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Original Research Article

CARDIOPROTECTIVE AND ANTIOXIDANT PROPERTIES OF THE ARILS OF THE FRUIT OF *BLIGHIA SAPIDA* KD KOENIG (SAPINDACEAE) IN ISOPRENALINE-INDUCED MYOCARDIAL INFARCTION IN WISTAR RATS

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ABSTRACT

Coronary artery disease and myocardial infarction are major global health issues, with myocardial infarction becoming more common. In the current study, the cardioprotective effects of methanol extract from the aril of the *Blighia sapida* fruit were assessed in relation to certain cardiac function biomarkers, endogenous antioxidant activity, and the pathology of the heart in Wistar rats that had undergone isoprenaline-induced myocardial infarction. Phytochemical screening was according to methods described by Trease and Evans. The LD_{50} of the extract was calculated using Lorke's approach. The LD_{50} was calculated using nine (9) Wistar rats. For cardioprotective and antioxidant activities, six groups of seven Wistar rats each were formed from forty-two (42), which were divided at random. Group 1 were administered 1ml of normal saline daily, group 2 through 4 were pretreated with 250 mg/kg, 500 mg/kg, and 750 mg/kg of the extract, respectively, for 28 days. Group 5 were administered 85 mg/kg of isoprenaline on days 26 and 27, 24 hours apart to cause myocardial infarction. Group 6 served as the positive control (pretreated with 2 mg/kg carvedilol for 28 days). The plant contains bioactive substances like alkaloids, flavonoids, polyphenols, cardiac glycosides, cyanogenic glycosides, steroid glycosides, cardenolides, terpenes, and tannins. No mortality even at a dose of 5000mg/kg. Glutathione peroxidase, reduced glutathione, catalase, and superoxide dismutase all show considerable increases. Total troponin, troponin c, troponin I, creatine kinase, creatine kinase-MB, and myoglobin, significantly decreased when compared to the isoprenaline group. Additionally, there was a significant drop in the levels of some biochemical markers in the extract treated groups compared to the isoprenaline control. Total cholesterol, high-density lipoprotein, low-density lipoprotein, triglycerides, and ALT, AST, and ALP significantly decreased. Histological analysis revealed some degree of cardiac modification and a notable improvement. According to the data, the extract contains modest antioxidant and cardioprotective characteristics.

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INTRODUCTION

In the treatment of cardiovascular disorders, synthetic drugs are quite helpful. However, negative side effects restrict their effectiveness [1]. For many years, medicinal plants have been employed in traditional medicine across the world [2]. Due to their fewer toxic effects and higher efficacy, particularly in illness situations that are frequently drug-resistant, the presence of natural antioxidants in plants has facilitated the invention of functional foods and medications with health-promoting qualities [3]. A rising market exists for fruit-based products with superior nutritional value and practical applications. Due to their high content of antioxidants such as phenolic compounds,

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carotenoids, anthracyclines, and tocopherols, studies have shown that eating fruits and vegetables lowers the chance of developing chronic diseases [4]. Because of their capacity to inhibit the production of free radicals following the consumption of a substance containing them, phenols have been linked to a reduction in degradation processes in the human body [4].

An oxidizing agent gains access to oxygen or hydrogen through the chemical process of oxidation. During this process, free radicals are produced, which causes cell damage [5]. Antioxidants prevent this process, preventing more oxidation reactions [6]. Oxidative stress is characterized by an imbalance between reactive oxygen species (ROS) levels (pro-oxidants) and cellular antioxidant defense systems able to counterbalance them, leading to molecular and cellular damage [7]. It is thought to contribute to a wide range of diseases, including cancer, chronic kidney disease, chronic obstructive pulmonary disease, and cardiovascular diseases (CVDs) [8]. The removal of harmful forms of oxygen and the halting of the radical oxygen cascade depends on antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPOX), glutathione reductase (GR), and glutathione-S-transferase (GST) and its peroxidase activity [9].

A common ischemia condition known as myocardial infarction is brought on by severe cardiac tissue destruction. The mismatch between blood supply and demand for oxygen in cardiomyocytes with or without atherosclerotic plaque is the cause of its development [10]. Endocardial lipid peroxidation is caused by excessive reactive oxygen species (ROS) production. Such damage triggers apoptosis, an increase in oxidative stress, and the loss of cardioprotective antioxidants [10]. Catecholamine also affects the heart in an inotropic and chronotropic manner. Increased myocardial oxygen demand and decreased myocardial blood supply brought on by excessive catecholamine cause coronary vasoconstriction and myocardial infarction [11].

Blighia sapida K.D. Koenig, often referred to as "Akee apple," is a member of the Sapindaceae plant family. When ripe, the pear-shaped fruit naturally splits into three pieces. The aril is the edible portion that is cream in color and has a sizable black seed at each end of each piece. "Ackee" (in English), "Isin" (in Yoruba), "Gwanja kusa" (in Hausa), and "Okpu" (in Igbo) are some of the most common names for it. The ackee fruit's ripe arils, which range in color from yellow to cream and have a nutty flavor, can be eaten [12]. According to traditional medicine, ackee arils contains medicinal qualities that can be used to treat or reduce symptoms of fever, constipation, skin infections, diarrhea, and other illnesses [12].

According to reports, *Blighia sapida* arils resemble numerous well-known legumes and oil seeds in terms of their phytochemical makeup [12–14]. However, ackee arils are not very important commercially or nutritionally in the West African sub-region, maybe because little is known about their health-promoting qualities, such as their cardioprotective and antioxidant activity. It would be ideal if more effective processes for turning fruit into goods with better medicinal applications could be developed. This study aims to ascertain whether the aril of fruits from *Blighia sapida* has cardioprotective and antioxidant properties.

MATERIALS AND METHOD

Drugs and Chemicals

Various drugs such as silymarin, acetaminophen and chemicals such as trichloroacetic acid (TCA), thiobarbituric acid (TBA), tris-HCL buffer, hydrogen peroxide (H_2O_2) - SRL, 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) and glutathione (GSH) standard - SRL NaOH - HiMedia, pyragallol - Ranbaxy, ethylene di-amine tetraacetic acid (EDTA), diethlene triamine pentaacetic acid (DTPA) were purchased from local medical store and kept under appropriate storage conditions.

All chemicals and reagents used for this experiment were from Sigma Company, St Louis, (USA) except for methanol which was of the analytical grade purchased from Macfes Chemicals limited, Jos, Nigeria.

Plant Collection and Authentication

The fruits of *Blighia sapida* were procured on January 25, 2022, from the Afana village in Kaduna State, Nigeria's Zangon Kataf Local Government Area. Taxonomist Joseph Azila at the Federal College of Forestry in Jos, Nigeria, authenticated the plants with voucher number FHJ 26022. The plant voucher was stored in the Federal College of Forestry's herbarium in Jos, Nigeria.

Preparation and Extraction of Plant Material

The *B. sapida* fruit arils were thoroughly cleaned, cut into pieces, and allowed to air dry for two weeks in the shade. They were extracted with 70 % methanol after being pulverized into a powder. Then, 300 g of the dried, coarse powdered particles were soaked in 2500 ml of methanol for 48 hours, while the mixture was periodically agitated, to produce the extract. Later, Whatman filter paper was used to refine it. The extracts were dried by evaporating them in a water bath at 40 ^{o}C. To avoid the chemical components from being damaged, this temperature was maintained. By evaporating the methanol in a rotavapor while under vacuum, the leftover methanol was eliminated. This was carried out right before drying the extract using evaporation.

Animal Care and Ethical Clearance

Wistar male rats weighing 160–200g were the animals used in this study. They were cared after in the Department of Pharmacology and Toxicology's Experimental Animal House in the Faculty of Pharmaceutical Sciences at the University of Jos in Nigeria. They were housed in rat cages, fed common pellet diets, and given unrestricted access to fresh, clean water. All experimental protocols adhered to the Faculty of Pharmaceutical Science at the University of Jos' Ethics on Animal Research as well as generally recognized standards for the use and care of laboratory animals. On March 16, 2020, the F17-00379 reference number for the ethical clearance was accepted and issued.

Phytochemical Screening

The qualitative phytochemical screening was carried out using the method as described by Trease and Evans [15].

Acute Toxicity (LD_{50})

Lethal dosage (LD_{50}) Determination

The concentration of *Blighia sapida* fruit aril extracts that caused 50% of the test animal population to perish was determined using the Lorke method [16]. The animals used in this experiment were male Wistar rats. Three animals per group were divided into three groups of nine (9) Wistar rats. Two phases made up the test. Each group of animals received doses of 10 mg/kg, 100 mg/kg, and 1000 mg/kg of the extract in the initial phase. After 24 hours of observation, mortalities in the animals were recorded. In the second phase, the extract was given to each group (of one animal each) in doses of 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg. Mortalities were monitored throughout the 24-hour observation period. Oral administration (gavage) was used for all extract administrations. The greatest dose that caused no mortality (D_o) and the lowest dose that resulted in mortality (D_{100}) were used to calculate the lethal dose (LD_{50}).

Methods for Evaluation of Antioxidant Activity in Rats

By measuring oxidative stress indicators and antioxidant enzymes in the blood of albino rats following the induction of oxidative stress by the method described by Galal et al. [17], the antioxidant activity of methanolic extract of arils of fruits of *Blighia sapida* was studied. Six groups of seven animals each were formed from the total number of animals. As a control group, the normal saline group was given 1 ml of normal saline orally. The Silymarin group served as the positive control group and received 100 mg/kg of silymarin orally, whereas the carvedilol group received 2 mg/kg of carvedilol. As test groups, Groups I, II, and III were given methanol extracts of the arils of *Blighia sapida* orally in doses of 250 mg/kg, 500 mg/kg, and 750 mg/kg, respectively. For seven days straight, the animals received the appropriate medications once daily by oral (gavage) administration. Animals from all groups were starved for 18 hours on the sixth day (the day before the final treatment). All the animals received paracetamol 600 mg/kg on the seventh day, one hour after the final dosage of drugs, to induce oxidative stress. Following a 24-hour administration period, urethane (10 mg/kg) anesthesia was used to kill all the animals. By puncturing the heart, blood samples were obtained that were used in subsequent laboratory tests.

Estimation of Malondialdehyde [18]

One of the aldehyde byproducts of lipid peroxidation that reacts with thiobarbituric acid (TBA) to produce a colored product and whose absorbance was measured spectro-photometrically at 530 nm is malondialdehyde in plasma. The test tubes included 0.5 ml of the serum from the test samples, which was combined with 3 ml of 10 % Trichloroacetic acid (TCA), allowed to stand for 10 minutes at room temperature, and then centrifuged for 15 minutes at 5000 rpm. Two sets of test tubes were labeled "test" and "blank." 1.5 ml of 0.67 % TBA and 2 ml of supernatant fluid were combined to create a test sample. 2 ml of distilled was mixed with 2 ml of 0.67 % TBA to create a blank sample. They were well combined, boiled for 10 minutes, and then quickly cooled under running water. At 530 nm, a colorimeter will measure the intensity of a light pink blue. Malonaldehyde (MDA) concentrations were expressed as n moles per 100 ml of serum using the molar extension coefficient (1.5×10^5) as the basis.
1.5 = 100 µmol/L (here, 100 is for conversion from ml to dl).
Then MDA = 100 × O.D. of unknown/1.5.

Estimation of Superoxide Dismutase [19]

This method utilizes the inhibition of auto-oxidation of pyrogallol by superoxide dismutase (SOD) enzyme. The assay mixture in a 3 ml volume consisting of 100 µL each of 0.2 mM pyrogallol, 1 mM EDTA, 1 mM DTPA, and varying concentrations of standard SOD enzyme or 100 µL of serum in air equilibrated tris-HCl buffer (50 mM; pH 8.2). The reaction mixture prepared in 3 sets includes standard, test, and control. Pyrogallol was added after the addition of all other reagents to start the reaction. The initial 10s period was considered as the induction period of the enzyme. So, after 10s, a change in absorbance at 420 nm at 10s intervals was recorded for a period of 4 min. The average change in the absorbance per minute was calculated. One unit of enzyme SOD was defined as the amount of enzyme received to cause 50% inhibition of pyrogallol auto-oxidation. Accordingly, the activity of the enzyme in different standards was expressed in units/ml.

Estimation of Reduced Glutathione [20]

The approach was based on the yellow color that results from a redox reaction between GSH and DTNB when DTNB (Ellman's Reagent) was introduced to sulfhydryl substances. The color that forms is stable for around 10 minutes, and temperature changes have no impact on the reaction. At 412 nm, the reaction was read. GSH levels in venous blood samples anticoagulated with ACD were maintained for up to 3 weeks at 4 °C, and GSH in red cells was comparatively stable. Only fresh lysates were utilized for the experiment since GSH slowly oxidizes in the solution. 200 ul of whole blood were thoroughly combined with 1.8 ml of distilled water and 3 ml of PPT solution, then allowed to stand for 5 minutes before being filtered. Taking two test tubes with the labels "test" and "blank." 2 ml of the clear filtrate from the mixture were put into an unlabeled test tube along with 1 ml of the DTNB reagent and 8 ml of disodium phosphate buffer. Rapid color development and stability for 10 minutes. 2 ml of distilled water, 8 ml of phosphate buffer, and 1 ml of DTNB reagent were combined to create a reagent blank. At 412 nm, the spectrophotometer recorded readings. A reading was recorded, and the curve was plotted with concentration on the X-axis and absorbance at 412 nm on the Y-axis. Using a standard curve, the concentration of the test samples was determined. GSH levels in blood samples are lower and are represented in mg/dl.

Estimation of Catalase [21]

The process works because, when heated in the presence of H_2O_2, dichromate in acetic acid reduces to chromic acetate, with perchromic acid forming an unstable intermediate. At 570 nm, the chromic acetate that results from this process was measured colorimetrically. H_2O_2 can be split using the catalase (CAT) preparation for various lengths of time. After heating the reaction mixture, the reaction was halted at a specific point by adding a dichromate/acetic acid mixture, and the amount of leftover H_2O_2 was then quantified by measuring chromic acid colorimetrically.

The appropriate reagent additions were made to three sets of tubes, which were arranged and labelled as blank, test (0 s), and test (60 s): Readings were collected at 570 nm after the tubes had boiled for 10 minutes and cooled to room temperature. From 10 to 160 moles of H_2O_2 at various concentrations were injected into tubes. The units/ml of the serum sample were used to express the CAT activity. One mole of H_2O_2/min of the enzyme is destroyed by one unit of CAT activity. Evaluation of Cardioprotective Activity of the Extract

Animal Screening and ECG Recording

To prevent the inclusion of any animals with a cardiac abnormality, the ECG was used to perform cardiac screening on all the experimental animals. Animals with a depressed ST segment, no P wave, an inverted P wave, a non-specific ST segment, or an elevated ST segment were not allowed to participate in the experiment. The study then comprised 42 adult Wistar rats with normal ECGs, who were placed into six groups (n = 7) each. This was accomplished after each rat was placed on an animal operating table and anesthetized with urethane (10 mg/kg). Rat limbs with clean-shaven surfaces had electrodes connected to them. The right hind limb was linked with a grounded electrode while the front limbs and left hind limb were used to capture ECG in conventional lines. Carefully applying conductive ECG gel over each electrode prevented the formation of a gel bridge between them. Lead II was utilized to record the ECG (BPL Cardiart 9108). Every animal had an ECG recorded for one minute, and the analysis only used the average of data from 7 consecutive ECG signals. Each experimental animal's ECG underwent quantitative and qualitative analysis, as well as additional validation by an interventional cardiologist. A quantitative analysis of the ST segment of the ECG was performed.

Experimental Design

The Wistar albino rats were distributed at random into 6 treatment groups, each with 7 rats, in a completely randomized design (CRD). Rats were given isoprenaline (ISO) (85 mg/kg) s.c. for two additional days, on days 26 and 27, at intervals of 24 hours while still receiving methanol extract for a total of 28 days, to cause myocardial infarction. Study groups were distributed as follows.

Group 1 were administered 1 ml of normal saline orally daily for 28 days.

Group 2 rats were administered methanol extract 250 mg/kg orally daily for 28 days and Isoprenaline (85 mg/kg) s.c. on the days 26 and 27

Group 3 rats were administered methanol extract 500 mg/kg orally daily for 28 days and Isoprenaline (85 mg/kg) s.c. on days 26 and 27.

Group 4 rats were administered methanol extract 750 mg/kg orally daily for 28 days and Isoprenaline (85 mg/kg) s.c. on days 26 and 27.

Group 5 were administered 1 ml of normal saline orally daily for 28 days and Isoprenaline (85 mg/kg) s.c. on days 26 and 27.

Group 6 were administered carvedilol (2 mg/kg) orally daily for 28 days and Isoprenaline (85 mg/kg) s.c. on days 26 and 27.

At the end of the experiment, the rats were sacrificed and blood samples for biochemical assays were collected in plain tubes and allowed to clot before centrifugation and the sera were separated thereafter and used for the assays. The heart was further harvested, fixed in 10 % buffered formalin, and used for histopathological studies.

Collection of Blood and Serum Preparation

Each anesthetized rat had 2 ml of blood drawn from the retro-orbital venous plexus after the ECG was recorded using capillary tubes. After clotting, blood was stored in microcentrifuge tubes. Using a Remi C-24 refrigerated centrifuge, the entire blood was centrifuged at 3,000 rpm for 20 minutes at 4°C to obtain serum. To conduct further biochemical analyses, serum was kept at 80°C.

Determination of Cardiac Biomarkers by Mesoscale Discovery Platform (MSD) [22]

Electro-chemiluminescent tests were used to analyze cardiac biomarkers. According to the manufacturer's protocol, 25ml of the diluted serum sample was dispensed and evaluated in duplicate. The serum samples were diluted in a ratio of 1:4. The plates were examined using an MSD Sector upon the end of the operation based on the Mesoscale Discovery (MSD) assay methodology. The outcomes were reported as the average of the two values. The manufacturer's protocol was followed while dispensing and testing each sample's ten microliter of serum in duplicate. A Molecular Device was used to analyze the plates. By using a specialized test, MPI Research (Mattawan, MI) measured the amount of myosin light chain 3 (Myl3) in rat serum. To ensure that every sample enters and flows optimally via the microfluidic channels of a Gyrolab CD, samples were diluted 1:4 in Rexxip A buffer prior to analysis. The work interpolated quantities from a standard curve using a rat-specific recombinant his-tagged myl3. Positive controls at six different concentrations on three distinct CDs were used to characterize the assay's precision and accuracy.

Determination of Total Troponin, Troponin I & C

Cardiac troponin I and C were measured using the troponin-Ultra assay. It is a three-site sandwich immunoassay that uses direct chemiluminometric technology and was developed to detect human cardiac troponin I, although it has been

demonstrated to have cross-reactivity in several species, including rats [22]. According to the manufacturer's methodology, serum samples were examined.

Determination of CK and CK-MB Activity

The immuno-inhibition approach, which was employed and described by [23-25], was used to determine this. High Sensitivity C-Reactive Protein (hsCRP) measurement A technique described by [26] and [24] was used to measure the High Sensitivity C-Reactive Protein (hsCRP). Calculating the troponin level, The ELISA approach was used to assess troponin. According to [27], the UV kinetic technique was used to evaluate the activity of lactate dehydrogenase (LDH).

Histopathological Examination

The technique given by [28] was applied with a few minor modifications. At the conclusion of the research period, the experimental animals were put to death. Each group's cardiac tissue slices were obtained for histopathological research. Prior to tissue processing, the samples were fixed in 10 % phosphate-buffered formalin for a minimum of 48 hours. The tissues were then cut, dehydrated in four alcohol concentrations (70 %, 80 %, 90 %, and 100 % alcohol), cleaned in three xylene concentrations, and embedded in molten wax. The tissue-containing wax blocks were placed in water baths, allowed to set, and then cut into 5 m thick slices before being incubated at 60 °C for 30 minutes. After clearing in three xylene grades and rehydrating in three alcohol grades (90 %, 80 %, and 70 %), the 5 m thick sectioned tissues were processed. Hematoxylin was then applied to the sections for 15 minutes of staining. The bluing process used ammonium chloride. Before counterstaining with Eosin, differentiation was performed using 1 % acid alcohol. On degreased glass slides, permanent mounts were created using a mountant.

Slide Examination

Using x4, x10, and x40 objective lenses, the prepared slides were viewed using a MoticTM compound light microscope. The photomicrographs were taken at x40 magnifications with a MoticTM 9.0-megapixel microscope camera.

Statistical Analysis

The statistical analysis was done using the SPSS package (SPSS version 15.0). Data analysis was carried out using one-way ANOVA followed by Dunnett's post hoc test. The data were expressed as Mean ±SEM and valves of $p<0.05$ were considered significant.

RESULTS

Table 1 shows the results of phytochemical analysis.

Table 2 shows that the arils *Blighia sapida* does not have any acute toxicity since no mortality was recorded at the highest dose of 5000mg/kg.

Table 3 represents antioxidant activity of the various group when compared with the normal saline group. The extract-treated (Group 2-4) group significantly increased compared with normal saline group (Group 1).

Table 4 represents the ST-segment elevation of ECG in the normal saline, extract-treated groups, and Isoprenaline treated group. The extract treated groups (group 2-4) significantly decreased compared with the isoprenaline treated group (group 5).

Table 5 shows the cardiac biomarkers in normal saline, extract treated, isoprenaline group, and carvedilol group. The extract treated groups (groups2-4) significantly decreased when compared with isoprenaline treated group (group 5).

Table 6 represents some biochemical and lipid profile parameters of the different groups that were administered normal saline, methanol extract, isoprenaline, and carvedilol in different doses. The extract treated groups (groups 2-4) significantly decreased when compared with the isoprenaline treated group (group 5).

The ECG tracing in Figure 1 describes the different groups that were administered normal saline, methanol extract, and isoprenaline in different doses. There was decreased ST segment elongations of the ECG in groups (B-D) compared to the isoprenaline treated group (group E).

The plates A-F in Figure 2 represent histological photomicrographs of sections of the heart of the wistar rats after treatment with normal saline, methanol extract, carvedilol and isoprenaline to the respective groups as in figure 2. There was no significant damage to the heart tissues as presented in plates (c-d) as against necrotic heart tissue in plate (f).

DISCUSSION

This investigation aims to assess how cardiac function indicators, endogenous antioxidant activity, and the histological structure of the heart in isoprenaline-induced myocardial infarction in rats respond to *Blighia sapida* methanol extract.

Alkaloids, flavonoids, glycosides, polyphenols, tannins, terpenes, and cardenolides were discovered by phytochemical analysis (Table 1). These outcomes are comparable to those of [28–29] and to those attained by [30]. These components oversee their pharmacological effects, which forms the basis for their application in medicine [30].

Even at a dose of 5000 mg/kg of the methanol extract of the arils of *Blighia sapida*, no mortality was observed in the acute toxicity experiments (Table 2), making it impossible to calculate the extract's LD_{50}. This finding is comparable to that of [31], who found that the LD_{50} of an aqueous and ethanol extract of *Blighia sapida* leaves was larger than 5000 mg/kg, indicating that the substance was either safe or non-toxic. According to the Toxicity Scale Principle, any drug having an LD_{50} greater than 5000 mg/kg is essentially nontoxic [32].

The impact of the extract on a few oxidation parameters in acetaminophen-induced oxidative stress is shown in Table 3. Compared with all extract treated group (groups 2-4), the malondialdehyde concentration is significantly higher in

Table 1: Phytochemical Evaluation of the 70 % Methanol extract of the aril of the fruit of *Blighia sapida*

Constituents	Indications
Saponins	-
Polyphenols	+
Flavonoid	+
Steroid glycosides	+
Terpenes	+
Phlobatanins	-
Anthraquinone	-
Cyanogenic glycoside	+
Balsam	-
Resins	-
Alkaloids	+
Cardiac glycoside	+
Cardenolides	+
Tannins	+

Key: (+) = Present; (-) = Absent

Table 2: Acute toxicity test for the 70 % Methanol extract of the arils of the fruit of *Blighia sapida*

Phase	Dose (mg/kg)	No of Animals	Mortality	Mortality %
1	10	3	0	0
	100	3	0	0
	1000	3	0	0
2	1600	1	0	0
	2900	1	0	0
	5000	1	0	0

Table 3: The Effect of Methanol Extract of *Blighia sapida* on some oxidation parameters in Acetaminophen-induced oxidative stress in wistar rats

Group	MDA	CAT	SOD	GPX	GSH
Group 1	3.98±0.29	6.27±1.02	8.48±1.43	8.18±1.45	10.50±1.45
Group 2	1.060±0.01	6.868±0.16*	8.973±0.05*	7.948±0.25*	10.52±0.29*
Group 3	5.41±1.48*	10.61±0.30*	13.58±0.37*	11.99±0.57*	13.96±0.59*
Group 4	6.028±0.37*	16.98±0.05	20.25±0.46	16.58±0.60	18.27±0.42
Group 5	2.67±0.16*	14.19±0.25*	16.58±0.28*	12.99±0.16*	15.48±0.21*

*Values presented as mean ± SEM, Significance relative to control *p < 0.05, n=7;*
KEY: MDA= Malondialdehyde (Nm/MG); Protein=mg/dl; CAT=Catalase (µmol/mg protein); SOD= Superoxide dismutase (µMol epinephrine oxidized/min/mg protein); GPx= Glutathione peroxidase (µmol/mg protein); GSH=Reduced Glutathione (µg/mg protein); Group 1 were administered 1 ml of normal saline, Group 2 received 250 mg/kg of extract; Group 3 received 500 mg/kg of extract; Group 4 received 750 mg/kg of extract and Group 5 received 100 mg/kg of Silymarin

Table 4: Effect of administration of methanol extract of *Blighia sapida* on the ST-segment elevation of ECG of the Isoprenaline-induced myocardial infarction in Wistar rats

	Treatment (mg/kg)				
	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**
ST segment (mm)	0.05±0.02	0.05±0.02	0.02±0.03	0.03±0.02	0.08±0.03

Group 1 received 1 ml of normal saline; Group 2 received 250 mg/kg of methanol extract; Group 3 received 500 mg/kg of methanol extract; Group 4 received 750 mg/kg of methanol extract; Group 5 received isoprenaline 85 mg/kg

Table 5: Effect of Methanol Extract of *Blighia sapida* on Cardiac Biomarkers in Isoprenaline-induced Myocardial Infarction in Wistar Rats

Group	TP	TI	TC	MG	CK	CKMB
Group 1	2.75±0.05	0.08±0.00	2.66±0.05	66.20±1.20	195.80±3.90	28.22±0.57
Group 2	4.74±0.05 *	0.14±0.00 *	6.60±1.10 *	114.60±1.28 *	337.80±3.76 *	49.38±0.55 *
Group 3	2.96±0.02 *	0.09±0.00	2.86±0.02	71.50±0.39 *	210.80±1.15 *	30.82±0.17 *
Group 4	2.83±0.02	0.09±0.00	2.74±0.02	68.49±0.38	201.90±1.13	29.52±0.17
Group 5	6.45±0.11 *	0.19±0.00*	6.056±0.15 *	155.90±2.71 *	459.50±7.99 *	67.04±1.16 *
Group 6	2.68±0.02	0.08±0.00	2.60±0.02	64.78±0.38	191.00±1.13	28.04±0.19

*Values presented as mean ± SEM, Significance relative to control *p < 0.05, n=7;*
KEY: Group 1 received 1 ml of normal saline daily, Group 2 received 250 mg/kg of methanol extract; Group 3 received 500 mg/kg of methanol extract; Group 4 received 750 mg/kg of methanol extract; Group 5 received 85 mg/kg of Isoprenaline; Group 6 received 2 mg/kg of carvedilol; TP= Total Troponins (ng/ml); TI= Troponin I; TC= Troponin C; CK= Creatine Kinase (IU/L); CKMB= Creatine Kinase-MB (IU/L); MG= Myoglobin (ng/ml); ISO= Isoprenaline

Table 6: Effect of Methanol Extract of *Blighia sapida* on Some Biochemical Parameters in Isoprenaline-induced Myocardial Infarction in Wistar Rats

Group	TC	HsCRP	HDL	LDL	ALT	AST	LDH
Group 1	2.95±0.05	79.00±0.34	0.54±0.03	2.25±0.15	25.64±0.40	32.90±0.66	384.40±2.11
Group 2	6.06±0.04 *	70.87±1.10*	0.44±0.02 *	4.34±0.09 *	37.36±2.13 *	48.52±0.41 *	489.50±1.15 *
Group 3	4.13±0.02 *	68.33±2.18*	0.90±0.02 *	2.78±0.03 *	33.94±0.36 *	41.22±0.49 *	427.20±1.51*
Group 4	3.80±0.04 *	71.50±1.71*	1.08±0.01 *	2.40±0.05	29.94±0.34 *	37.08±0.22 *	415.20±1.66
Group 5	6.79±0.05 *	80.90±1.26	0.81±0.02 *	4.48±0.04 *	48.58±0.62 *	74.98±1.24 *	600.90±6.06 *
Group 6	3.47±0.04 *	76.69±0.26 *	0.76±0.03 *	2.31±0.03	28.90±0.17	37.32±0.26 *	440.60±23.73 *

*Values presented as mean ± SEM, Significance relative to control *p < 0.05, n=7*
KEY: TC=Total Cholesterol (mg/dl); TG=Triglyceride (mg/dl); HDL= High-Density Lipoprotein (mg/dl); LDL=Low Density Lipoprotein (mg/dl); ALT=Alanine aminotransferase (U/L); AST=Aspartate aminotransferase (U/L); LDH= Lactate Dehydrogenase (U/L); HSCRP= Highly Sensitive C- Reacting Protein (ng/ml); Group 1 received 1 ml normal saline daily, Group 2 received 250 mg/kg of methanol extract; Group 3 received 500 mg/kg of methanol extract; Group 4 received 750 mg/kg of methanol extract; Group 5 received 85 mg/kg of Isoprenaline; Group 6 received 2 mg/kg of carvedilol

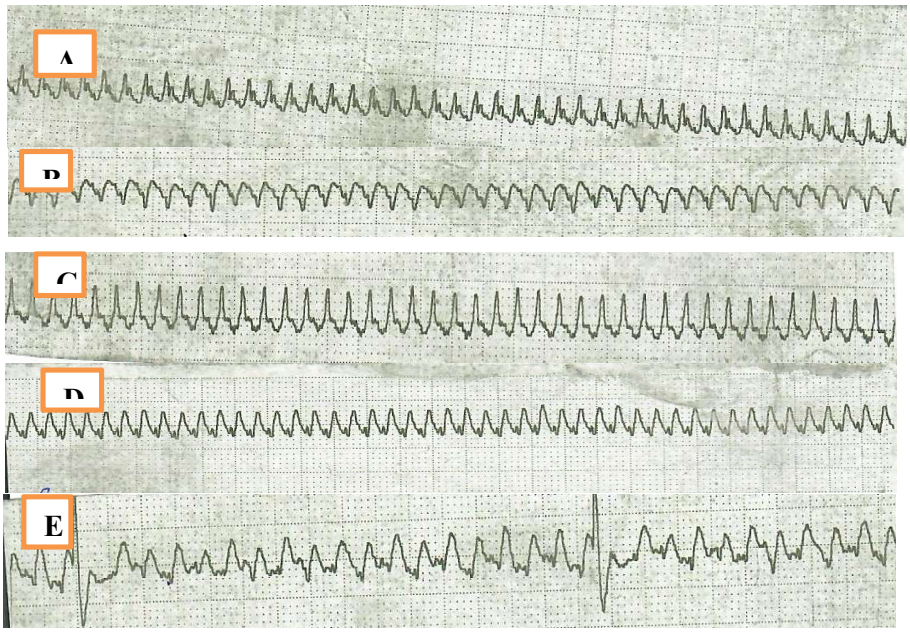

Figure 1: Electrocardiogram recording of the various groups
(*A) group of rats that received normal saline; (B) group of rats that were administered 250mg/kg of extract; (C) group of rats that were administered 500mg/kg of extract (D) group of rats that were administered 750mg of extract while (E) were group that were administered isoprenaline 85 mg/kg*

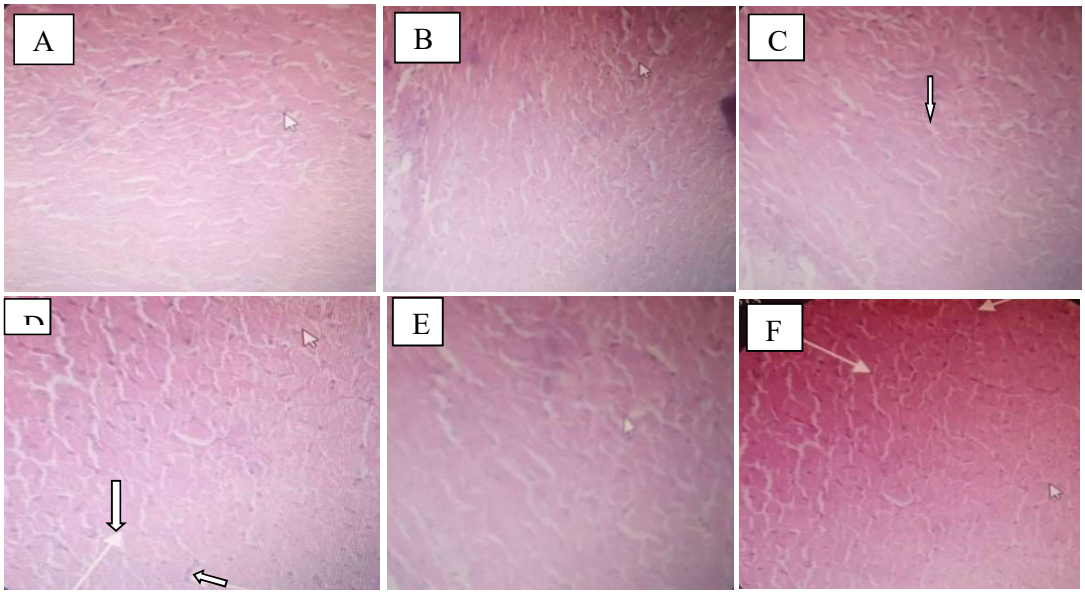

Figure 2: Photomicrographs of Heart sections from control and experimental rats after the administration of Methanol extract of *Blighia sapida*
*(**A**) show sections of the heart from rats in the normal saline group. The arrow shows a normal myocardial histomorphology with elongated nuclei of myocardiocytes (**B**) showing a section of the heart from rats in the carvedilol 2 mg/kg group. The arrow shows a normal myocardial histomorphology with elongated nuclei of myocardiocytes (**C**) shows a section of the heart collected from rats that were administered extract 750 mg/kg group with mild myocardial necrosis (**D**) shows a section of the heart from rats in the group administered 500 mg/kg. The up and down arrow shows moderate necrosis (**E**) shows the section of the heart collected from rats that were administered 250 mg/kg with moderate myocardial necrosis (**F**) shows the section of the heart collected from rats administered isoprenaline 85 mg/kg with widespread myocardial necrosis (down arrow), fragmentation, and loss of striation (up arrow)*

isoprenaline treated groups. This would suggest the extract has antioxidant properties. Because the hydroxyl group in antioxidant compounds is the reactive form of ROS and can produce lipid peroxidation by attacking polyunsaturated fatty acids, lipid peroxidation is helpful in determining oxidative stress [33]. One of the often-utilized substances or biomarkers to assess lipid peroxidation is malondialdehyde. Table 3 indicates that lipid peroxidation most likely took place. Enzymes that are endogenous antioxidants and play significant roles in oxidative stress include catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and reduced glutathione [34].

Table 3's presentation of glutathione peroxidases reveals that there is a considerable increase in the extract-treated groups (groups 2-4) compared to the normal saline group. It is a crucial intracellular enzyme that converts lipid peroxides into alcohol and hydrogen peroxide to water in the mitochondria and cytoplasm [35]. This is crucial in preventing oxidative stress from damaging cells [36]. This may indicate that the extract possesses cardioprotective and antioxidant effects. Additionally, as compared to the normal saline group, catalase concentrations in the extract-treated animals considerably increased. This suggests that the extract can prevent oxidative damage brought on by acetaminophen. Glutathione peroxidase concentration (table 3) significantly decreased as compared to the normal saline group (group 1), suggesting that the extract could prevent cell damage caused by hydroxyl and superoxide radicals. Glutathione plays an important role in the antioxidant defense system as well as in maintaining membrane protein thiols [38]. This could also imply that it could find use in the management of many age-related degenerative diseases [37].

Table 4 and Figure 1 show that the ST segment of the ECG in the isoprenaline group (group 5) is elongated than in the extract-treated groups (groups 2-4). According to [39], a ventricular aneurysm that forms after an infarction can cause chronic ST elevation, loss of the S wave, and T wave inversion.

In large doses, the non-selective beta agonist isoprenaline causes myocardial necrosis that resembles an infarct [23]. Additionally, several publications have demonstrated that isoprenaline can result in myocardial infarction at high doses [39–43]. The method of action has not yet been established, though. The possible mechanisms include intracellular calcium overload, altered myocardial cell membrane permeability brought on by lipid peroxidation [41], hypoxemia brought on by increased cardiac work and oxygen demand, production of free oxygen radicals by catecholamine auto-oxidation, interruption of mitochondrial oxidative phosphorylation by free fatty acids, and changes in electrolyte content [42].

The myocardium experiences high oxidative stress from isoprenaline, which leads to cardiac muscle necrosis that resembles an infarct [44]. Additionally, it has been shown to produce free radicals and promote lipid peroxidation, which may be the cause of irreparable harm to the cardiac membrane. As a result of Table 4, an elongated ST segment may indicate a myocardial infarction [44].

According to a study [45], cardiovascular diseases are responsible for most of the morbidity and mortality in the modern era. Myocardial infarction is one of the cardiovascular diseases and it occurs when there is a decrease or complete blockade in the blood (oxygen) supply to part of the heart, resulting in degeneration of that part of the myocardium which triggers a cascade of cellular inflammation and biochemical events, leading to the irreversible death (necrosis) of the muscle cells [45].

Cardiology relies heavily on proteins and enzymes known as cardiac function biomarkers for the main and secondary prevention, diagnosis, and treatment of acute myocardial infarction and other heart-related conditions [46]. Table 5 lists the outcomes of the heart function biomarkers. According to Table 5, all cardiac indicators significantly decreased in the extract-treated groups (group 2-4) compared to the isoprenaline group (group 5). Troponins are regulatory proteins that are essential for muscular contraction and can be found in skeletal and cardiac muscle. Actin and myosin's interaction with calcium is regulated by cardiac troponin 1 [47]. Troponin I is frequently utilized to detect damaged cardiac muscle [47–48]. The reversible conversion of creatine and ATP to creatine phosphate and ADP is catalyzed by the enzyme creatine kinase (CK) [49]. Three other kinds of CK, or isoenzymes, exist CKMM, which is found in skeletal muscle and the heart; CK-MB, which is in the heart; and CK-BB, which is mostly found in the brain [50]. 4 to 9 hours after the onset of chest discomfort or cardiac cell injury, the serum level of CK-MB increases.

Additionally, according to Table 5's findings from the study, the isoprenaline treated group (group 5) exhibits myocardial necrosis/cardiotoxicity and has a significantly higher level of total troponin, troponin I, C, myoglobin, CK, and CK-MB activity than the groups who received extract treatment. This is consistent with the findings of numerous authors' studies [51][41][43]. The significant rise in the isoprenaline treated group's (group 5) total troponin, troponin I, C, myoglobin, CK, and CK-MB levels demonstrates that isoprenaline-induced cardiac tissue damage occurred.

Troponin I level rise when cardiac cells are harmed, and cardiac marker enzymes are also released into the extracellular fluid during myocardial necrosis of the heart, according to [52]. When a cell membrane is damaged due to hypoxia or another sort of injury, CK-MB is produced [53]. The fact that the cardiac indicators are not significantly decreased by the methanol extract of *Blighia sapida* arils compared with the isoprenaline group (group 5) suggests that it may be cardioprotective.

Table 6 shows that, when compared to the isoprenaline-treated group (group 5), the extract-treated groups' (group 2-4) lactate dehydrogenase (LDH) levels dramatically decreased. The carvedilol-treated group (group 6), however, is within the same range as the groups that received extract treatment. This suggests that the extract may function in a cardioprotective manner. Skeletal muscles, the heart, the liver, the kidney, the brain, the lungs, and red blood cells all contain lactate dehydrogenase. An increase in LDH levels is a sign of cell damage linked to a wide range of disorders [54]. This is evident in the isoprenaline-treated group, where it is known that large dosages of isoprenaline can result in myocardial infarction [39].

The liver, muscles, and heart contain both AST and ALT. According to Table 6, the extract-treated groups (group 2-4) showed a substantial decrease compared to the isoprenaline treated group (group 5). They are typically employed as biomarkers in determining the level of damage to any of the organs [55]. They only rise when the liver, muscles, and heart are damaged and eventually leak damage into the blood. When compared to the isoprenaline-treated group (group 5), the triglyceride level in the extract-treated groups (group 2-4) reduced considerably. However, when there is cell injury, the level of triglycerides changes in the other direction. Triglyceride levels may rise after myocardial infarction due to increased fatty acid flow and compromised plasma clearance of very low-density lipoprotein (VLDL) [56]. In research [57], patients with acute myocardial infarction had significantly higher levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (HDL).

Table 6 shows that when compared to the isoprenaline-treated group (group 5), highly sensitive C-reacting protein (hsCRP) levels in the extract-treated groups (groups 2-4) considerably decreased. According to a study's data, most myocardial infarction patients have increased hsCRP [58]. This is because it has been identified as a systemic inflammatory marker. The following risk of fatal major adverse cardiovascular events and HsCRP are linked [59]. Both the use of hsCRP for predicting the risk of vascular events in cardiovascular prophylaxis [50] and the role of inflammation in the pathophysiology of atherosclerosis are well known [60–61]. The isoprenaline group's (group 5) considerable increase in hsCRP levels in this study is proof that myocardial infarction and inflammation are related, as stated [47].

The normal saline group (group 1) is the only one in the histopathological investigation plates A through F of Figure 2 that does not exhibit any cardiac abnormality. The heart damage significantly improved after receiving the extract treatment. The cardiac necrosis, myocytolosis, and occasionally the loss of cell striation typical of Zenker's necrosis were seen in the isoprenaline group.

CONCLUSION

Some isoprenaline-induced biochemical alterations in myocardial infarction can be avoided by using the methanol extract of *Blighia sapida's* arils. The extract may have cardioprotective and antioxidant potentials that might be used for therapeutic purposes, according to the significant ($p<0.05$) rise in several endogenous enzymes and cardiac function biomarkers.

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CONFLICT OF INTEREST DISCLOSURE

The authors declare that there is no conflict of interest in this study.

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