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# Aqueous extract of *Hibiscus Sabdariffa calyx* modulates gentamicin activity in rats

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## ABSTRACT

Gentamicin have been shown to generate free radicals .This study investigated modulatory potential of aqueous extract of *Hibiscus Sabdariffa calyx* with gentamicin in rats. Thirty rats weighing between 150-200g were randomly selected into 6 groups: Group A( control), group B received 200mg/kg body weight of extract, group C received 80mg/ kg body weight of gentamicin intra peritoneally for six days while group D, E and F received 200mg/kg, 400mg/kg and 600mg/kg body weight of extract for six days before and eight days following administration of 80mg/kg gentamicin. Antioxidants and biochemical indices were determined using standardized methods . Rats treated with extracts at 200mg/kg elicit significant ($p<0.05$) decreases in total bilirubin (TB), conjugated bilirubin (C.BIL), urea, creatinine, Malondialdehyde (MDA) concentrations, Aspartate aminotransferase (AST), Alanine aminotansferase (ALT) and Alkaline phosphatase (ALP) activities as well as significant( $p<0.05$) increases in Superoxide Dismutate (SOD) activity , levels of reduced glutathione (GSH) ,albumin and total protein (TP). Gentamicin treated rats however showed significant ($p<0.05$) decreases in GSH, TP, albumin and SOD activities with elevated levels of MDA, TB,C.BIL, urea, creatinine and AST, ALT and ALP activities . Furthermore , animals treated with various concentrations of extract after gentamicin administration showed significant increases ($p<0.05$) in concentrations of TP, ALB, GSH and SOD activities with corresponding decreases in TB, C.BIL, urea, creatinine and MDA concentrations as well as AST,ALT and ALP activities compared with gentamicin group in a dose dependent manner. Properties exhibited by extract is antioxidative suggestive of its modulatory effects on gentamicin activity .

**Key words:** Antioxidative, Gentamicin, *Hibiscus Sabdariffa calyx*, Modulatory effect.

## Introduction

Gentamicin is an aminoglycoside with broad bactericidal activity against many aerobic gram negative and some aerobic gram positive organisms [1]. It is the most commonly used aminoglycoside antibiotic and is indicated for moderate to severe bacterial infections caused by sensitive agents, primarily gram negative bacteria. Like other aminoglycosides, gentamicin is thought to act by binding to bacterial ribosomes and inhibiting protein synthesis and considered to have both bacteriocidal and bacteriostatic activities [2]. Gentamicin and other aminoglycosides are typically used in combination with a penicillin or cephalosporin for treatment of severe infections. Gentamicin is most commonly used for septicemia, bacterial endocarditis, peritonitis, meningitis, pelvic inflammatory disease and pneumonia[1]. It was first approved for use in the United States in 1970 and remains in wide use available in multiple generic parenteral and topical formulations [3] . Topical formulations are also available for local wound, tissue and ophthalmologic anti-bacterial therapy [4], common side effects of gentamicin include dizziness, headache, confusion,

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nausea and skin rash. Important, dose related adverse effects include ototoxicity, hepatoxicity and nephrotoxicity which are shared by all aminoglycosides[5] .Despite its usefulness ,it has been shown that gentamicin treatment induces oxidative damage which can be attenuated by addition of antioxidant agents as lipid peroxidation have been shown to increase in the course of gentamicin administration giving rise to free radical generation which are highly toxic to tissues [6] . One of the exogenous sources of ROS is gentamicin which has been reported to induce oxidative stress both in vivo and in vitro [7] , as they have been shown to be generated in small amounts during the body s metabolic reactions as they can react with and damage complex cellular molecules such as fats, protein or DNA [8] .The incidence of hepato-nephrotoxicity from aminoglycosides has increased from 2 to 3% in 1969 to 20% in the past decade [9] ,and despite this , aminoglycosides are being continuosly used in clinical practices because of their bacteriocidal efficacy ,synergism with β lactam agents ,low cost ,limited bacterial resistance and a post antibiotic effect [9] .Free radicals generated as by products or intermediates of aerobic metabolism of drugs or environmental toxins has cumulative effects resulting in oxidative stress to surrounding cells and tissues as products of ROS/RNS through either endogenous or exogenous sources which may be responsible for their tissue toxicity has widely been reported[10];[11] . Hence induced tissue toxicity by gentamicin through free radical productions and oxidative damage can be attenuated by antioxidants as they counteract the deleterious actions of these reactive oxygen species and protects against cellular damage[12] . Source for antioxidants has therefore become imperative in treatment involving certain orthodox chemotherapeutic agents as most of them exerts their toxic effects majorly by production of ROS and free radicals in their mode of action hence usage of some of these antioxidant are sourced from natural sources especially of plant origin.However the use of medicinal plants has been a central of health care in many culture for centuries .More pharmaceutical drugs in the market contain extract from medicinal plants [13] .World health organisation ( WHO),estimates that up to 80percent of the world now rely on medicinal plants as their source of therapeutics [2], hence the justification for this study which made an attempt to see if certain extract of Hibiscus sabdariffa callyx consumed locally and generally as tea, or local soft drink and beverages in many areas of West Africa especially in South West Nigeria may have positive effects on gentamicin activity .*Hibiscus Sabdariffa* (of the Malvaceae family) is a plant where its leaves and stems are commonly referred to as *hibiscus* tea and sometimes it is referred to as the colloquial name of roselle or Sour Tea [14] . The calyx (flowers) of the plants are commonly used for teas while the white calyx of *hibiscus sabdariffa* has the name '*Isakpa*' by the yoruba's of southwestern Nigeria, '*krachiap daeng*' in Thailand, '*flor de jamaica*' in mexico and Lo-Shen. It has limited traditional medicinal use and tends to be a treatment for high blood pressure, gastrointestinal disorders, purgative and diuretic diaphoresis and anuria among others [15] . The red flowers have sometimes been used traditionally for their pigmentation as a dye which may have a role in histological studies . The roselle (*Hibiscus sabdariffa*) is a species of *hibiscus* native to West Africa [16] . It is an annual or perennial herb or woody-based sub shrubs, growing to 2-2.5m (7-8ft) tall. The leaves are deeply three- five-lobed, 8-15cm long.*Hibiscus sabdariffa* (Roselle or Sour Tea) is a tea where the usually dark colored flowers are used to brew, it appears to inhibit carbohydrate absorption to a degree and appears to be effective in reducing blood pressure , it is a supplemental herb that is derived from the plant's calyces, which are the collection of sepals separating the blooming flower from the stem. The calyces have traditionally been steeped into tea where the anthocyanins (red-blue pigmentation) is steeped into the water and drank for medicinal purposes [17] .Although several researches and findings have the plant extracts to possess anti-hypertensive ,anti cancer,anticlastogenic, hypolipidemic,anti-stress ,antiplasmodic, diuretic ,antidiarrheal and antioxidant activities [18] .This study was therefore designed to investigates the possible modulatory effects of aqueous extract of Hibiscus Sabdariffa calyx on gentamicin activity in tissues using certain antioxidants, liver and kidney function indices.

**Fig 1: Dried calyx of *Hibiscus sabdariffa* flower (Ezeani, N.F., 2000)**

## Materials and Methods

### Materials

Measuring cylinders, beakers, washing brushes, test tubes, disposable gloves, spectrophotometer, automatic micropipette, refrigerator, pipettes, spatula, incubator, filter paper,surgical blades, electric weighing balance,universal bottles, funnel, petri dishes, water bath, detergent, wooden rat cage, permanent marker, hand gloves, feeding trough, needles and syringes, aqueous extract of *hibiscus sabdariffa*, mortar and pestle, cotton wool, commercial rat feed, centrifuge and water.

### Reagents

Reagents used in this study includes; Laboratory assay kits for total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP), Washing buffer, Homogenising buffer, Ellman's reagent, bromocresol green, biuret reagent, normal saline, thiobarbituric acid, Sulphanilic acid, Sodium nitrite, Caffeine benzoate reagent, Alkaline tartrate reagent, Trichloroacetic Acid (TCA), Tris Hcl Buffer ($P^H$ 7.4), carbonate buffer (pH 10.2), Adrenalin, GSH working standard, Phosphate buffer ($P^H$ 7.4), all of which were of analytical grade and products of Sigma chemical company USA.

### Plant material and preparation of aqueous extract of *hibiscus sabdariffa* calyx.

Dried calyx of *Hibiscus sabdariffa* was purchased at Igbona market, Osogbo identified at the Department of Botany, Obafemi Awolowo University, Ile-Ife thereafter authenticated at the department of pharmacognosy Obafemi Awolowo University, Ile-Ife, Nigeria with herbarium voucher number 16750 issued. The dried calyx was powdered and used for the extraction.The extraction of the red pigment from the calyx of *Hibiscus sabdariffa* was performed by the method described by [19] . Hundred grams (100g) of dried *Hibiscus sabdariffa* calyx was added to 500ml of distilled water and boiled for 15minutes. The boiled material was then filtered after cooling. The filtrate was evaporated to dryness and was stored in a sealed bottle at $4^0$C until required.

### Experimental animals and groupings

Thirty wistar rats weighing between 150g-200g were obtained from a breeding stock in the animal House of the College of Health Sciences, Ladoke Akintola University of Technology, Osogbo. The animals were housed in well ventilated metal cages in the animal facility of the Department of Biomedical Science . The rats were maintained under hygienic conditions and were allowed to acclimatize for a period of two weeks with free access to rat feed and water before the commencement of the experiment. The animals were treated and handled based on the general rules and guidelines for handling of laboratory animals as obtained in my institution and were randomly divided into 6 groups (A-F) . The dried extract was dissolved in distilled water and administered orally at different concentrations ( 200,400 and 600 mg/kg body weight) while gentamicin (80mg/kg) was administered intraperitoneally as shown in the table1.

**Table 1 : Protocol table for various groups and treatments**

| GROUPS | Treatments |
|---|---|
| A | Negative group (Feed and water only) |
| B | 200mg/kg b.w of extract only. |
| C | Positive group (80mg/kg. b. w gentamicin only) |
| D | 80mg /kg. b .w gentamicin+200 mg/kg b .w of extract |
| E | 80mg/kg b.w gentamicin+400mg/kg b.w of extract |
| F | 80mg/kg b.w gentamicin+600mg/kg b.w of extract |

**Animal sacrifice, sample collection and preparation of tissue homogenates.**

The experimental animals were sacrificed 12 hours after the last treatment by cervical dislocation. The animals were carefully cut open and blood drained from the heart using a syringe and needle (heart puncturing), which was then collected into lithium heparin bottles. Liver and kidneys were removed and dissected free from the fat and connective tissues. They were washed using washing buffer (1.15% KCl, pH 7.4) to remove haemoglobin which may inhibit enzyme activity. They were then sectioned and homogenized with mortar and pestle in 4ml of cold Trisbuffer (0.06M Tris, 1.12%KCl). The homogenates were centrifuged at 5000rpm for 10 min. The resulting supernatant were used for the determination of MDA, GSH and the activities of SOD.

**Methods**

**Biochemical analysis**

The activities of plasma ALT, AST were estimated using the method described by [20] , while determination of plasma activities of alkaline phosphatase (ALP) was estimated as described by [21]**.** Estimation of plasma bilirubin was done using Jendrassik and Grof method as described by [22] . Biuret method was used for the estimation of plasma total protein as described by [23], while plasma albumin estimation was carried out using bromocresol green binding method as described by [24] .Also plasma urea concentrations were determined according to the method of Berthelot-Searcy as described by [25] , while creatinine was determined by the method described by [26 ].

**Antioxidant parameters and marker of lipid peroxidation**

Lipid peroxidation in liver and kidney were estimated spectrophotometrically by thio barbitturic acid-reacting substances (TBARS) as described by the procedure of [27] . Determination of GSH concentration was done using the method described by [28], while SOD activity was determined by the method of [29].

**Statistical Analysis**

The SPSS (Statistical package for social sciences) software version 16.0 was used for the statistical analysis. Data obtained was analysed using analysis of variance (ANOVA), value of $p<0.05$ was considered statistically significant.

**Results**

**Table 2: Plasma total protein (TP) and albumin ( ALB) concentrations in various treatment groups**

| Treatment groups | Total Protein ± SD( g/l) | Albumin ± SD (g/l) |
|---|---|---|
| Group A (control ) | 81.97 ± 3.10 | 36.55 ± 3.54 |
| Group B(200mg/kg of extract only | 82.50 ± 4.12 | 38.92 ± 3.53 |
| Group C (80mg/kg Gentamicin only. | 33.45 ± 3.23 | 12.19 ± 1.88 |
| GroupD(80mg/kg gentamicin+200mg/kg extract | 40.39 ± 3.80 | 14.64 ± 1.73 |
| Group E (80mg/kg gentamicin +400mg/kg extract | 54.89 ± 3.72 | 21.67 ± 1.61 |
| Group F(80mg/kg gentamicin + 600 mg/kg extract | 67.27 ± 1.68 | 25.71 ± 1.55 |

Values were given as mean ± Standard deviation of five determinations with p value <0.05 considered statistically significant.
*P-value <0.05 Considered significant when compared with Gentamicin only group.

**Table 3: Plasma total bilirubin (T.BIL) and conjugated bilirubin (C.BIL) concentrations in various treatment group**

| Treatment groups | T.BIL(µmol/L) | C.BIL(µmol/L) ±SD |
|---|---|---|
| Group A (control ) | 11.25±3.57 | 3.55±1.05 |
| Group B(200mg/kg of extract only | 11.09±3.74 | 3.29±1.55 |
| Group C (80mg/kg Gentamicin only. | 33.40±4.15 | 14.95±4.28 |
| GroupD(80mg/kg gentamicin+200mg/kg extract | 24.93±3.16 | 10.71±1.40 |
| Group E (80mg/kg gentamicin +400mg/kg extract | 24.92 ±5.03 | 6.98±1.19 |
| Group F(80mg/kg gentamicin + 600 mg/kg extract | 17.96 ±4.52 | 5.48±0.96 |

Values were given as mean ± Standard deviation of five determinations with p value <0.05 considered statistically significant.
*P-value <0.05 Considered significant when compared with gentamicin only group.

**Table 4: Plasma Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP )activities in various treatment groups**

| Treatment Groups | AST ±SD (U/L) | ALT±SD (U/L) | ALP ± SD (U/L) |
|---|---|---|---|
| Group A (control ) | 22.57±3.13 | 21.15±2.24 | 53.91±4.01 |
| Group B (200mg/kg of extract only | 25.55±4.88 | 21.52±2.74 | 53.21±3.48 |
| Group C (80mg/kg Gentamicin only. | 116.75±3.75 | 116.86±2.89 | 99.28±1.95 |
| Group D (80mg/kg gentamicin+200mg/kg extract | 108.88±1.43 | 100.98±6..58 | 93.99±0.77 |
| Group E (80mg/kg gentamicin +400mg/kg extract | 100.10±1.37 | 91.29±2.79 | 89.44±2.31 |
| Group F (80mg/kg gentamicin + 600 mg/kg extract | 89.05±0.67 | 82.39±1.80 | 81.45±074 |

Values were given as mean ± Standard deviation of five determinations with p value <0.05 considered statistically significant.
*P-value <0.05 Considered significant when compared with Gentamicin only group.

**Table 5: Malondialdehyde (MDA), Reduced glutathione (GSH) concentrations and Superoxide dismutase activities (SOD) in the liver homogenates of different treatment group**

| Treatment Groups | MDA± SD (µg/mg) | GSH ±SD (µmol/mg) | SOD±SD (U/mg) (µmol/mg) |
|---|---|---|---|
| Group A (control | 33.12±2.09 | 6.86±1.11 | 25.81±2.11 |
| Group B (200mg/kg of extract only | 31.54±1.54 | 7.17±1.32 | 27.23±1.33 |
| Group C (80mg/kg Gentamicin only. | 71.00±1.20 | 1.06±0.58 | 16.43±2.68 |
| Group D (80mg/kg gentamicin+200mg/kg extract | 68.60±1.24 | 1.99±0.21 | 17.96±2.41 |

| | | | |
|---|---|---|---|
| **Group E (80mg/kg gentamicin +400mg/kg extract** | 64.74±1.91 | 2.26±0.60 | 21.78±1.35 |
| **Group F (80mg/kg gentamicin + 600 mg/kg extract** | 53.19±2.25 | 5.99±0.79 | 27.74±2.24 |

Values were given as mean ± Standard deviation of five determinations with p value <0.05 considered statistically significant.
*P-value <0.05 Considered significant when compared with Gentamicin only group.

**Table 6: Plasma total protein ,Urea and Creatinine concentrations in various treatment groups**

| Treatment Groups | Plasma Protein concentration (g/l) | Plasma Urea Concentration(mmol/l) | Plasma Creatinine Concentration (µmol/L) |
|---|---|---|---|
| **Group A (control** | 81.97 ± 3.10 | 5.89±0.36 | 130.60±2.10 |
| **Group B (200mg/kg of extract only** | 82.50 ± 4.12 | 6.11±0.74 | 127.10±2.10 |
| **Group C (80mg/kg Gentamicin only.** | 33.45 ± 3.23 | 52.27±9.86 | 692.10±120.70 |
| **Group D (80mg/kg gentamicin+200mg/kg extract** | 40.39 ± 3.80 | 40.19±5.40 | 455.70±28.00 |
| **Group E (80mg/kg gentamicin +400mg/kg extract** | 54.89 ± 3.72 | 36.35±6.35 | 396.80±27.00 |
| **Group F (80mg/kg gentamicin + 600 mg/kg extract** | 67.27 ± 1.68 | 34.62±6.95 | 376.90±59.00 |

Values were given as mean ± Standard deviation of five determinations with p value <0.05 considered statistically significant.*P-value <0.05 Considered significant when compared with Gentamicin only group.

**Table 7: Superoxide dismutase activity(SOD) ,Reduced glutathione (GSH) and Malondialdehyde (MDA) Concentrations in the kidney homogenate of various treatment groups**

| Treatment Groups | SOD Activity (U/mgProtein) ±SD | GSH Concentration(µmol/mg protein )±SD | MDA Concentration (µg/mg protein)±SD |
|---|---|---|---|
| **Group A (control** | 5.39±0.41 | 5.78±0.44 | 8.88±1.23 |
| **Group B (200mg/kg of extract only** | 5.85±0.29 | 6.78±0.77 | 8.39±0.89 |
| **Group C (80mg/kg Gentamicin only.** | 4.11±0.58 | 4.00±0.50 | 26.61±3.62 |
| **Group D (80mg/kg gentamicin+200mg/kg extract** | 5.44±1.14 | 5.51±0.74 | 19.04±1.43 |
| **Group E (80mg/kg gentamicin +400mg/kg extract** | 6.18±1.02 | 5.97±0.89 | 14.59±3.28 |
| **Group F (80mg/kg gentamicin + 600 mg/kg extract** | 6.94±0.78 | 7.27±0.93 | 13.41±0.48 |

Values were given as mean ± Standard deviation of five determinations with p value <0.05 considered statistically significant.
*P-value <0.05 Considered significant when compared with Gentamicin only group.

**Discussion**

Aminoglycoside antibiotics have long been used in antibacterial therapy, one of which include gentamicin. Gentamicin is effective against most of the life threatening Gram negative bacterial infections with reported incidence of hepato-nephrotoxicity [9]. The incidence of hepato-nephrotoxicity associated with aminoglycosides has increased in clinical practice despite their bactericidal efficacy such that efforts are made through intakes of other drugs or supplements to ameliorates this effects. The liver is the major site of synthesis of protein which includes albumin and all other proteins in the blood. Albumin is an important blood protein that is made only by the liver and excreted by the kidneys [30]. In this study, Administration of gentamicin (80mg/kg for 6 days) resulted in a significant ($p<0.05$) decreases in plasma total protein and albumin concentrations ( group C) (33.45±3.23g/l; 12.19±1.88g/l) compared with the controls (81.97±3.10g/l; 36.55±3.54g/l). However, treatment with different doses (200mg/kg, 400mg/kg and 600mg/kg) of the extract showed modulatory effects as it causes a dose dependent increases in plasma total protein and albumin levels ( table 2). The observed decrease in albumin could be a result of a decline in the number of cells responsible for albumin synthesis in the liver through necrosis and the direct interference with the albumin-synthesizing mechanism in the liver as may also be implicated for decrease in albumin.Bilirubin is a waste product of the normal breakdown of red blood cells, it passes through the blood into the liver where it is being conjugated to form a less toxic form which is eventually excreted through the kidney. Total Bilirubin and Conjugated bilirubin level were found to be significantly increased ($p<0.05$) in gentamicin treated rats ( table 3). The mean total bilirubin and conjugated bilirubin in this group were (33.40±4.15µmol/L; 14.95±4.28µmol/L) , compared with the healthy controls (11.25±3.57µmol/L; 3.55±1.05µmol/L). However, a significant dose dependent reduction was observed in the rats treated with *Hibiscus sabdariffa* extract (200mg/kg, 400mg/kg and 600mg/kg with gentamicin ) This improvement is attributed to the hepatoprotective effect of the extract. Accumulation of bilirubin is a measure of alterations in binding, conjugation and excretory capacity of hepatocytes. The elevated level of bilirubin is usually an indication of biliary obstruction, hemolysis, and in some cases renal failure [31] . The observation of increased level of plasma bilirubin in gentamicin treated rats are suggestive of hepatic damage which was modulated with the administration of the extracts as shown in the results.Liver transaminases, ALT and AST are enzymes produced by the hepatocytes, the working unit of the liver. The level of these enzymes in the blood is increased in conditions in which the hepatocytes are damaged. Aspartate amino transaminase (AST), and Alanine amino transaminase (ALT) , still remained the gold standards for the assessment of liver injury, and have been used as biomarkers of choice for decades [32] . In this study, there were significant ($p<0.05$) increases in AST and ALT activities in the gentamicin treated group (116.75±3.73U/L; 116.86±2.89U/L) ,compared with the controls (Group A) (22.57±3.13U/L; 21.15±2.24 U/L) , which were in agreement with several studies on these enzymes response to toxins or infectious agents as they are highly expressed in the blood with the presence of these agents [33] . However, a dose dependent reduction in the activity of these enzymes were observed in groups ( D,E and F) treated with different doses (200mg/kg, 400mg/kg and 600mg/kg) of the extract with gentamicin . These activities may eventually be reduced to the controls with increased dosage of the extract suggestive of the modulatory effects of the extract and its protective ability as a factor to decrease liver damage.Alkaline phosphatase (ALP), is an enzyme found in several tissues throughout the body including liver, bone, kidney, bowel (intestine), and in the placenta. Alkaline phosphatase (ALP) test is often used to detect liver damage (blocked bile ducts) because ALP is especially high in the edges of cells that join to form bile ducts. In this study, the results showed a remarkable elevation in the plasma activities of ALP following gentamicin administration ( group C) (99.28±1.95U/L) compared to rats in group A (53.91±4.01U/L). The significant increase in plasma activities of this enzyme could be attributed to the damaged structural integrity of the liver by gentamicin. However, groups treated with the extract (D, E and F) showed a dose dependant decrease (93.99±0.77U/L; 89.44±2.31U/L; 81.45±0.74U/L respectively) in plasma activities of this enzyme ( table 4). In this study ,the elevated levels of these marker enzymes in the plasma is suggestive of

oxidative stress induced by ROS production by gentamicin and possible antioxidative effects of the extract in reversing this trends. Malondialdehyde (MDA) results from lipid peroxidation of polyunsaturated fatty acids, the degree of lipid peroxidation can be estimated by the amount of malondialdehyde in tissues [34] . In this study, MDA was assessed as clinical biomarkers for oxidative stress in gentamicin treated rats. MDA level was found to be significantly ( $p<0.05$)increased in gentamicin administered rats ( Group C ) ;(71.00±1.20µg/mg) compared to controls (Group A); (33.12±2.09µg/mg). The Increased MDA level with gentamicin is an indication of oxidative stress while significant dose dependent reduction was observed in the rats treated with the extract (200mg/kg, 400mg/kg and 600mg/kg) as 68.60±1.24µg/mg; 64.74±1.91µg/mg and 53.19±2.25µg/mg respectively . This improvement is attributed to the anti oxidant and modulatory effect of *the extract.* Reduced glutathione (GSH) is a key component of the antioxidant system which protects the body from free radicals at the cellular level. It is well established that reduced glutathione (GSH), the most important bio molecule protecting against chemically induced cytotoxicity, can participate in the elimination of reactive intermediates by conjugation and hydroperoxide reduction, or of free radicals by direct quenching. GSH plays a key role in the detoxification of gentamicin reactive toxic metabolites, while liver necrosis begins when GSH stores are markedly depleted [35] . The results ( table 5) demonstrated a significant ($P<0.05$) decrease in hepatic GSH content and increase in MDA content. There is reduction in GSH in group intoxicated with gentamicin (1.06±0.58 µmol/mg) compared with healthy controls ( Group A) (6.86±1.11 µmol/mg) and significant ($p<0.05$) increase in level of MDA (71.00±1.20µmol/L) compared with control (33.12±2.09), these results are in agreement with some studies suggesting that aminoglycoside antibiotics can stimulate the formation of reactive oxygen species (ROS) which may directly be involved in gentamicin induced liver failure and membrane lipid peroxidation [36] , while rats treated with *the extract* in group D (200mg/kg), E (400mg/kg) and F (600mg/kg) showed significant ( $p<0.05$) increases in level of GSH (1.99±0.21 µmol/mg; 2.26±0.60 µmol/mg; 5.99±0.79 µmol/mg)) respectively and significant reduction in MDA (68.60±1.24µmol/L; 64.74±1.91µmol/L; 53.19±2.25µmol/L) . Also, significant ($p<0.05$) decrease in Superoxide dismutase (SOD) activity was observed in the group intoxicated with gentamicin (16.43±2.68) when compared with the control( group A ) ;(25.81±2.11). However, dose dependent increases were observed in groups treated with 200mg/kg (17.96±2.41), 400mg/kg (21.78±1.35) and 600mg/kg (27.74±2.24) of the extract. The ability of the extract to increase GSH levels and boost SOD activity may not be unconnected with its antioxidant potential and the ability to boost the antioxidant status of the animals there by modulating the activity of gentamicin.Further investigation of some kidney biochemical indices and antioxidant status also revealed similar results as it was earlier reported that the clinical usage of the drug have been limited due to the development of nephrotoxicity[37] . Also, intakes of gentamicin arbitrarily without prescription has led to increase in the rate of kidney damage due to the ability of the drug to induce generation of superoxide ,hydroxyl radical and hydrogen peroxide free radicals which have been associated with its mode of action [38]. The uncontrolled presence and generation of these radicals and oxidants may over power the natural antioxidant system leading to oxidative stress .

Results on the renal indices( table 6) showed slight decreases in creatinine concentration in group B treated with extract only, compared with group A. Also, a significant increase ($p<0.05$) in creatinine level in gentamicin treated group (group C) were observed which could possibly be a result of accumulation of gentamicin in the proximal tubular cells which are protonated in the body and bind to negatively charged phosphotidyl inositol or phoshotidyl serine. This binding causes the inhibition of lysosomal phospholipidosis. This excessive phospholipid overload causes proximal tubular necrosis as reported by [39] . However, treated groups D, E and F shows significant decreases ($p<0.05$) in creatinine level when compared with group C. Evidence from this study suggests strongly that *H. sabdarifja* calyx aqueous extract may have effect on kidney by mechanisms which includes the stabilization of plasma creatinine and a modulatory effect in synergy with gentamicin . Therefore, further increases in concentration of the extract will show nephroprotective effects as creatinine level decreases significantly in a concentration dependent manner.Furthermore ,similar results were obtained for the determination of urea concentrations as gentamicin treated group elicit significant increases, ($p<0.05$) in urea level in group C, when compare with the control (group A), which may be a result of gentamicin inducing lysosomal phospholipidosis that disrupts normal renal function [40], given evidenced that the renal accumulation of gentamicin is implicated in the induction of nephrotoxicity as reported by [41] . However, treated groups D, E and F showed significant decrease in urea level compared with group C. Also , all the antioxidant

parameters examined such as MDA,GSH concentrations and SOD activities in the kidney homogenates elicit similar effects as obtained in the liver (Table 7), all pointing to the side effects of the drug at the treated dosage suggestive of the ability of the drug to induce renal oxidative stress [42] . However in all the treated groups with different concentrations of the extract , their modulatory effects on the drug activity were identified as these effects were ameliorated in a concentration –dependent manner.

## Conclusion

Properties exhibited by the extract is majorly anti-oxidative which helps in modulating the activity of gentamicin and also convered hepatoprotective and nephroprotective effects by mediating potent antioxidant effects that helped to preserved intra cellular levels of biological pathways that supportively enhance amelioration of toxic levels of gentamicin. This modulatory effects is attributable to the rich excellent bioactive components of the plants with immense dietary importance and a credence to its wide use as local soft drinks in most West African villages and cities and this may play an important role as supplements on antibiotic treatment such as gentamicin and other derivatives. It is therefore recommended that further study be carried out to determine the specific mechanism of modulatory-antioxidant activity of this plant and also determine whether different forms of extract from this plant can perform the same function.

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