) animals each and were treated as follows:

- Group I. Rats + 2% (v/v) DMSO (control);
- Group II. Rats + Gentamicin (80 mg/kg bwt)
- Group III. Rats +Methanolic extract of *G. kola* (250 mg/kg bwt) only;
- Group IV. Rats + DCMF of *G. kola* (250 mg/kg bwt) only;
- Group V. Rats +Methanolic extract (250 mg/kg bwt) 3 h before Gentamicin (80 mg/kg bwt);
- Group VI. Rats+ DCMF (250 mg/kg bwt) 3 h before Gentamicin (80 mg/kg bwt);
- Group VII. Rats + Gentamicin (80 mg kg bwt) 3 h. before Methanolic extract (250 mg/kg bwt)
- Group VIII. Rats + Gentamicin (80 mg kg bwt) 3 h. before DCMF (250 mg/kg bwt).

Sacrificing of experimental animals

On day 15, the animals were sacrificed after overnight fasting with light ether anaesthetics. The animals were dissected, blood was collected by cardiac puncture into heparinized bottles and mixed gently. The kidneys were aseptically removed and washed with physiological saline (0.9% (w/v) NaCl), dried between filter paper. Portions of the kidney was preserved in 10% (v/v) buffered formalin for histopathological assessment. The remaining kidney tissues were stored frozen in deep freezer for further analysis.

Preparation of blood plasma

Blood samples collected were centrifuged at 3000 rpm on bench centrifuge (Model 90-2, Microfield Instrument, Essex, England) for 10 min. at room temperature. The plasma (supernatant) was carefully removed with pasture pipette, kept in the deep freezer and used for the analysis of biochemical parameters. Aliquots (1 ml) of blood samples were reserved for the estimation of haemoglobin concentrations.

Preparation of kidney homogenates

Ten percent (10% w/v) kidney homogenates (post mitochondrial fraction) was prepared according to the procedure reported by Olagunju et al. (2000). 1 g of the kidney was weighed, cut into bits and thoroughly homogenized with 10 ml of freshly prepared 100 mM Phosphate buffer, pH 6.8. The homogenates were transferred into centrifuge tubes and centrifuged at 3000 rpm for 10 min. at room temperature as above. The supernatants were carefully transferred into vial bottles, and stored frozen and used for biochemical assays.

Biochemical analyses

The plasma creatinine was estimated as described by Bartels and Bohmer (1972) using the Randox Diagnostic kit. The creatinine concentration in the sample was calculated from the expression:

$$\text{Concentration of Creatinine (mg/dL)} = \frac{\Delta \text{abs}_{\text{sample}}}{\Delta \text{abs}_{\text{standard}}} \times \text{standard conc. (mg/dL)}$$

The plasma uric acid and urea concentrations were estimated as described by Fossati et al. (1980) and Weatherburn (1967), respectively using the Randox Diagnostic kits. The estimation of plasma and kidney homogenate total proteins was estimated using biuret method (Tietz, 1995) using Randox Diagnostic kit. Haemoglobin was quantified as cyanmethaemoglobin (Mattenther, 1971) with slight modification. The reaction mixture contained whole blood (50 µl) and 4.5 ml of haemoglobin reagent. The blank contained distilled water (50 µl) and 4.5 ml of haemoglobin reagent. The reaction mixtures were mixed and incubated at room temperature for 20 min. The absorbance was read against blank at 540 nm. Haemoglobin concentration was calculated using the expression:

$$\text{Hb concentration (g/dL)} = \frac{\text{Abs test} \times 16114}{11.0 \times 10^3} \times 0.1 \times \frac{\text{tv}}{\text{sv}}$$

Plasma Gamma glutamyltransferase was estimated as described by Teitz (1987) using the Randox Diagnostic kit. The assays of acid and alkaline phosphatases activities were carried out according to the method of Saini and Van Etten (1979) as slightly modified

Table 1. Levels of concentrations of urea, uric acid, creatinine and blood haemoglobin of normal and treated animals.

Group	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)	Haemoglobin (mg/dL)
I Rats + 2% (v/v) DMSO	11.61 ± 0.72	1.55 ± 0.24	2.46 ± 0.14	12.98 ± 0.89
II Rats + Gentamicin (80 mg/kg bwt)	31.81 ± 3.56 ^a	3.85 ± 0.38 ^a	2.89 ± 0.33	8.56 ± 1.87
III Rats + ME (250 mg/kg bwt) only	17.65 ± 6.37	2.37 ± 0.46 ^b	2.73 ± 0.05	9.48 ± 1.54
IV Rats + DCMF (250 mg/kg bwt) only	13.70 ± 5.87	1.91 ± 0.22 ^b	2.29 ± 0.18	11.46 ± 2.42
V Rats + ME (250 mg/kg bwt) + gentamicin (80 mg/kg bwt)	14.34 ± 2.77	1.83 ± 0.15 ^b	2.55 ± 0.13	13.65 ± 0.60
VI Rats + DCMF (250 mg/kg bwt) + gentamicin (80 mg/kg bwt)	12.91 ± 3.21 ^b	1.99 ± 0.24 ^b	2.78 ± 0.11	11.06 ± 0.59
VII Rats + gentamicin (80 mg/kg bwt) + ME (250 mg/kg bwt)	20.35 ± 4.05	1.99 ± 0.40 ^b	2.20 ± 0.14	10.52 ± 1.24
VIII Rats + gentamicin (80 mg/kg bwt) + DCMF (250 mg/kg bwt)	12.04 ± 2.22 ^b	1.89 ± 0.13 ^b	2.31 ± 0.12	10.73 ± 2.25

Each value represented Mean ± SEM, n=5 readings, Value of P ≤ 0.05 was considered statistically significant. The value with superscript (a) was significantly different from that of control, values with superscript (b) were significantly different from nephrotoxic induced group (Group II).

(Oyedapo, 1996). The substrate was 10 mM p-Nitrophenyl phosphate (Na salt) in 10 mM acetate buffer, pH 4.5 containing 5 mM NaCl and 5 mM Tris- HCl buffer, pH 8.2 containing MgCl₂.6H₂O for AciPase and AlkPase respectively. The reaction was initiated by the addition of 1 ml of substrate and the reaction mixture was further incubated at 37°C for 15 min. then 2 ml of 0.02 M NaOH was added to terminate the reaction. The reaction mixture was allowed to cool thoroughly and absorbance was read at 410 nm against the reagent blank. The activities of acid and alkaline phosphatases were calculated using the expression:

$$\text{Activity } (\mu\text{mole p - nitrophenol/ml}) = \frac{\Delta\text{Abs}(410 \text{ nm})}{EXd} \times \frac{1}{t} \times 10^6 \times \frac{Tv}{Sv}$$

Abs (change in absorbance at 410 nm); t = 15 min; E (molar extinction coefficient (18.8 × 10³ M⁻¹ cm⁻¹); d = path length (1 cm); Sv = (sample volume); and Tv = (total volume).

RESULTS AND DISCUSSION

The acute toxicity studies of ME and DCMF of *G. kola* root bark revealed that, the animals that received varying concentrations (10 to 5000 mg/kg bwt) of ME and DCMF did not exhibit any clinical signs of toxicity or mortality. Thus, the results suggested that the LD₅₀ of ME and DCMF of the root-bark of *G. kola* could be higher than 5000 mg/kg bwt.

Moreover, treatment of rats with 80 mg/kg bwt gentamicin caused increase in the plasma levels of kidney function markers such as creatinine, urea and uric acid concentrations as compared to the control animals indicating renal dysfunction (Table 1). The renal dysfunction could be due to, the production of free radicals and involvement of oxidative stress caused by toxic effects of gentamicin. The urea concentration was markedly increased in the group treated with gentamicin alone from 11.61 to 31.81 mg/dl (173.99%) which is in agreement with the earlier observations of Safa et al. (2010) who showed that serum urea levels were higher in the rats treated with gentamicin alone. This could be due to inability of kidney to excrete excess urea. Both the pre

and post treatments with 250 mg/kg bwt of ME showed 54.92 and 36.03% decrease in plasma urea concentrations respectively compared to the group treated with gentamicin alone. Similarly, the pre and post treatments of the animals with 250 mg/kg bwt of DCMF showed 59.42 and 62.15% decrease in plasma urea concentrations respectively compared to the group treated with gentamicin alone. Increase in blood concentration of urea might indicate renal dysfunction (Stevens and Levy, 2005).

The pre and post treatments of the animals with 250 mg/kg bwt of ME showed 52.47 and 47.31% decrease in plasma uric acid concentrations respectively compared to the group treated with gentamicin alone. Similarly, pre and post treatments of the animals with 250 mg/kg bwt of DCMF showed 48.31 and 50.91% decrease in plasma uric acid concentrations respectively compared to the group treated with gentamicin alone.

Creatinine, a marker of muscle mass is released into the plasma at a relatively constant rate, freely filtered at the glomerulus, and not reabsorbed or metabolized by the kidneys (Dirkes, 2011). Measurement of the serum level of creatinine is a widely used method of assessing renal function (Stevens and Levy, 2005). In this study, the exposure of rats to gentamicin (80 mg/kg bwt) caused increase in the plasma creatinine concentrations. Earlier studies (Safa et al., 2010) showed that plasma creatinine and urea levels were highest in the rats that received gentamicin alone. Both the pre and post treatments with 250 mg/kg bwt of ME showed 11.78 and 23.88% decrease in plasma creatinine concentrations respectively compared with gentamicin treated group. Also, pre and post treatments of the animals with 250 mg/kg bwt of DCMF showed 3.81 and 20.07% decrease in plasma creatinine concentrations respectively compared to the group treated with gentamicin alone. In addition, administration of gentamicin (80 mg/kg bwt) caused reduction in blood haemoglobin concentrations from 12.98 ± 0.89 to 8.56 ± 1.87 mg/dl that is, 51.64% reduction compared to the normal animals (Table 1). This

Table 2. Plasma and kidney protein concentrations (g/dL).

Group	Plasma protein (g/dL)	Kidney protein (g/dL)
I Rats + 2% (v/v) DMSO	4.83 ± 0.34	0.79 ± 0.06
II Rats + Gentamicin (80 mg/kg bwt)	1.23 ± 0.40 ^a	0.69 ± 0.06
III Rats + ME (250 mg/kg bwt) only	2.87 ± 0.64 ^c	0.75 ± 0.07 ^c
IV Rats + DCMF (250 mg/kg bwt) only	2.72 ± 1.02 ^c	0.76 ± 0.20 ^c
V Rats + ME (250 mg/kg bwt) + gentamicin (80 mg/kg bwt)	3.15 ± 1.10 ^c	0.81 ± 0.08 ^c
VI Rats + DCMF (250 mg/kg bwt) + gentamicin (80 mg/kg bwt)	3.46 ± 1.05 ^c	0.73 ± 0.05 ^c
VII Rats + gentamicin (80 mg/kg bwt) + ME (250 mg/kg bwt)	3.91 ± 0.77 ^c	0.77 ± 0.07 ^c
VIII Rats + gentamicin (80 mg/kg bwt) + DCMF (250 mg/kg bwt)	3.29 ± 0.59 ^c	0.78 ± 0.19 ^c

Each value represented Mean ± SEM of n = 5 readings. Value of P ≤ 0.05 was considered statistically significant. The value with superscript (a) was significantly different from normal while the values with superscript (c) were not significantly different from nephrotoxic induced group (Group II).

might be due to the breakdown of erythrocyte in the treated animals. It might due to inhibitory effect of gentamicin on protein synthesis or secretion of erythropoietin which is responsible for haemoglobin synthesis (Sundin et al., 2001). The pre-treatment of rats with ME (250 mg/kg bwt) and DCMF (250 mg/kg bwt) showed increase of 59.46 and 29.21% respectively in haemoglobin concentrations compared to the group treated with gentamicin alone. The post-treatment of rats with ME (250 mg/kg bwt) and DCMF (250 mg/kg bwt) showed increase of 22.9 and 25.35% respectively in haemoglobin concentrations compared to the group treated with gentamicin alone (8.56 ± 1.87 mg/dL). Table 2, is the summary of the effects of gentamicin (80 mg/kg bwt), ME (250 mg/kg bwt) and DCMF (250 mg/kg bwt) on the plasma and kidney protein concentrations. It was observed that oral administration of gentamicin (80 mg/kg bwt) significantly reduced plasma and kidney protein concentrations by 74.5 and 12.7% respectively when compared to the control animals. This might be due to the inhibitory effects of gentamicin on protein synthesis. This could also be due to destructive effect of gentamicin on golgi apparatus and endoplasmic reticulum which could cause mistranslation or block incorporation of amino acids and microsomal protein synthesis (Sundin et al., 2001). These results were in agreement with the results of Sundin et al. (2001) who reported significant decreased in total protein concentrations in the gentamicin-induced nephrotoxicity. Both the pre and post treatments of rats with 250 mg/kg bwt of ME showed 156.10 and 217.89% caused increase in plasma protein concentrations respectively when compared to the group treated with gentamicin alone. Similarly, pre and post treatments of the rats with 250 mg/kg bwt of DCMF reversed the inhibitory effects of gentamicin on the treated rats.

Gamma-glutamyl transferase (GGT) is a membrane-anchored enzyme that functions, to transfer a glutamyl group onto certain amino acids, important in liver function.

GGT is also found in the spleen, kidneys, pancreas, heart, brain, and seminal vesicles (Gogoi et al., 2012). It has been shown recently to be a good marker for potential type 2 diabetes developments, as a dysfunction and sensitive marker of oxidative stress (Gautier et al., 2009).

However, little information exists on its association with the prospective development of chronic kidney diseases (Ryu et al., 2007). Table 3, is the summary of the effects of ME and DCMF of root bark of *G. kola* on the activities of some metabolic enzymes. It was observed that administration of gentamicin (80 mg/kg bwt) slightly increased plasma GGT enzymatic activity by 31.36% when compared to the normal. This might be due to the released of the enzymes from kidney cells as a result of oxidative stress exerted on the kidney tubules.

Moreover, AlkPase activity was found to be highly concentrated in plasma membrane, whereas AciPase is associated with lysosomes (Abraham and Wilfred, 2000). The plasma AlkPase activities in gentamicin treated rats showed a slight increase (8.56 ± 0.68 µmol/mg protein) as compared to the normal rats (8.28 ± 2.99 µmol/mg protein) while the plasma AciPase activities showed a higher increase (37.71 ± 11.72 µmol/mg protein) (p ≤ 0.05) of 75.18% as compared to the control rats (9.36 ± 0.42 µmol/mg protein). The increase in AciPase activities might be due to the destruction of lysosome of intact membrane. The treatment with ME and DCMF before administration of gentamicin (80 mg/kg bwt) caused a significant decrease in plasma AciPase activities by 48.61% and 42.24% respectively. While the treatment with ME and DCMF 3 h after administration of gentamicin (80 mg/kg bwt) exerted a very highly significant decrease in plasma AciPase activities by 67.81% and 67.99% respectively. The increase in the activities of AciPase and AlkPase revealed that the animals were under oxidative stress and this had been attributed to the damaged to the structural integrity of the kidney cell membrane. These results were in agreement with those of Hozayen et al.

Table 3. Summary of the activities of plasma GGT, ALP, ACP and kidney ACP of nephrotoxic rats treated with ME and DCMF of *G. kola* root-bark.

Group	Plasma GGT U/L	Plasma AlkPase ($\mu\text{mol/unit/mg protein}$)	Plasma AciPase ($\mu\text{mol/unit/mg protein}$)	Kidney AciPase ($\mu\text{mol/unit/mg protein}$)
I Rats + 2% (v/v) DMSO	1.51 \pm 0.14	8.28 \pm 2.99	9.36 \pm 0.42	55.46 \pm 9.73
II Rats + Gentamicin (80 mg/kg bwt)	2.20 \pm 0.12	8.56 \pm 0.68	37.71 \pm 11.72	36.46 \pm 5.46
III Rats + ME (250 mg/kg bwt) only	2.08 \pm 0.30	9.10 \pm 3.10	14.19 \pm 3.87	44.07 \pm 4.68
IV Rats + DCMF (250 mg/kg bwt) only	2.32 \pm 0.24	10.01 \pm 1.73	25.45 \pm 8.11	45.91 \pm 10.45
V Rats + ME (250 mg/kg bwt) + Gentamicin (80 mg/kg bwt)	2.20 \pm 0.22	10.21 \pm 0.70	19.38 \pm 7.75	44.93 \pm 13.33
VI Rats + DCMF (250 mg/kg bwt) + Gentamicin (80 mg/kg bwt)	2.61 \pm 0.50	8.97 \pm 2.15	21.78 \pm 12.12	45.15 \pm 6.84
VII Rats + Gentamicin(80 mg/kg bwt) + ME (250 mg/kg bwt)	2.08 \pm 0.30	8.56 \pm 1.19	12.14 \pm 3.91	45.05 \pm 7.48
VIII Rats + Gentamicin (80 mg/kg bwt) + DCMF (250 mg/kg bwt)	3.01 \pm 0.38	9.94 \pm 1.87	12.07 \pm 2.93	50.09 \pm 9.29

Each value represented Mean \pm SEM, n = 5 readings, Value of P \leq 0.05 was considered statistically significant.

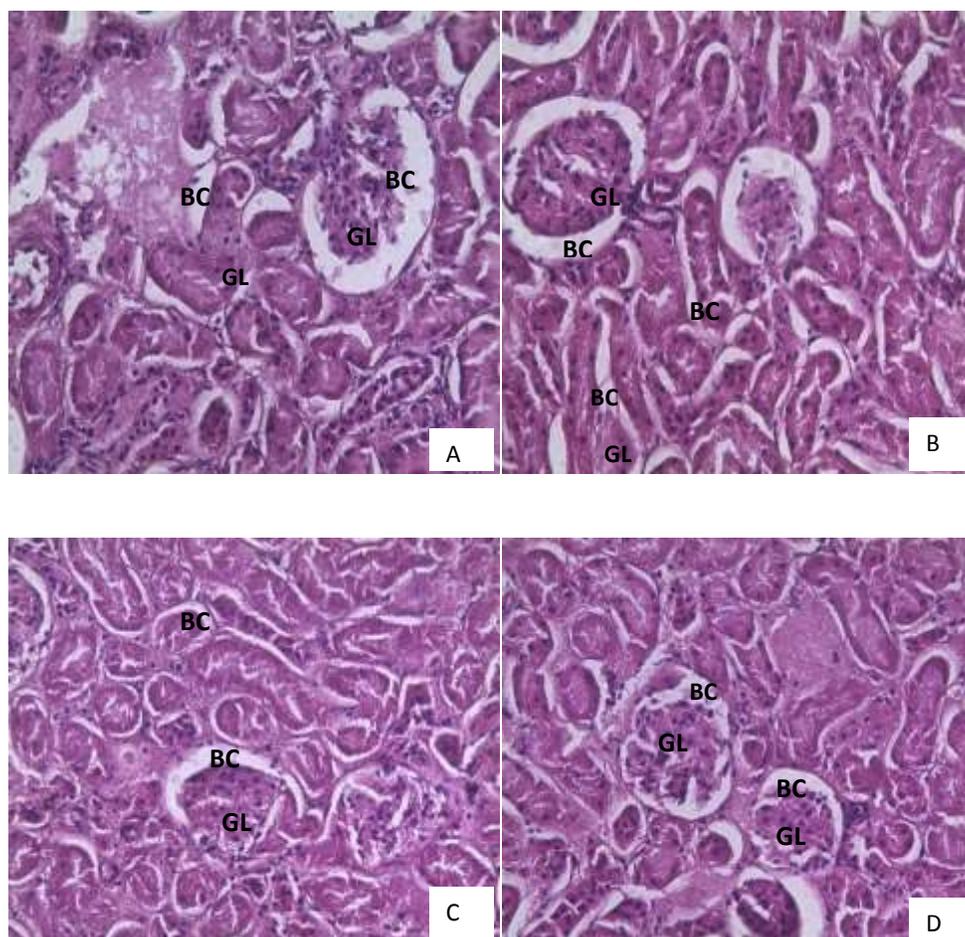


Plate 1. Microscopic section of kidney tissues (H & E. Magnification \times 200). A: Cross section of a normal kidney showing normal tubular brush-borders and intact glomerulus (GL) and surrounding Bowman's capsule (BC); B: Cross section of 80 mg/kg bwt of gentamicin-treated rat kidney showing a glomerulus (GL) with degenerating of surrounding Bowman's capsule (BC); C: Cross section of kidney of rat treated with ME only showing normal tubular brush-borders and intact glomerulus (GL) and surrounding Bowman's capsule (BC); D: Cross section of a kidney of rat treated with DCMF only showing normal tubular brush-borders and intact glomerulus (GL) and surrounding Bowman's capsule (BC).

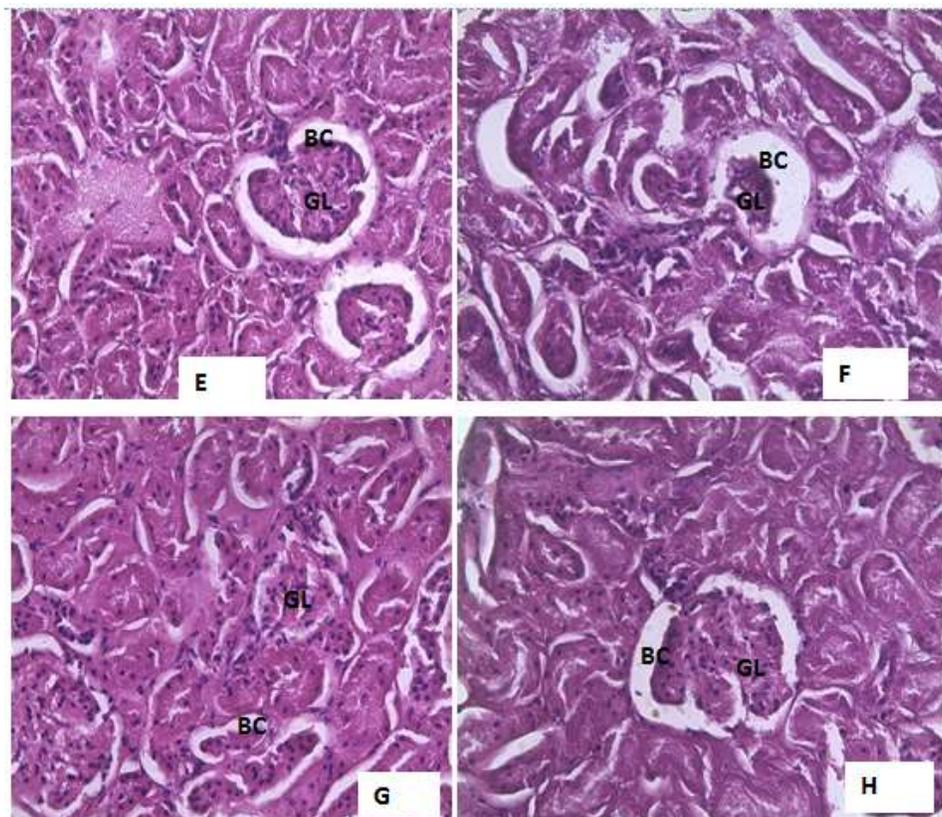


Plate 2. Microscopic Section of Kidney Tissues (H & E. Magnification $\times 200$). E: Cross Section of Kidney of rats pretreated with 250 mg/kg bwt ME 3 h before gentamicin (group V) showed glomerulus (GL) with an intact Bowman's capsule (BC); F: Cross Section of Kidney of rats pretreated with 250 mg/kg bwt DCMF 3 h before gentamicin (group IV) showed glomerulus (GL) with regenerating Bowman's capsule (BC) G: Cross Section of Kidney of rats post-treated with 250 mg/kg bwt of ME showed glomerulus (GL) with an intact Bowman's capsule (BC); and H: Cross Section of Kidney of rats post-treated with 250 mg/kg bwt DCMF showed glomerulus (GL) with a regenerating Bowman's capsule (BC).

(2011) who observed that the levels of both AlkPase and AciPase were elevated in gentamicin induced nephrotoxic rats. Also the administration of gentamicin to rats reduced kidney homogenates AciPase activities and is in agreement with its increase in plasma level. Moreover, the histopathological results revealed that kidney of rats treated with gentamicin (80 mg/Kg bwt) alone showed a significant kidney injury (plate 1-2). The treatments of rats with ME and DCMF (250 mg/kg bwt) was able to reduced kidney injury significantly caused by gentamicin (80 mg/Kg bwt) when compared with the control. These findings are additionally in agreement with the above mentioned biochemical results.

Conclusion

Gentamicin, elicited deleterious nephrotoxic effects by causing severe damage to kidney and altered its biological functions and biomarkers. The altered levels of

biochemical parameters were adequately restored as a result of treatment. These effects probably involved multiple molecular and biochemical mechanisms regulated by the polyphenolics one of the phyto-constituents of the ME and DCMF. The bioactive components of the ME and DCMF of *G. kola* root-bark ameliorated the oxidative damage elicited by gentamicin and increased regenerative and reparative capacities of the kidneys.

Conflict of Interests

The authors have not declared any conflict of interests.

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