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Cardio-protective potentials of *Bryophyllum pinnatum* against doxorubicin-induced heart impairment in Wistar rats

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Abstract

Doxorubicin (Dox), an antibiotic used to treat a variety of tumors, has several substantial adverse effects that limit its clinical utilization. Dox administration induces myocardial damage and impairs cardiac functions, *Bryophyllum pinnatum* is an effective anti-oxidant and anti-inflammatory plant that treats many ailments. This study was therefore set to ascertain the cardioprotective role of *Bryophyllum pinnatum* (*Crassulacaea*) against Dox-induced cardiac damage in rats. Five groups of 7 rats each were used for the study. The control received normal rat feed and drinking water. Group 2 (Dox only) received 50 mg/kg of Doxorubicin i.p. once daily. Group 3 (Extract group) took 600mg/kg of the ethanolic extract of *Bryophyllum pinnatum* orally once daily. Group 4 (Dox + extract) had Doxorubicin + extract and group 5 (Dox + Bis) received Doxorubicin + 5mg/kg of Bisoprolol orally once daily. The feeding regimens lasted 28 days. Blood samples and the heart were collected. Our results revealed that administration of doxorubicin induced cardiac injured in rats with significant increases in Apolipoprotein-E, CRP and IL-6 and reduction in Apolipoprotein-A concentrations. Also, Dox-only group had significant ($p<0.05$) elevation of serum cardiac biomarkers (Troponin, myoglobin, CK-MB, ACE) as well as significant ($p<0.05$) reduction in NO concentration. Doxorubicin also significantly ($p<0.05$) reduced serum antioxidant enzymes (SOD, CAT, GPx and TAC) concentrations. Furthermore, all the photomicrographs featured damage to cardiomyocytes induced by doxorubicin administration. All these anomalies were ameliorated following treatment with ethanolic extract of *Bryophyllum pinnatum* or Bisoprolol. These findings reveal the cardio-protective potentials of *Bryophyllum pinnatum* extract against doxorubicin-induced heart damage in rats.

Keywords: Doxorubicin; *Bryophyllum pinnatum*; Bisoprolol; Cardio-protection; Apolipoproteins

1. Introduction

Cardiovascular diseases (CVDs) are a leading cause of mortality worldwide, with various factors contributing to their onset and progression. Among these factors, drugs particularly, doxorubicin has been implicated in cardiac dysfunction [1]. Cardiovascular diseases (CVDs) encompass a range of conditions affecting the heart and blood vessels, including coronary artery disease, myocardial infarction, heart failure, and stroke [2]. These conditions collectively represent a significant global health burden, contributing to millions of deaths annually. Various risk factors predispose individuals to CVDs, including genetic predisposition, lifestyle factors (such as diet and physical activity), hypertension, diabetes, and environmental exposures to toxins like heavy metals [3].

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Doxorubicin is an anthracycline group antibiotic derived from the *Streptomyces peucetius* bacterium. It has had wide use as a chemotherapeutic agent. Other anthracyclines include daunorubicin, idarubicin, and epirubicin. Commonly, doxorubicin is an agent used in the treatment of solid tumors in adult and pediatric patients. Doxorubicin may be used to treat soft tissue and bone sarcomas and cancers of the breast, ovary, bladder, and thyroid. It is also used to treat acute lymphoblastic leukemia, acute myeloblastic leukemia, Hodgkin lymphoma, and small cell lung cancer. The liposomal formulation of doxorubicin has FDA approval for the treatment of ovarian cancer in patients who have failed platinum-based chemotherapy, AIDS-related Kaposi sarcoma, and multiple myeloma [4].

The primary mechanism of action of doxorubicin involves the drug's ability to intercalate within DNA base pairs, causing breakage of DNA strands and inhibition of both DNA and RNA synthesis. Doxorubicin inhibits the enzyme topoisomerase II, causing DNA damage and induction of apoptosis. When combined with iron, doxorubicin also 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 [5]. Adverse reactions are common after doxorubicin administration, including fatigue, alopecia, nausea and vomiting, and oral sores. Bone marrow suppression and an increased risk of secondary malignancy diagnoses may occur. Doxorubicin extravasation during intravenous administration can result in severe tissue ulceration and necrosis, which worsens over time. Doxorubicin is also associated with significant cardiac toxicity, which limits the long-term use of the drug. The mechanism of action of doxorubicin-induced cardiac toxicity differs from the drug's antitumor mechanism. It involves increased oxidative stress, down-regulation of cardiac-specific genes, and induction of cardiac myocyte apoptosis by doxorubicin, [6].

Natural products, including plant-derived compounds, have emerged as potential candidates for cardiovascular protection due to their antioxidant, anti-inflammatory, and lipid-

modulating properties. Crassulaceae, a family of succulent plants comprising over 1,400 species distributed across various regions worldwide, has attracted scientific interest due to its diverse pharmacological properties [6]. Extracts derived from Crassulaceae plants contain a plethora of bioactive compounds, including flavonoids, phenolic acids, saponins, polysaccharides, and alkaloids, which contribute to their medicinal properties. These phytochemicals exert antioxidant, anti-inflammatory, anti-cancer, immunomodulatory, and cardioprotective effects, making Crassulaceae extracts promising candidates for therapeutic interventions in various diseases, including cardiovascular disorders [7]. These extracts contain various phytochemicals, such as flavonoids, phenolic acids, saponins, and polysaccharides, which contribute to their therapeutic properties. Studies have demonstrated the ability of Crassulaceae extracts to scavenge free radicals, inhibit inflammatory pathways, reduce oxidative stress, and improve lipid profiles in experimental models of cardiovascular disease [6].

Bisoprolol is a β1-selective adrenergic receptor antagonist belonging to the class of beta-blockers, widely used in the management of cardiovascular conditions such as hypertension, angina pectoris, and heart failure [8]. Its mechanism of action involves the selective blockade of β1 adrenergic receptors, primarily located in the heart. Bisoprolol exerts its pharmacological effects by competitively binding to β1 adrenergic receptors on cardiac myocytes [9].

2. Materials and method

2.1. Collection and preparation of plant materials

The plant *Bryophyllum pinnatum (Ccrassulaceae)* (Africa never die) was obtained from Ugep local market, in Yakurr Local Government Area of Cross River State, on March, 2024. It was identified and authenticated in the Department of Botany, University of Calabar. Its ethanolic leaf extract was prepared in University of Cross River State (UNICROSS), Okuku Campus.

2.2. Preparation of *Crassulaceae* (*africa never die*) extract

Bryophyllum pinnatum (African never die) leaves were cleaned, washed to remove debris and dried. They were then grinded into powder using Allyson blender (model FY-999), after grinding. It was soaked in 98% ethanol solution (BDH chemical Ltd Poole England) for 24 hours and was then filtered, after 24 hours the supernatant was extracted, first using second time using white cellulose material, and then by Whatman number 1 filter paper, oven dried at 45 °C into paste form and stored at normal room temperature. The extraction gave a percentage yield of about 5.3%. The extract was stored at 4 °C till further use.

2.3. Experimental animal

Thirty-five (35) Wistar rats weighing between 150-250 were obtained from the animal house of physiology Department, faculty of Basic Medical Sciences, University of Cross River State (UNICROSS) Okuku campus. The animals were allowed to acclimatize for one week in the medical Physiology Department where the experiment was carried out. The animals were housed in well-ventilated cages. The beddings, feed and water were replaced every day, and kept under controlled environmental conditions (room temperature of about 27 ℃ and 12-hour light/dark cycle). The animals were given feed and water freely. Ethical approval was obtained from the Faculty of Basic Medical Sciences University of Calabar Animal Research and Ethics Committee with ethical approval number No: 024PHY20324.

2.4. Experimental design

The animals were group into five group containing 7 rats in each group.

- Group 1 (Control): received normal rat chow and drinking water
- Group 2 (Extract fed): took 600 mg/kg body weight of *Bryophyllum pinnatum* extract orally
- Group 3 (Dox only): Received 50 mg/kg body weight of doxorubicin intraperitoneally
- Group 4 (Dox + Extract): took 50 mg/kg body weight of doxorubicin + 600 mg/kg body weight of *Bryophyllum pinnatum* extract orally
- Group 5 (Dox + Bis): took 50 mg/kg body weight of doxorubicin + 5 mg/kg body weight of bisoprolol orally

Animals were also fed with normal salt and water *ad libitum*. Treatment lasted for 28 days.

2.5. Collection of blood samples

After 28 days of experimental regimens, the animals were sacrifice under 60mg/kg body weight ketamine intraperitoneally. Blood was collected via cardiac puncture using a 5 ml syringe into plain capped sample bottles, which was then centrifuged at 3000 RPM for 10 minutes). Serum was extracted from the supernatant and taken for laboratory assay

2.6. Harvesting of heart

The heart was harvested from each animal and put into a 10% formaldehyde for preservation, which would later be taken for further analysis to determine the cardiac biomarkers and histological study.

2.7. Determination of biochemical parameters

2.7.1. Superoxide dismutase (SOD) activity

Superoxide dismutase activity was determined spectrophotometrically [10].

Principle:

Superoxide dismutase uses the photochemical reduction of riboflavin as an oxygen generating system and catalyzes the inhibition of NBT reduction, the extent of which can be assayed spectrophotometrically.

Procedure

The incubation medium contained a final volume of 3.0 ml, 50 mM potassium phosphate buffer (pH 7.8), 45 M methionine, 5.3 mM riboflavin, 84 M NBT and 20 M potassium cyanide. The amount of homogenate added to this medium was kept below one unit of enzyme to ensure sufficient accuracy. The tubes were placed in an aluminum foil-lined box maintained at 25 °C and equipped with 15W fluorescent lamps. Reduced NBT was measured spectrophotometrically at 600 nm after exposure to light for 10 minutes. The maximum reduction was evaluated in the absence of the enzyme. One unit of enzyme activity was defined as the amount of enzyme giving a 50% inhibition of the reduction of NBT. The values were calculated as units/mg protein.

2.7.2. Catalase (CAT) activity

Catalase activity was assayed spectrophotometrically following standard method [11].

Principle:

The UV light absorption of hydrogen peroxide can be easily measured between 230 – 250 nm. On decomposition of hydrogen peroxide by catalase, the absorption decreases with time. The enzyme activity can be arrived at from this decrease

Reagents

- Phosphate buffer: 0.067 M (pH 7.0)
- Hydrogen peroxide in phosphate buffer (2mM)

Procedure:

A 20% homogenate was prepared in phosphate buffer (0.067M, pH 7.0) and the homogenate was employed for the assay. The samples were read against a control without homogenate, but containing the H_2O_2-phosphate buffer. To the experimental cuvette, 3.0 ml of H_2O_2-phosphate buffer was added, followed by the rapid addition of 40µl enzyme extract and mixed thoroughly. The time interval required for a decrease in absorbance by 0.05 units was recorded at 240nm. The enzyme solution containing H_2O_2-free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240 nm by 0.05 units.

Glutathione (GSH) level

Total glutathione level was measured following the method [12, 13] which is based on the reduction of 5-thio-2-nitrobenzoic acid formed by the reaction of 5,5-dithiobis-2-nitrobenzoic acid (DTNB) with the sulfhydryl group of GSH. To deproteinize the samples, 100 µL of each sample was pipetted into a 1.5 mL Eppendorf tube, followed by the addition of 100 µL of 10 % (w/v) MPA. The content was vortexed for 10 sec and allowed to stand at room temperature for 5 min, followed by centrifugation at 1200 g for 15 min at 4 ℃. 100 µL of the supernatant was pipetted into a separate 1.5 mL Eppendorf tube, followed by the addition of 5 µL of 4 M triethanolamine solution, and vortexed to mix. The samples were kept at -80 ℃ until use. This procedure was also performed for the diluted standards to eliminate the need for any dilution factor.

To access GSH level, 10 µL of samples and standards were pipetted into separate wells of a 96-well plate, followed by the addition of 140 µL of working reagent. The plate was vortexed for 5 sec, followed by the addition of 10 µL of 1 mM DTNB solution. The increase in absorbance was measured at 412 nm every min, for 5 min, using ELISA plate reader. The net absorbance for each sample was obtained by subtracting the absorbance of blank (0.2 M potassium phosphate buffer containing 0.01 M EDTA, pH 7.5) from that of the sample. Total GSH level in each sample was estimated from a 6-points standard curve (0 - 25 µg/mL) obtained using GSH standard solutions, and results were normalized with protein concentration, and expressed as nmol GSH equivalent mg^{-1} protein.

2.8. Determination of total antioxidant capacity

Total antioxidant capacity was determined following standard method [3], using the cell Biolabs' OxiSelect™. Total Antioxidant Capacity assay kit manufacturer's procedure was used to estimate total antioxidant capacity. 20 µl of the diluted uric acid and sample were added to the 96-well microtiter. The reaction buffer (180 µl) was also added to each well using a multichannel pipette and shake vigorously. The initial absorbance was obtained by reading the plate over the spectrophotometer at 490nm. To initiate the reaction, 50 µl of copper ion reagent was added to each well and incubated for 5 minutes. 50 µl of the buffer reagent was again added to terminate the reaction, and the plate read off at 490nm.

Total antioxidant capacity = [(corrected absorbance) – (y-intercept)/slope].

2.8.1. Malondialdehyde (MDA) level

Lipid peroxidation was estimated by measuring spectrophotometrically the level of the lipid peroxidation product, malondialdehyde (MDA) as described previously [14].

Principles:

Lipid degradation occurs forming such products as malondialdehyde (from fatty acids with two or more double bonds), ethane and pentane (from the n – terminal carbons of 3 and 6 fatty acids, respectively). malondialdehyde reacts with thiobarbituric acid to form a red or pink coloured complex which in acid solution absorbs maximally at 532 nm.

Procedure:

Into three test tubes labeled test, serum and blank were added 10 µl of serum, and 10 µl distilled water respectively. Then, 0.5 ml of 25 % TCA (trichloroacetic acid) and 0.5 ml of 1 % TBA (thiobarbituric acid) in 0.3 % NaOH were added. The mixture was boiled for 40 minutes in a water bath and cooled in cold water. Then, 0.1 ml of 20 % sodium dodecyl sulfate (SDS) was added to the cooled solution and mixed properly. The absorbance was taken at wavelengths of 532 nm and 600 nm against a blank.

- **Creatine Kinase activity** was determined by the PNPG calorimetric method [15,16].
- **Troponin-c reactivity** was determined using a rapid assay, Card-I Kit Combo Test, following the manufacturer's instructions. A commercially available enzyme-linked immunosorbent assay (ELISA) kit can be used to determine cTnI concentration in all animals [17].
- **Angiotensin Converting Enzyme (ACE) activity:** The enzyme-linked immunosorbent assay (ELISA) (Quantikine, Rat ACE Immunoassay, R&D Systems, Minneapolis, USA) was used to determine the concentration of Angiotensin Converting Enzyme (ACE) activity in the Heart tissue homogenate [18].
- **Nitric oxide** assay, spectrophotometric method was utilized. Total nitrite and nitrite levels were represented as total nitrite oxide metabolites (NOx) and measurement of NOx was considered a direct marker of in vivo NO production [19,20].

2.9. Determination of C-reactive peptide concentration (Using Enzyme-linked rat serum CRP ELISA kit).

The number of wells for the assay run was first determined. Duplicates were used including 10 standard wells for each sample and control were equally assayed. This was followed by the removal of the appropriate number of microwell strips from the pouch and later, the unused strips were returned to the pouch. The pouch was then resealed and stored in a refrigerator. Then, 200-300ul Working Wash Solution was added to each well and allowed to stand for 5 to 30 minutes before sample addition.

100ul of standards, samples and controls were added to pre-determined wells. The plate was then tapped gently to mix reagents and incubated for 60 minutes. Wells were washed four times (4 x) and pat dried on fresh paper towels (an automatic plate washer could be used sometimes).

Furthermore, 100ul of diluted Anti-Rat CRP-HRP conjugate was added to each well. The wells were washed five times after being incubated for 30 minutes. 100ul TMB Substrate was added to each well and as the liquid in the well began to turn blue, incubation was done for 15 minutes in the dark (in a closet). Meanwhile, caution was taken to ensure the microplate reader did not register the optical density (OD) above 2.0 for the top standard.

Then, 100ul of Stop Solution was added to each well and tapped gently to mix in order to stop the enzyme reaction. The liquid in the wells eventually turned yellow and absorbance read off at 450nm with a microplate reader using a single wavelength within 30 minutes following the addition of Stop Solution. OD reading was obtained and subtracted at 630nm to normalize the well background. Caution was taken to prevent improper washes which could lead to falsely elevated signals and poor reproductivity.

2.10. Determination of serum myoglobin concentration (Using Enzyme-linked rat serum Myoglobin ELISA kit).

Each standard was pipetted (100uL) including zero control and duplicated into pre designated wells. Also, 100ul of sample (in duplicate) was pipetted into pre designated wells. A microtiter plate was then incubated at room temperature for sixty (60 ± 2) minutes and the plate was covered and levelled during the incubation.

Following incubation, the contents of the wells were aspirated while each well was completely filled with diluted IX Wash Buffer. This was repeated three times for a total of four washes. 100ul IX Enzyme-Antibody Conjugate was pipetted to each well and then incubated at room temperature for 30 minutes. The plate was covered in the dark and levelled during incubation.

Further, the wells were then washed and blotted. After that, 100ul of TMB Substrate Solution was pipetted into each well. It is then incubated in the dark at room temperature for precisely ten minutes before the absorbance of each well content was read off at 450nm using a plate reader.

Interleukin-6, apolipoprotein-a and apolipoprotein-e concentrations were determined in the laboratory following standard laboratory procedure and guidelines according to manufacturer's instructions.

2.11. Histological analysis of heart

Tissues fixed in Bouin's solution were transferred to 70% (vol/vol) ethanol for histological analysis. Samples were then dehydrated at increasing ethanol concentrations, cleared in xylene, embedded in paraffin wax, and sectioned (7 μm) using a microtome. Sections were sustained with hematoxylin–eosin, and pictures were taken with an optical microscope (Zeiss, Axio Imager A2, Oberkochen, Germany).

2.12. Data Analysis

Results are expressed as mean ± SEM. Data was analyzed using the GraphPad Prism software (version 6.0). Analysis of variance (ANOVA) followed by Tukey comparison test where F value was significant. Probability level of $p<0.05$ was accepted as significant.

3. Results

3.1. Comparison of Apolipoprotein concentration in the different experimental groups

Apolipoprotein-a concentration in the control group was 360.00 ±1.20 pg/mL, it reduced significantly ($p<0.05$) in Dox-only (218.00 ±0.58 pg/mL) compared with control. Values obtained for the other experimental groups (Extract, 321.00 ±1.00; Dox + Extract, 356.00 ±1.30 and Dox + Bis, 370.00 ±3.00 pg/mL) were significantly ($p<0.05$) higher compared with Dox only groups, Figure 1.

Apolipoprotein-e concentration in the control group was 82.00 ±2.10 ng/mL, it increased significantly ($p<0.05$) in Dox only (152.00 ±0.86 ng/mL) compared with control. Values obtained for the other experimental groups (Extract, 104.00 ±1.00; Dox + Extract, 95.00 ±0.86 and Dox + Bis, 79.00 ±1.70 ng/mL) were significantly ($p<0.05$) lower compared with Dox-only groups, Figure 2.

3.2. Comparison of cardiac function biomarkers in the different experimental groups

The troponin concentration (mg/mL) in the control, Dox only, Extract, Dox + extract and Dox + Bis groups were 375.00 ±1.80, 70.00 ±3.30, 624.00 ±4.20, 557.00 ±3.20 and 486.00 ±2.80 respectively. It was significantly ($p<0.05$) higher in Dox-only group compared with control. Values obtained for other experimental groups were significantly ($p<0.05$) lower compared with the Dox only group, Table 1.

Myoglobin concentration (mg/mL) in the control group was 500.00 ±4.20, it increased significantly ($p<0.05$) in the Dox only group compared with control and other experimental groups, Table 1.

Creatine kinase concentration in the control and Dox-only groups were 0.76 ±0.08 and 13.00 ±0.51 IU/L, showing significant ($p<0.05$) increase in the Dox-only group compared with control. Values obtained for the other experimental groups were in turn significantly lower ($p<0.05$) compared with Dox only group, Table 1.

Angiotensin converting enzyme concentration (IU/L) was significantly ($p<0.05$) raised in the Dox only group compared with control. The extract and Bisoprolol treated groups had significantly ($p<0.05$) lower levels of ACE compared with Dox only group. ACE concentration in the control, Dox only, Extract, Dox + extract and Dox + Bis were 4.00 ±0.29, 22.00 ±0.86, 11.00 ±0.51, 2.40 ±0.39 and 1.30 ±0.07 respectively, Table 1.

Also illustrated in Table 1 are results for nitric oxide concentration (pg/mL). NO concentration decreased significantly ($p<0.05$) in the Dox-only group compared with control and other experimental groups. Significantly ($p<0.05$) higher levels of NO were observed in extract, and Dox + Bis groups compared with control, Table 1.

3.3. Comparison of anti-oxidant enzymes and lipid peroxidation product concentration in the different experimental groups

The different anti-oxidants, catalase, superoxide dismutase, glutathione and total antioxidant capacity, assayed in the study, were significantly ($p<0.05$) reduced in Dox-only group compared with control and other experimental groups, Table 2.

CAT concentration (umol/mg) in the control group was 169.00 ±4.20, values obtained for Dox only groups was 53.00 ±1.40, while values obtained for Extract, Dox + extract and Dox + Bis were 250.00 ±2.40, 176.00 ±2.00 and 174.00 ±1.70 respectively.

SOD concentration (IU/L) for control, Dox only, Extract, Dox + Extract and Dox + Bis groups were 59.00 ±2.40, 43.00 ±0.68, 91.00 ±1.20, 55.00 ±1.50 and 50.00 ±1.20 respectively.

GPx concentrations (IU/L) were 124.00 ±2.00, 103.00 ±4.30, 188.00 ±2.50, 160.00 ±1.60 and 151.00 ±2.40 respectively for control, Dox only, Extract, Dox + Extract and Dox + Bis groups.

TAC for control and Dox-only groups were 5.60 ±0.51 and 2.00 ±0.16 respectively.

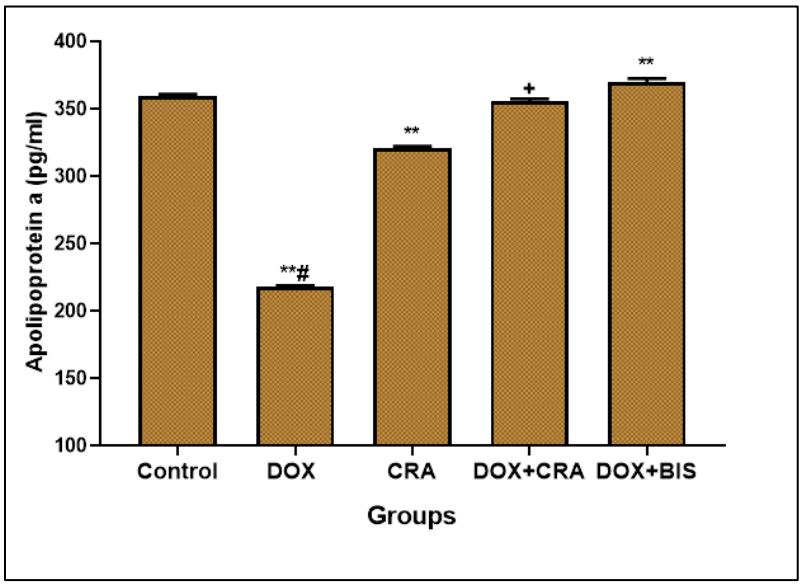
The concentration of MDA (mmol/mg) in the Dox-only group (39.00 ±1.80) was significantly ($p<0.05$) raised compared with control (19.00 ±1.10). It was significantly ($p<0.05$) lower in extract, Dox + extract and Dox + Bis groups compared with Dox only group, Table 2.

3.4. Comparison of inflammatory biomarkers concentration in the different experimental groups

The inflammatory biomarkers (CRP and IL-6) studied were also significantly ($p<0.05$) raised in the Dox-only group compared with control and other experimental groups. CRP and IL-6 concentrations in the control group were 226.00 ±5.80 ng/mL and 138.00 ±2.90 pg/mL respectively. While commutations of CRP and IL-6 in Dox-only group were 415.00 ±3.50 ng/mL and 317.00 ±3.10 pg/mL respectively, the extract group had CRP and IL-6 concentrations of 266.00 ±5.90 ng/mL and 159.00 ±0.71 pg/mL respectively, Table 2.

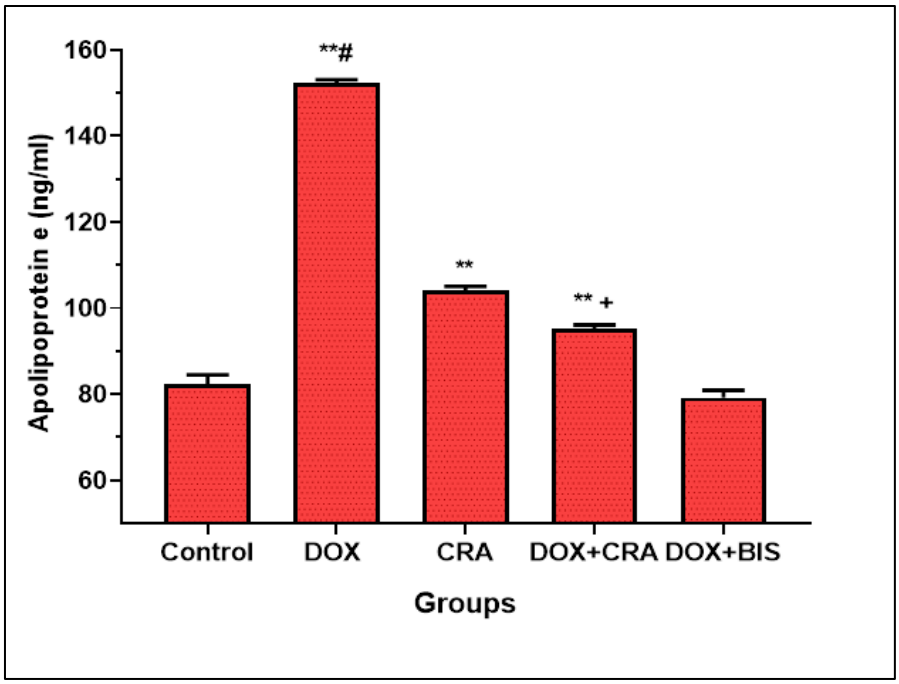
3.5. Histological study

Figure 3 shows photomicrograph of the heart tissue of the control group showing myocardial fibers and adjoining connective tissue. Microstructure appears normal. Figure 4 shows the photomicrograph of the heart tissue of dox-only showing myocytolysis of myocardial fibers (arrow). Figure 5 shows the photomicrograph of the heart tissue of dox + extract group showing myocardial fibers and adjoining connective tissue with microstructure appearing normal. Figure 6 shows the photomicrograph of the heart tissue of do-bis group showing normal myocardial fibers and adjoining connective tissue. Microstructure appears normal.



Values are expressed as mean ±SEM, n = 7. ** = $p<0.01$ versus control; # = $p<0.01$ versus other treated groups; + = $p<0.05$ compared with DOX+BIS group

Figure 1 Comparison of Apolipoprotein-a concentration in the different experimental groups



Values are expressed as mean ±SEM, n = 7. ** = p<0.01 compared with control; # = p<0.01 versus other treated groups; + = p<0.05 compared with DOX+BIS group

Figure 2 Comparison of Apolipoprotein-e concentration in the different experimental groups

Table 1 Comparison of some cardiac function parameters among the different experimental groups

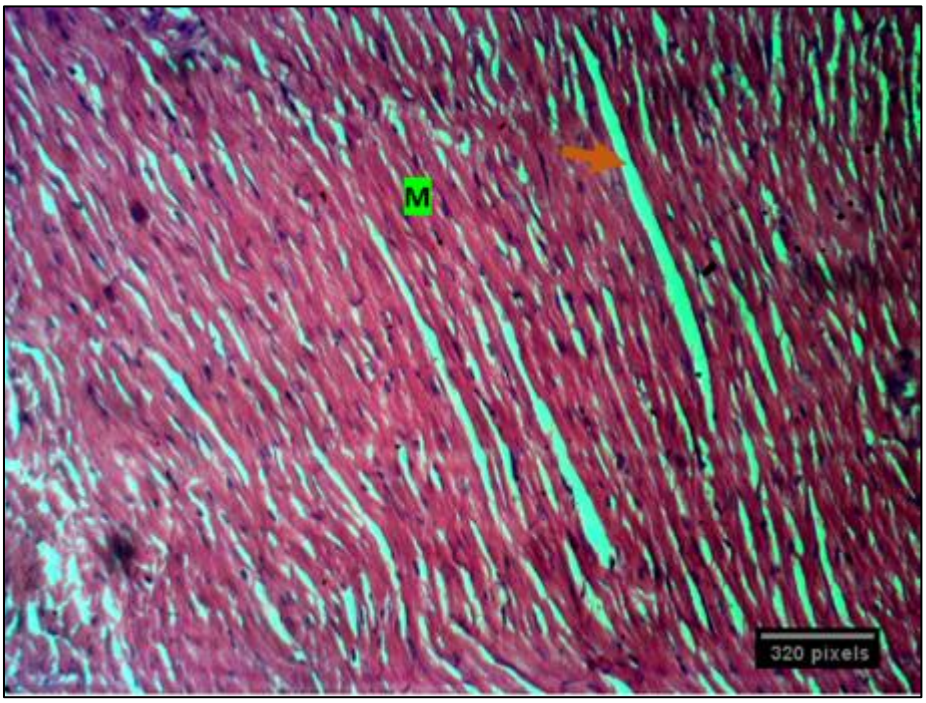
	Troponin (mg/mL)	**Myoglobin (mg/mL)**	**CK-MB (IU/L)**	**ACE (IU/L)**	**NO (pg/mL)**
Control	375.00	500.00	0.76	4.00	201.00
	±1.80	±4.20	±0.08	±0.29	±8.60
Dox	708.00	1134.00	13.00	22.00	172.00
	±3.30*	±12.00*	±0.51*	±0.86*	±4.40*
Extract	624.00	775.00	2.30	11.00	227.00
	±4.20*,a	±6.10*,a	±0.04*,a	±0.51*,a	±6.20*,a
Dox + Extract	557.00	731.00	±0.66	2.40	187.00
	±3.20*,a,b	±3.10*,a,b	±0.06*,a,b	±0.39*,a,b	±2.60*,a,b
Dox + Bis	486.00	627.00	±0.46	1.30	249.00
	±2.80*,a,b,c	±4.60*,a,b,c	±0.07*,a,b,c	±0.07*,a,b,c	±3.40*,a,b,c

Values are expressed as mean ±SEM, n = 7. * = p<0.05 vs control; a = p<0.05 vs Dox; b = p<0.05 vs Extract; c = p<0.05 vs Dox + Extract

Table 2 Comparison of some antioxidant enzymes, lipid peroxidation and inflammatory biomarkers among the different experimental groups

	CAT (umol/mg)	**SOD (IU/mL)**	**GPx (IU/mL)**	**TAC (umol/mg)**	**MDA (mmol/mg)**	**CRP (ng/mL)**	**IL-6 (pg/mL)**
Control	169.00	59.00	124.00	5.60	19.00	226.00	138.00
	±4.20	±2.40	±2.00	±0.51	±1.10	±5.80	±2.90
Dox	53.00	43.00	103.00	2.00	39.00	415.00	317.00
	±1.40*	±0.68*	±4.30*	±0.16*	±1.80*	±3.50*	±3.10*
Extract	250.00	91.00	188.00	6.00	25.00	266.00	159.00
	±2.40*,a	±1.20*,a	±2.50*,a	±0.32*	±0.89*,a	±5.90*,a	±0.71*,a
Dox + Extract	176.00	55.00	160.00	4.80	22.00	232.00	136.00
	±2.00*,a,b	±1.50*,a,b	±1.60*,b	±0.37*,a,b	±0.92*,a	±7.60a,b	±2.50a,b
Dox + Bis	174.00	50.00	151.00	4.00	17.00	202.00	124.00
	±1.70*,a,b	±1.20*,a,b	±2.40*,b	±0.32*,a,b	±0.86a,b,c	±2.50*,a,b,c	±1.40*,a.b.c

Values are expressed as mean ±SEM, n = 7; * = p<0.05 vs control; a = p<0.05 vs Dox; b = p<0.05 vs Extract; c = p<0.05 vs Dox + Extract



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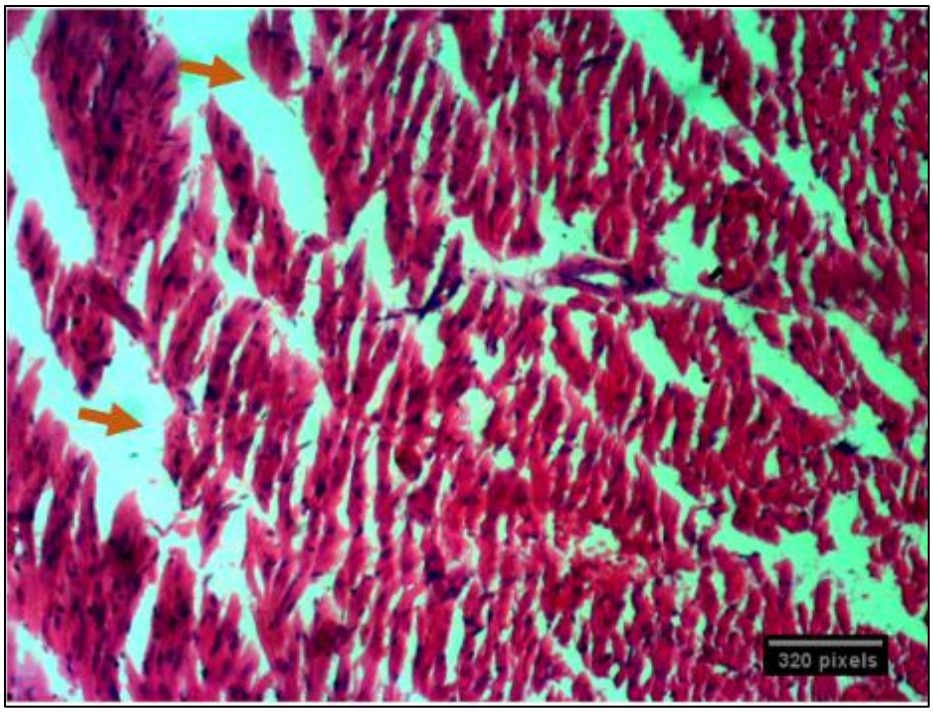
M = myocardial fibers (M) and adjoining connective tissue (arrow).

Figure 3 Control group



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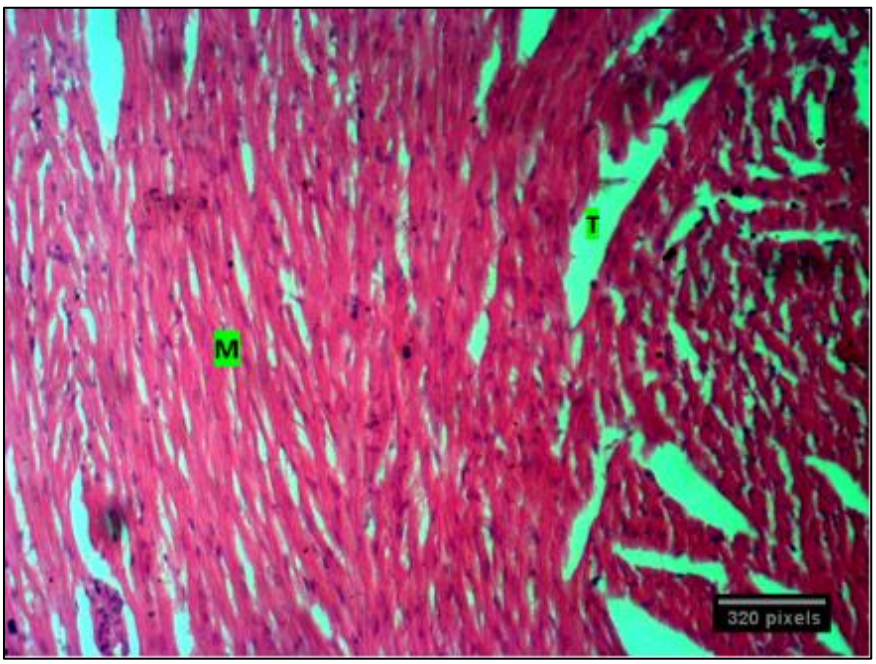
M = myocardial fibers (M) and adjoining connective tissue (arrow).

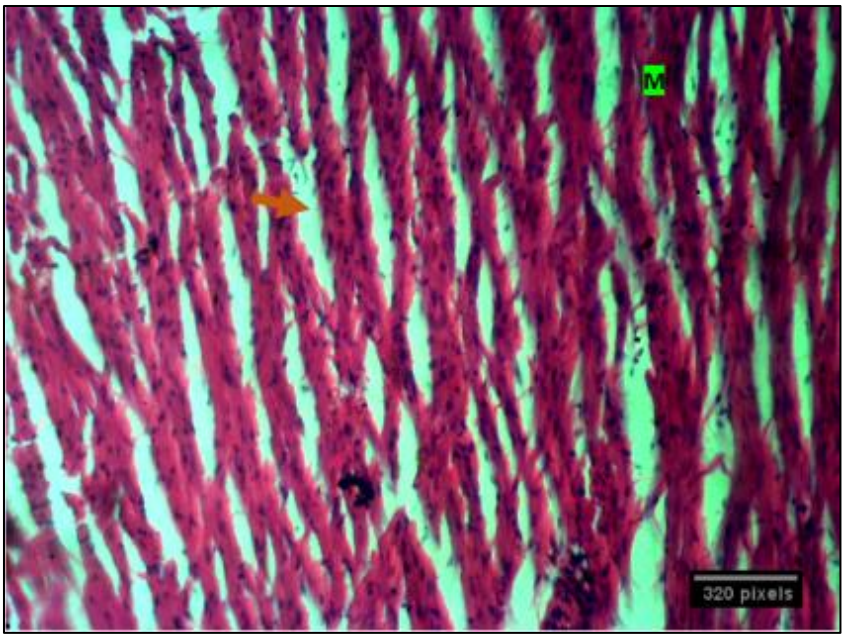
Figure 4 Dox-only group



H & E. x300

M = myocardial fibers (M) and adjoining connective tissue (arrow).

Figure 5 Dox + Extract group



H & E. X300

M = myocardial fibers (M) and adjoining connective tissue (arrow).

Figure 6 Dox and bis group

4. Discussion

This research work aimed to assess the ameliorative effect of ethanolic extract of *Bryophyllum pinnatum* of the family *Crassulaceae* on some cardiac function indices in Doxorubicin induced cardio-toxicity in rats.

Results obtained from this present study reveals cardiotoxic effect of doxorubicin with attendant increase in cardiac function biomarkers (apolipoprotein-a, troponin, ACE, myoglobin, creatine kinase, MDA, CRP and IL-6) concentrations, with a reduction in the levels of antioxidant enzymes (SOD, CAT, GPx and TAC). These deleterious effects were ameliorated by administration of *B. pinnatum* extract.

Doxorubicin, an anthracycline antineoplastic and a potent cytotoxic agent, used in the treatment of various malignancies, solid tumors and hematologic malignancies, including breast cancer, lymphoma, and sarcoma, doxorubicin has significantly improved cancer treatment outcomes [21]. Doxorubicin exerts its potent cytotoxic effects by interactions with DNA and cellular membranes, its intercalating between DNA base pairs, disrupts the structure and function of DNA molecules thereby inhibiting the activity of topoisomerase II, an essential enzyme involved in DNA replication and repair processes. By stabilizing the topoisomerase II-DNA complex, doxorubicin prevents DNA resealing after strand cleavage, leading to the accumulation of double-stranded DNA breaks and subsequent cell cycle arrest [22]. Doxorubicin-induced cardiotoxicity is characterized by dose-dependent myocardial damage, leading to cardiomyopathy, congestive heart failure, and compromised cardiac function. The mechanisms underlying doxorubicin-induced cardiotoxicity include oxidative stress, mitochondrial dysfunction, DNA damage, and apoptosis in cardiomyocytes [21].

On the other hand, the medicinal potentials of *Bryophyllum pinnatum* has been reported variously reported in treating high blood pressure, stroke, convulsion, pain, epilepsy, candidiasis, bladder infection and also as an anti-poison. In traditional medicine, *B. pinnatum* has been used to treat infections, rheumatism, inflammation, hypertension and kidney stones, [23,24].

Bisoprolol is a cardio-selective beta1-blocker (B1-blocker). Selective B1-blockers are used to treat multiple heart diseases, such as congestive heart failure, without having the unwanted effect of the B_2 receptor blocking, which can affect various systems in the body. Bisoprolol is recommended to reduce mortality and hospitalizations in patients with cardiac diseases. Selective B1- blockers are used as the first-line treatment for chronic stable angina and patients with coronary artery disease [25].

Apolipoproteins are crucial in lipid metabolism, functioning as essential mediators in the transport of cholesterol and triglycerides and being closely related to the pathogenesis of multiple systems, including cardiovascular. Apolipoprotein A is the main component of HDL-c [26]. Apolipoprotein B, is the primary apolipoprotein in LDL-c, playing a role in LDL-C metabolism and binding to LDL receptors [27]. While Apolipoprotein E is primarily present in HDL and remnant particles [28]. Myocardial Apolipoprotein-B is upregulated post myocardial infarction, DOX-induced heart failure and in DOX-induced myocardial injury and has been shown to be cardioprotective in this setting by unloading excessive lipid [29].

Our present study showed reduction in levels of Apolipoprotein-A and elevated levels of Apolipopotein-E in doxorubicin administered rats. These were ameliorated following *B. pinnatum or* bisoprolol treatment. Showing the ability of *B. pinnatum* in preventing cardiovascular risk in the face of doxorubicin administration.

Cardiac biomarkers are utilized to assess the functionality of the heart. Troponin, Myoglobin, Serum CK-MB and angiotensin converting enzyme are sensitive marker of myocardial injury, and these biomarkers were significant elevation in the serum following Doxo-only administration, indicative of the cardiotoxic effect of Doxorubicin. Similar findings, where Doxorubicin led to elevated troponin levels have been documented [30]. Increase myoglobin concentrations has been reported [31]. While the elevated CK-MB levels in the Doxo-only group align with previous findings [32].

The groups treated with either the extract or bisoprolol showed a marked reduction in these cardiac biomarkers, indicating their cardioprotective effects. Bisoprolol, a beta-blocker, is known to reduce oxidative stress and improve cardiac function in heart failure, and these are consistent with previous findings, [33]. The cardioprotective effect of the *B. pinnatum* extract could be attributed to its antioxidant properties, as seen in the elevated levels of anti-oxidant enzymes assayed in this study. This is in agreement with previous findings [34] that *B. pinnatum* has cardioprotective effects. *B. pinnatum* likely plays a role in mitigating oxidative stress, which is in line with earlier findings [35]. The antioxidative and anti-inflammatory properties of this extract as also been demonstrated [34].

The role of the antioxidants is to neutralize the free radicals in biological cells. Superoxide dismutase (SOD), glutathione (GPx) and catalase (CAT) specially play this role. The result of the study findings showed that the serum SOD, GPx, CAT levels and totalanti-oxidant capacity (TAC) in the Dox-only group were significantly lower compared with the control group. These decreases indicate oxidative stress-induced damage in the heart, as these are primary antioxidant enzymes responsible for scavenging reactive oxygen species (ROS) generated during oxidative stress. These findings correlate with earlier studies that have shown reduced SOD levels as a marker of oxidative damage in doxorubicin-induced cardiotoxicity [31]. This also agree with a study that reporting that reduced SOD levels are a common feature of doxorubicin treatment, as ROS generated in the mitochondria overwhelm the antioxidant defense system, contributing to cellular injury and apoptosis in cardiomyocytes [36].

Previous report also pointed to reduced GPx and CAT activities and increased cardiac damage induced by doxorubicin administration [36].

The groups that were administered *B. pinnatum* extract had improved levels of SOD, CAT and GPx. Supporting the antioxidant potentials of *B. pinnatum* extract. Bisoprolol was also observed to mitigates doxorubicin-induced oxidative stress by upregulating antioxidant SOD activity. However, the extract of *B. pinnatum* was more effective in elevating the GPx levels compared with Bisoprolol, this correlate with previous studies that plant extracts have superior antioxidant effects compared to conventional pharmaceuticals. Kang et al., (2016) found that plant extracts containing a wide range of bioactive compounds provided greater protection against drug-induced oxidative stress than single-compound medications.

The MDA level in the Dox-only group was significantly higher compared to the normal control. This result is consistent with previous studies, where doxorubicin treatment caused excessive ROS production and lipid peroxidation, resulting in a marked increase in MDA levels. This rise in MDA correlates with oxidative damage and cardiotoxicity, as recorded in other studies [31], where increased MDA levels were a hallmark of doxorubicin-induced oxidative stress and myocardial damage. *B. pinnatum* extarct significantly lower MDA level compared to Dox-only group. This result suggests that Crassulaceae extract prevented lipid peroxidation, by keeping MDA levels low. This finding correlate with study on the antioxidant capacity of various plant extracts, particularly those containing phenolic compounds and flavonoids, which are known to scavenge ROS and inhibit lipid peroxidation [37]. Previous study showed the cardioprotective role of Bisoprolol, via reduction in ROS and lipid peroxidation in models of oxidative stress [38].

Elevated levels of circulating proinflammatory cytokines are associated with disease progression and adverse outcomes in patients with chronic heart failure, which can also be observed after chemotherapy. Cytokines are released by the host myocardium to modulate tis-

sue repair and adaptation after injury. Cytokines such as CRP or IL-6 are elaborated soon after myocardial ischemia injury and can acutely regulate myocyte survival or apoptosis and trigger additional cellular inflammatory responses [39].

Doxorubicin was observed to cause increased levels of CRP and IL-6 in this present study, indicative of cardiac injury. Administration of *B. pinnatum* extract or bisoprolol ameliorated these menaces.

From the histological examination, the Doxo-treated group showed significant myocardial damage, while the groups treated with bisoprolol and *B. pinnatum* extract exhibited relatively normal myocardial architecture, with fewer signs of damage. These findings are consistent with previous [40] where antioxidants and beta-blockers have been shown to protect against Doxorubicin-induced histopathological changes in the heart.

In conclusion, the administration of ethanolic extract of *B. pinnatum* and bisoprolol effectively ameliorates the cardiotoxic effects of Doxorubicin, as evidenced by the reduction in serum levels cardiac biomarkers (Apolipoprotein-A, and Apolipoprotein-E, Troponin, Myoglobin, ACE, CK) and inflammatory markers (CRP and IL-6) along with improved levels of serum anti-oxidants enzymes (SOD, CAT, GPx and TAC) and cytoarchitecture of the heart. The antioxidant properties of *B. pinnatum* coupled with the established cardioprotective effects of bisoprolol, provide a promising therapeutic approach in managing Doxorubicin-induced cardiotoxicity.

Compliance with ethical standards

Acknowledgement

The authors of this article wish to acknowledge all those who assisted to make this research a success. We appreciate the staff of Chemical Pathology, University of Calabar Teaching Hospital, Calabar, Nigeria, especially Dr. Iya Bassey and Dr. Bassey Icha for their assistance to run the biochemical assay for this study. Mr Ededet Umoh is also appreciated for sourcing and providing the rats used for this study.

Disclosure of conflict of interest

The author of this research paper declares no conflict of interest

Statement of ethical approval

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Statement of Informed Consent

The research was carried on experimental animals and not humans, hence informed consent was not necessary.

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