October 2024, Volume 18, Number 4

Iranian Journal of Toxicology
ARAK UNIVERSITY OF MEDICAL SCIENCES

Research Paper

Cardiomodulating Activity of *Gongronema latifolium* and Lisinopril in Doxorubicin-induced Cardiotoxicity in Wistar Rats

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How to cite this paper:
Ujong G, Beshel J, Okon I, Ejim C, Benedict I. Cardiomodulating Activity of *Gongronema latifolium* and Lisinopril in Doxorubicin-induced Cardiotoxicity in Wistar Rats. **Iranian Journal of Toxicology.** 2024; 18(4):210-216. doi: 10.32592/IJT.18.4.210

doi: 10.32592/IJT.18.4.210

Article info
Received: 15/04/2024
Accepted: 04/11/2024
Published: 04/11/2024

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ABSTRACT

Background: A major side effect of some cancer drugs, including Doxorubicin, is cardiotoxicity. This study was designed to evaluate the cardioprotective role of ethanolic leaf extract of *Gongronema latifolium* **(**GL) compared to Lisinopril in doxorubicin-induced cardiotoxicity in rats.

Methods: Forty Wistar rats of both sexes (150-200 g) were divided into 5 groups (n=8 each). Group 1 (control) took normal rat chow; Group 2 received 25 mg/kg Doxorubicin; Group 3 received Doxorubicin + GL (200 mg/kg orally); Group 4 received Doxorubicin + Lisinopril (10 mg/kg orally); and Group 5 received Doxorubicin + Lisinopril + GL. The regiment lasted for 28 days. Blood samples were collected from each animal via cardiac puncture for biochemical assays.

Results: The results of the study showed a significant decrease in superoxide dismutase (SOD) concentration in the doxorubicin group as compared to the control group. Intervention with GL and Lisilopril caused a significant increase in SOD concentration. Total antioxidant capacity, catalase, SOD, and angiotensin-II levels were significantly decreased with a corresponding increase in malondialdehyde (MDA) in the Doxorubicin group. Treatment with GL and Lisinopril significantly reversed these changes by increasing the levels of TAC, SOD, CAT, and angiotensin-2 to normal while lowering the MDA levels to normal. Cardiac biomarkers, namely troponin and creatine kinase levels, were significantly increased in the doxorubicin group as compared to the normal control. However, the coadministration of GL and Lisinopril decreased the troponin and creatine kinase concentrations to normal levels.

Conclusion: *Gongronema Latifolium* and Lisinopril provided better cardioprotective and antioxidant effects versus other agents against cardiotoxicity induced by Doxorubicin.

Keywords: Antioxidants; Cardiac biomarkers; Doxorubicin; *Gongronema Latifolium* extract; Lisinopril

Introduction

Doxorubicin is a chemotherapy drug used to treat cancers (e.g., breast and bladder), Kaposi's sarcoma, lymphoma, and acute lymphocytic leukemia. Doxorubicin is marketed under the brand name Adriamycin. It is a cytotoxic anthracycline antibiotic widely used in oncology practice. It functions by slowing down or inhibiting the growth of cancer cells by blocking an enzyme called topoisomerase. However, the clinical use of Doxorubicin is restricted because of its serious toxicity, which frequently leads to irreversible degenerative cardiomyopathy and heart failure [1-3]. Of note, the cumulative dose of anthracycline is the major cause of induced cardiotoxicity.

The major predisposing high-risk factors for anthracycline-based, chemotherapy-induced cardiotoxicity are age, body weight, female gender, radiotherapy, and such diseases as diabetes and hypertension. It has also been shown that free radicals are involved in doxorubicin-induced toxicity. Doxorubicin causes the generation of free radicals and the induction of oxidative stress, which is associated with cellular injury. Pre-existing arterial hypertension has a highly important role in the development of anthracycline-based, chemotherapy-induced cardiotoxicity despite its positive effect on arterial hypertension control [4].

Gongronema latifolium (GL) is one of the medicinal plants used in the treatment of ailments, including cardiovascular disorders [5]. This herbaceous climbing plant features yellow flowers and a stem that yields distinct milky exudates. It is widespread in tropical Africa and can be found in Senegal, Chad, and the Democratic Republic of Congo. It occurs in rainforests, deciduous and secondary forests, mangroves, and disturbed roadside forests, from sea level up to 900 m altitude [6, 7].

This leafy plant can be propagated by seed. Its

common name is 'amaranth globe'. In Nigeria, *G. latifolium* is known by different local names, such as '*utasi*' by the Efiks/Ibibios, '*utazi*' by the Igbos, and *arokeke* by the Yorubas [8]. *G. latifolium* crude leaf extract is used in the treatment of malaria, diabetes, and hypertension and as a laxative [7]. It also reduces renal and hepatic oxidative stress [8-11].

Doxorubicin cardiotoxicity can be acute, occurring during and within 2-3 days of its administration. The incidence of acute cardiotoxicity is approximately 11% [1, 12]. The manifestations are usually chest pain due to pericarditis and/or heart palpitations due to sinus tachycardia, paroxysmal non-sustained supraventricular tachycardia, and premature atrial and ventricular beats. In such cases, the electrocardiogram may reveal nonspecific ST-T changes, left axis deviation, and decreased amplitude of QRS complexes. The mechanisms for these acute changes are not clear but may be due to doxorubicin-induced myocardial edema, which is reversible. Acute left ventricular failure is a rare manifestation of acute cardiotoxicity; however, it can be reversible with appropriate treatments [1].

The incidence of chronic doxorubicin cardiotoxicity is much lower, with an estimated incidence of about 1.7%. It typically manifests within 30 days following the administration of its last dose; nevertheless, it may occur even after 6-10 years later. The incidence of doxorubicin cardiomyopathy is primarily related to its dosage, which is about 4% when the dose is 500-550 mg/m^2, 18% at 551-600 mg/m^2, and 36% at doses exceeding 600 mg/m^2. Other risk factors are combination therapy with cardiotoxic antitumor drugs and mediastinal radiation therapy. Cancer therapy in childhood and adolescence predisposes to the development of doxorubicin cardiomyopathy in adults. Age influences the risk of developing doxorubicin cardiomyopathy. Very young and very old individuals are more prone to develop this complication. Further, a history of cardiovascular disease, such as hypertension and reduced left ventricular ejection fraction before therapy, is also a risk factor for developing this complication [13].

The primary mechanism of action of Doxorubicin involves its ability to intercalate within DNA base pairs, causing breakage of DNA strands and inhibition of both DNA and RNA synthesis. It also inhibits the enzyme topoisomerase II, causing DNA damage and an induction of apoptosis. When combined with iron, Doxorubicin causes free radical-mediated oxidative damage to DNA, further limiting DNA synthesis. Iron chelators, such as dexrazoxane, may prevent free radical formation by limiting the binding of Doxorubicin with iron [14].

Materials and Methods

Experiment Animals: Forty adult Wistar rats of both genders, weighing 150-200 g, were used in this study. The animals were purchased from the animal house of the Department of Human Physiology, Faculty of Basic Medical Sciences, Cross River University of Technology (CRUTECH), Okuku Campus, Cross River State, Nigeria. They were kept under experimentally controlled conditions (27±2°C, with a 12-hour light-dark cycle). All animals were acclimatized for one week before the experiment. They were kept in plastic cages and fed with normal rat chow and tap water *ad libitum*. Ethical approval was obtained from the Faculty of Basic Medical Sciences, University of Calabar, Animal Research, and Ethical Committee (approval code: 019PY20317).

Preparation of *Gongronema latifolium* Extract: The extract was prepared according to the standard method [8]. *G. latifolium* was harvested at a local farm in Ugep, Yakurr Local Government, Cross River State, Nigeria. It was identified and authenticated in the Department of Botany and Ecological Studies, University of Calabar, Calabar (Figure 1).

Figure 1: Image of *Gongronema latifolium* Plant.

The leaves were first washed and then dried in the shade for seven days before being ground into a fine powder using a home choice blender model KD-313A. This powder was stored in a cool, dry place away from light until further use. The powdered leaf (400 g) was dissolved in 1250 ml of 70% ethanol (BDH Ltd Poole, England) and allowed to stay overnight. The mixture was then centrifuged the following morning, and the supernatant was collected. The supernatant underwent two filtration steps: first with Whatmann filter paper (#1) and then again with cellulose filter paper. The filtrate was evaporated to dryness at 30°C in a vacuum rotatory evaporator (Caframo, VV2000, Ohio) and water bath (Caframo, WB2000). The extraction resulted in a percent yield of 4.3%, using a digital sensitive weighing balance. The extract was stored at 4°C until further use.

Induction of Cardiotoxicity: Cardiotoxicity was induced using the intraperitoneal injection of a freshly prepared solution of 25 mg/kg doxorubicin and was administered over 16-day intervals. Doxorubicin is incompatible with heparin and fluorouracil and can cause precipitation if mixed with these drugs. While Doxorubicin may be administered rapidly (over 15-20 min), slow administration of the liposomal formulation is recommended to reduce the risk of infusion reactions [14].

Administration of the Ethanolic Extract and Lisinopril: The plant extract reconstituted in distilled water (vehicle) was administered via oral gastric intubation at 200 mg/kg, while Lisinopril was given at 10 mg/kg of body weight. Doxorubicin (25 mg/kg) was administered intraperitoneally four times per week for 12 days. The dosage of plant extracts was administered following a previously reported method [8]. The treatment regimen lasted for 28 days.

Experimental Design: Forty Wistar rats were divided into five groups of eight each and were placed individually in rat cages:

Group 1: Control
Group 2: Doxorubicin
Group 3: Doxorubicin + *G. latifolium*
Group 4: Doxorubicin + Lisinopril
Group 5: Doxorubicin + *G. latifolium* + Lisinopril

Collection of Blood Samples: After 28 days of treatment, the animals were anesthetized using chloroform vapor, and their blood samples were collected into plane and EDTA sample bottles via cardiac puncture using sterile needles. The blood samples in plane tubes were then centrifuged at 1000 rpm for 10 min. After that, the serum was collected and stored for the subsequent biochemical analysis of cardiac biomarkers, antioxidants, lipid peroxidation, and angiotensin-II (Ang-II) enzyme activities.

Determination of Angiotensin-II: The Ang-II enzyme immunometric assay is a complete kit for the quantitative measurement of Ang-II in serum and plasma samples. Other matrices, such as urine and tissue, may be suitable; however, we did not validate them in this study. Angiotensin can be present in very low concentrations in some biological samples. In such cases, dilution of samples to avoid “nonspecific” interference by any present factors is not productive because the angiotensin analyte is also diluted to levels far below the minimum detection concentration. Thus, investigators have used several procedures for extracting angiotensin from biological samples prior to using them for immunoassays.

Determination of Malondialdehyde Level: The standard method [15] for the determination of malondialdehyde (MDA) was adopted by following the TBARS procedure provided by the manufacturer (Calbiochem Chemicals, USA). For this purpose, 100 µl of each sample or standard and 100 µl of sodium dodecyl sulfate solution were added to a 5 ml vial and vortexed. Next, 4 ml of the reagent was added to each vial. The vials were placed in vigorously boiling water for 1 h, and then removed and placed in an ice bath for 10 min. A 96-well plate was loaded with 150 µl of the sample or standard, and the absorbance was read at 540 nm, using the microplate reader within 30 min of the reaction based on the following equation: MDA (µM) = [(corrected absorbance) – (y-intercept)/slope].

Total Antioxidant Capacity Determination: Total antioxidant capacity was determined following the standard method supplied by the Cell Biolabs’ Oxiselect™ [16]. For this purpose, 20 µl of diluted uric acid plus the sample were added to 96-well microtiter plates. The reaction buffer (180 µl) was also added to each well using a multichannel pipette and was shaken vigorously. The initial absorbance was obtained by reading the plate on a spectrophotometer at 490 nm. To initiate the reaction, 50-µl copper ion reagent was added to each well and incubated for 5 min. A volume of 50 µl of the buffer reagent was again added to terminate the reaction, and the plate was read at 490 nm as follows: Total antioxidant capacity = [(corrected absorbance) – (y-intercept)/slope].

Determination of Creatine Kinase: The creatine kinase (CK) activity was determined based on three different methods, and the results were compared. The colorimetric (diacetyl-e-naphthol) method of Hughes, using kit No. 520 (Sigma Chemical Co.), and the spectrophotometric method of Rosalki were employed, using CK-NAC UNIKITS (Roche). The resultant data were compared based on the PNPG method [17, 18]. The incubation condition for the CK determination based on the PNPG method was as described for the diacetyl-naphthol method (Sigma Bulletin No. 520). The incubation mixture contained 7 mmol/L creatine phosphate, 0.015 mo1/L MgS0 4, 4 mmol/LADP, 0.25 mmo1/L reduced glutathione, and 10 µL serum in 0.8 mL of 0.1 mol/L Tris, at pH 7.5. This mixture was incubated at 37°C for 30 min. Following the incubation,

the reaction was stopped by adding 0.2 ml 0.05 mol/L p-hydroxymercurybenzoate. The creatine concentration was determined as described above, using the molar extinction coefficient for the colored product. In this study, one unit of CK activity was defined as the amount of enzyme that can produce 1 µmol creatine per minute at 37°C and pH 7.5.

Determination of Troponin: Serum troponin was determined using a rapid assay, Card-I Kit Combo test, following the manufacturer's instructions. A commercially available enzyme-linked immunosorbent assay was used to determine troponin-c concentration for all animals [19].

The test device, specimen, and/or controls were allowed to reach room temperature (15-30°C) prior to testing. The pouch was brought to room temperature before opening it. The test device was removed from the sealed pouch and used as soon as possible and was then placed on a clean and level surface. For serum specimens, the dropper was held vertically, and 2 drops of serum (approximately 100 L) were transferred to the specimen well(s) of the test device, and the timer was started until the colored line(s) appeared. The results were read at 10 min. Caution was taken not to interpret the results after 20 min.

Interpretation of the Findings

***Positive*:** Two distinct colored lines appeared. One line should be in the control line region (C) and another line should be in the test line region (T).

Negative: One colored line appeared in the control line region (C). No apparent colored line appeared in the test line region (T).

Invalid: Control line (C) failed to appear. Insufficient specimen volume or incorrect procedural techniques were the most likely reasons for control line failure. The procedure was reviewed, and the test was repeated with a new test device. If the problem persisted, we discontinued using the test kit immediately and contacted the local distributor.

Data Analyses: Results were expressed as the means ± SEM. Data were analyzed using GraphPad Prism software (version 6.0). Analysis of variance (ANOVA) followed by Tukey's comparison test was done where the F value was significant. The probability level of $P < 0.05$ was set as significant.

Results

Superoxide Dismutase Activity: The mean serum superoxide dismutase (SOD) activities in the control; Doxorubicin; Dox + GL; Dox + Lisilopril; and Dox + GL + Lisilnopril groups were 32.40±3.58 ng/mL, 61.00±4.08 ng/mL, 70.00±2.47 ng/mL, 76.33±3.47 ng/mL and 96.00±3.91 ng/mL, respectively. The results showed a significantly decreased SOD in the doxorubicin group compared to the control group. The administration of GL and Lisilopril caused a significant increase in SOD concentration compared to Doxorubicin. Coadministration of Lisinopril and GL resulted in a significant increase in SOD level (P<0.01) above normal control (Figure 2).

Catalase Concentration: The mean serum CAT concentrations in control, Doxorubicin, Dox + Gl, Dox + Llisilopril, and Dox + GL + Lisilnopril groups were 25.90±6.27 ng/mL, 48.77±2.46 ng/mL, 49.93±2.47 ng/mL, 55.03±3.06 ng/mL, and 69.00±3.84 ng/mL, respectively. Showing a significant increase (P<0.05) in catalase concentration in the GL and/or Lisinopril when compared with Doxorubicin (Figure 3).

Total Antioxidant Capacity: The mean serum total antioxidant capacity **(**TAC) in the control, Doxorubicin, Dox + GL, Dox + Lisinopril, and Dox + GL + Lisinopril groups was 1.45±0.09 mmol/L, 1.88±0.05 mmol/L, 1.01±0.01mmol/L, 0.15±0.01mmol/L, and 0.08±0.04mmol/L, respectively. A significant increase (P<0.05) was observed in TAC concentration in the doxorubicin group when compared with the control group. The administration of *G. latifolium* decreased TAC activity when compared with the doxorubicin group (Figure 4).

Malondialdehyde Concentration: The mean serum concentrations of MDA in the control, Doxorubicin, Dox + GL, Dox + Lisinopril, and Dox + GL + Lisinopril groups were 0.73±0.27 nmol/mL, 2.30±0.12 nmol/mL, 2.09±0.51 nmol/mL, 0.88±0.12 nmol/mL, and 0.76±0.47 nmol/mL, respectively. A significant increase was observed in MDA concentration in the doxorubicin group compared to the control group. GL + Lisinopril reduced MDA significantly (P<0.05) compared with Doxorubicin (Figure 5).

Angiotensin-II Concentration: The result of the Ang-II concentrations in the control, Doxorubicin, Dox + Gl, Dox + Lisinopril, and Dox + Gl + Lisinopril groups were 465.33±19.28 pg/mL, 571.40±92.41 pg/mL, 550.83±52.71 pg/mL, and 560.53±66.16 pg/mL, and 474.83±12.35 pg/mL, respectively. No significant change was observed in Ang-11 concentration among the various groups (Figure 6).

Troponin Level: The mean serum troponin concentrations in the control, Doxorubicin, Dox + GL, Dox + Lisinopril, and Dox + GL + Lisinopril group were 68.67±4.06 pg/mL, 345.33±16.18 pg/mL, 113.33±6.12 pg/mL, 172.67±11.85 pg/mL, and 168.67±7.86 pg/mL, respectively. A significant increase (P<0.05) in serum troponin concentration in the doxorubicin group was observed when compared with the control group. Treatment with GL and/or Lisinopril reduced troponin levels significantly (P<0.05) compared with the doxorubicin group (Figure 7).

Creatine Kinase Concentration: The mean serum CK concentrations in the control, Doxorubicin, Dox + GL, Dox + Lisinopril, and Dox + GL + Lisinopril groups were 5.83±0.32 ng/mL, 58.73±1.62 ng/mL, 51.73±3.28 ng/mL, 38.80±3.13 ng/mL, and 12.17±1.58 ng/mL, respectively. The findings showed a significant increase in serum CK concentration in the doxorubicin group as

compared with the control group. The administration of GL and/or Lisinopril significantly reduced (P<0.05) serum CK level compared with Doxorubicin (Figure 8).

Discussion

This study investigated the ameliorative potentials of ethanolic leaf extract of *G. latifolium* and Lisinopril on the cardiac biomarkers and antioxidant levels on doxorubicin-induced cardiotoxicity in Wistar rats. *G. latifolium* is an edible nutritional/medicinal plant that produces white latex and yellow flowers and can be propagated by seed or stem cuttings [20]. The plant is used to lower blood pressure in hypertensive patients [21]. Flavonoids, the phytochemicals present in the *G. latifolium* extract, are known antioxidants that mitigate inflammation and cardiac myopathy [22, 23]. *G. latifolium* has been shown to reduce some cardiovascular risk factors. Its ability to lower blood pressure has also been reported [24, 25].

Lisinopril is an angiotensin-converting enzyme (ACE) inhibitor that prevents the conversion of Ang-I to Ang-II (a powerful vasoconstrictor) by inhibiting angiotensin-converting enzymes. This inhibition results in the relaxation of blood vessels so the circulation can flow easily. Angiotensin-converting enzyme inhibitors effectively lower the mean arterial blood pressure and systolic and diastolic blood pressure in hypertensive patients. ACE inhibitors have been evaluated as antihypertensive drugs in multiple randomized controlled trials [26]. In high-risk patients who may benefit from an anthracycline-containing regimen, the use of Lisinopril is justified and should be considered to offset cardiotoxic events [27].

Doxorubicin is an effective anticancer drug that causes acute ventricular dysfunction and induces cardiotoxicity and heart failure [28]. Serial monitoring of circulating biomarker levels can evaluate cardiotoxicity during and after chemotherapy accurately and efficiently [29]. Troponin concentration was significantly increased in the group induced with Doxorubicin when compared to the control group, which is an indication of cardiotoxicity. Intervention with GL and Lisinopril significantly decreased serum troponin concentration. Creatine kinase concentration significantly declined by the administration of GL, which was caused by Doxorubicin administration. The increase signified cardiotoxicity.

The results of Ang-II showed a significant increase in its concentration in the Doxorubicin-only group as compared to the control group, which showed doxorubicin-induced cardiotoxicity. However, there was a significant decrease in Ang-II concentration due to the administration of GL and Lisinopril in treated groups.

The increase in the concentration of cardiac biomarkers in the Doxorubicin-only group was in line with an earlier report that Doxorubicin, as an anticancer anthracycline, presents a dose-dependent and cumulative cardiotoxicity, which is considered one of its most serious side effects [30]. The increase in troponin concentration was in agreement with previous findings that damage to heart muscle cells leads to the release of a greater concentration of troponin into the blood [31]. An increased concentration of CK was in line with previous reports that elevated levels of CK are observed when the heart muscle is damaged [17]. The increase in Ang-II concentration in this study agreed with the report that elevated Ang-II levels contribute to hypertension and other cardiovascular diseases [32].

The decrease in the concentration of the Ang-II in GL and Lisinopril groups is consistent with the report stating that *G. latifolium* leaf extract is cardioprotective and thus provides a basis for the use of the plant as an alternative for the prevention and management or control of cardiovascular diseases [23]. This finding also aligns with reports indicating that Lisinopril is effective in preventing and managing cardiotoxicity in cancer patients who are treated with anthracyclines, such as doxorubicin [27, 33].

Antioxidants are the molecules that prevent cellular damage caused by oxidation of other molecules. The main function of antioxidants is to protect the body against the destructive effects of free radical damage [34]. In any case, an increase in free radical production can lead to oxidative damage. Oxidation reactions are known to produce free radicals. These free radicals are highly reactive species that contain one or more unpaired electrons in their outermost shell. Once they are formed, the chain reaction starts. Antioxidant reacts with these free radicals and terminates this chain reaction by removing free radical intermediates and inhibiting other oxidation reactions by oxidizing themselves. Plants and animals have a complex system of multiple types of antioxidants, such as vitamin C and vitamin E, as well as enzymes, such as catalase, SOD, and various peroxidases [35].

Superoxide dismutase constitutes the first line of defense mechanism against ROS. It is produced at any location where an electron transport chain is present in the cell, such as mitochondria, chloroplasts, microsomes, glyoxysomes, peroxisomes, and the cytosol. The results of our present study showed a significant increase in SOD activity in Dox + GL and/or Lisinopril groups as compared to the oxidative stress-induced group (Doxorubicin only). The SOD activity also increased significantly in the Dox + Lisinopril + GL group as compared to the normal control group. Our results were in agreement with those reported by a previous study [8]. The significant decrease in SOD activity in the Doxorubicin-only group is probably due to the increased production of free radicals causing its inhibition. The increase in SOD activity is probably due to the antioxidant property of *G. latifolium*, which can also minimize the extent of damage caused by free radicals [36, 37].

Malondialdehyde, a biomarker of lipid peroxidation, is found in biological tissues and fluids [18] and is one of the end products of oxidative reactions. The results for MDA level showed a significant increase in doxorubicin-induced oxidative stress group. The rise in MDA levels in the animals induced by Doxorubicin suggests that there was increased lipid peroxidation. The result in the Doxorubicin + GL and/or Lisinopril groups compared to the Doxorubicin group indicates the ability of *G. latifolium* and/or Lisinopril to combat lipid peroxidation. This lowered lipid peroxidation may be due to the presence of active antioxidant phytochemicals, such as coumarins and polyphenols, in the *G. latifolium* leaf extract [25, 38].

Conclusions

he findings of this study have shown that cardiotoxicity is associated with alteration in cardiac biomarkers, Ang-II enzyme activity, and oxidative stress biomarkers. Administration of G. latifolium and Lisinopril significantly inhibit the elevated levels of Ang-II, troponin, and CK levels induced by Doxorubicin. Upon administration of Lisinopril and G. latifolium in rats with induced cardiotoxicity, the antioxidant markers, such as superoxide dismutase, catalase, and total antioxidant capacity, increased significantly while MDA declined significantly. Hence, G. latifolium, when combined with Lisinopril, offers a better cardioprotective effect against Doxorubicin-induced cardiotoxicity in rats.

Conflict of Interests

None.

Funding

This study was funded by TETFund CRUTECH of Nigeria.

Acknowledgement

The authors wish to acknowledge the following persons for their valuable contributions to the success of this research project and subsequent publication of the manuscript. Mr. Ededet Umoh, the Department of Physiology, University of Calabar, for his assistance in feeding, euthanizing, and collecting blood samples from the animals. Dr. Iya Eze Bassey and Dr. Bassey Icha, the Department of Chemical Pathology, University of Calabar, Teaching Hospital, for carrying out the different biochemical assays in this study.

Compliance with Ethical Guidelines

Compliance with ethical guidelines: This study was approved by the Ethical Committee of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria (No: 019PY20317).

Authors' Contributions

Gabriel Otu Ujong - conceived the research work and drafted the design. Justin Atiang Beshel - Edited the design of the study and manuscript.

Idara Asuquo Okon - involved in the bench work and wrote the initial draft.

Clement Ikani Ejim - involved in the bench work.

Idam Benedict was involved in the bench work and edited the draft.

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