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ABSTRACT

Cardiovascular disease is the world's leading cause of death. Lipoxidation plays a relevant role in the onset of cardiovascular diseases (CVD) causing about17 to 19 deaths annually. The usage of traditional drugs was influenced by the need for effective medications for the treatment of cardiovascular disease without side effects. The study aimed at investigating antilipidemic and antioxidative protection offered by Adansonia digitata leaf extract on doxorubicin-induced cardiotoxicity in wistar rats. Thirty male wistar rats were grouped into five of six (6) each:(1) normal control, received 0.5 mL of distilled water (2) received 20 mg/kg of DOX only (3) received 20 mg/kg of DOX +100 mg/kg of A. digitata leaf extract (4) received 20 mg/kg of DOX + 200 mg/kg of A. digitata leaf extract and (5) received 20 mg/kg of DOX+400 mg/kg of A. digitata DOX was administered subcutaneously weekly while extract was given orally/day for three weeks. Results revealed significant ($p>0.05$) increased in Troponin T, Creatinekinase and Lactate dehydrogenase in group2 against normal control. Reduction of these parameters was observed across the groups administered.

Keywords: lipid peroxidation; doxorubicin; cardiotoxicity; oxidative makers adnsonia digitata.

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Uhuo E.N Aaduaka[α], E.G[σ], Chinedu, N.P[σ], Alaebo, P.O[ρ] & Chiemeziem, O.A[Ꮗ]

ABSTRACT

Cardiovascular disease is the world's leading cause of death. Lipoxidation plays a relevant role in the onset of cardiovascular diseases (CVD) causing about17 to 19 deaths annually. The usage of traditional drugs was influenced by the need for effective medications for the treatment of cardiovascular disease without side effects. The study aimed at investigating antilipidemic and antioxidative protection offered by Adansonia digitata leaf extract on doxorubicin-induced cardiotoxicity in wistar rats. Thirty male wistar rats were grouped into five of six (6) each:(1) normal control, received 0.5 mL of distilled water (2) received 20 mg/kg of DOX only (3) received 20 mg/kg of DOX +100 mg/kg of A.digitata leaf extract (4) received 20 mg/kg of DOX + 200 mg/kg of A .digitata leaf extract and (5) received 20 mg/kg of DOX+400 mg/kg of A.digitata .DOX was administered subcutaneously weekly while extract was given orally/day for three weeks. Results revealed significant (p>0.05) increased in TroponinT, Creatinekinase and Lactate dehydrogenase in group2 against normal control. Reduction of these parameters was observed across the groups administered. A digitata extract. Elevation of MDA was recorded in group2 compared with normal control. This parameter decreased upon treated with the extract. Significant (p<0.05) reductions in Chol, TG, and LDL levels were obtained in group fed 400 mg/Kg b.wt of the extract in comparison with group 2 (DOX–induced only) and normal control. Significant variations of Chol, TG and LDL levels were observed within the text groups in dose dependent manner. HDL increased in all the text groups except group2.CAT and GPx activities decreased significantly (p>0.05) while SOD activity was non- significantly (p>0.05) decreased. Increase activities of these antioxidant enzymes were recorded across the test groups administered extract against group2 (DOX –induced only) and normal control. In this study A.digitata leaf extract has demonstrated its potential to delaying lipid peroxidation and protects oxidative damage induced by doxorubicin in the experimental rats.

Keywords: lipid peroxidation; doxorubicin; cardiotoxicity; oxidative makers adnsonia digitata.

Author α σ ρ Ꮗ: Department of Biochemistry, College of Natural Science, Michael Okpara University of Agriculture Umudike.

σ: Department of Biochemistry, University of Nigeria, Nsukka.

I. INTRODUCTION

The heart is distinctively prone to oxidative damage. Heart attack, also called myocardial infarction (MI), and related complications are the main causes of deaths throughout the world [1]. Doxorubicin (DOX) is one of the most efficient anticancer agents. The use of DOX in clinical setting is limited due to the extensive adverse effects. It is has been shown that 41% of cancer patients who received DOX are affected with various cardiac problem. DOX treatment increases the morbidity and mortality of cancer patients due to the heart failure [2]. Doxorubicin-induced cardiomyopathy is strongly linked to an increase in cardiac oxidative stress, as indicated by the depletion of endogenous antioxidant enzymes, and accumulation of free radicals in the myocardium which increases the chance of DOX-induced cardiomyopathy [3].

Heart uses large amounts of fatty acids (FAs) as energy-providing substrates. Lipoxidation plays a relevant role in the onset of cardiovascular diseases (CVD), mainly in the atherosclerosis-based diseases in which oxidized lipids and their adducts have been extensively characterized and associated with several processes responsible for the onset and development of atherosclerosis, such as endothelial dysfunction and inflammation. More than 70% of all substrates used for ATP generation are derived from FAs, with the remaining sources being glucose, lactate, ketone bodies, and amino acids. The relatively tight coupling between lipid uptake and oxidation prevents accumulation of excess lipids in the cardiomyocyte. Several processes that affect heart function, including ischemia, sepsis, and heart failure, are associated with a reduction in FA oxidation (FAO) with a relative increase in anaerobic glycolysis and, in some cases, accumulation of nonoxidized FA derivatives in the form of lipids. In addition, excess circulating FA levels in type 2 diabetes mellitus and its precursor, the metabolic syndrome, also cause cardiac lipid accumulation.

Further investigations revealed that the correlation of high cholesterol with increased risk of cardiovascular events was related mainly to low-density lipoprotein (LDL)-cholesterol (LDL-C) [4,5]. In contrast, high-density lipo-protein (HDL-C) was shown to be inversely correlated to mortality from coronary heart disease [4, 5]. Based on these findings, the"cholesterol hypothesis was born, which proposed that LDL-C is causal for the development of atherosclerosis, and consequently, lowering of LDL-C would reduce the risk of myocardial infarction and other cardiovascular events.Compared to a healthy heart, lipid metabolism is altered in a stressed heart, with for example a reduced fatty acid β-oxidation in heart failure as well as during myocardial ischemia [6].

Andonsonia digitata (Baobab) plants are tropical trees, native to Africa, Australia and Madagascar but dispersed widely by humans. A. digitata is commonly found in the woodlands of African savannah, it is a very long-lived tree with multipurpose use. A. digitata contain glycosides, saponins, steroids and flavonoids while alkaloids, tannins and resins were not detected [7]. The medicinal property of A. digitata is based on some bioactive compounds that produce a definite physiological action on the human body, these chemical substances have a potential or established biological activity that has been identified and they are known as phytochemicals [8] There is, however, paucity of scientific evidence on antilipidemic properties of A.digitata leaf extract. As a result, the aim of the study was to determine lipid profiles and oxidative stress markers in doxorubicin-induced cardiac toxicity in rats administered Adansonia digitata extract.

II. MATERIAL AND METHODS

2.1 *Plants materials*

Plant material Fresh leaves of A.gigitata were collected from Kaduna State, Northern part of Nigeria. The leaves obtained were carefully rinsed under running water, dried under room temperature (250C) in the laboratory. It was milled and weighed before extraction.

2.2 *Assay kits and Chemicals*

Most of the chemical materials utilized in the study came from Sigma Chemicals Co. (St Louis, Mo, USA). Doxorubicin was obtained from Pfizer Global Pharmaceutical Limited in Nigeria. Assay kits for Creatinekinase, Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidise (GPX), Lactate dehydrogenase (LDH) were purchased from Randox Laboratories Ltd, Co-Atrim, United Kingdom. Troponin T research kit was supplied by Glory Science co ltd, www.glorybio.com.

2.3 *Experimental animals*

Thirty (30) male wistar rats (mean weight 105±0.35g) were ordered from the animal house of Department of Zoology and Environmental Science, University of Nigeria, Nsukka. Rats were housed using clean iron cages under standard environmental conditions of temperature (24± 10C) and relative humidity (45-50%) under a 12 h dark-light cycle. Acclimatization of the rats took 7 days before dosing and allowed free access to drinking water and standard pellets feed. The research protocols were approved by the Animal Ethics Committee, College of Natural Science, Michael Okpara University of Agriculture, Abia State, Nigeria. The approval was in line with the Guide for the Care and Use of Laboratory Animals.

2.4 Preparation of extract

A milled 500 g plant material was extracted in 2.0 L ethanol for 72 h at 300C on an orbital shaker (Stuart Scientific Orbital Shaker, UK) at room temperature. This was centrifuged at 1500 rpm for 5 min and the filtrate further filtered with wahtman No 4 filter paper. It was concentrated using rotary evaporator at 400C and the yield was 21.60 g. Reconstitution of the sample in distilled water was done to give the required concentrations of, 100, 200 and 400 mg /Kg body weight used in the study

2.5 Design of Experimental

A total of thirty male wistar rats were grouped into five of six (6) each:(1) Normal control, received 0.5 mL of distilled water (2) received 20 mg/kg of DOX only (3) received 20 mg/kg of DOX +100 mg/kg of A.digitata leaf extract (4) received 20 mg/kg of DOX + 200 mg/kg of A .digitata leaf extract and (5) received 20 mg/kg of DOX+400 mg/kg of A. digitata DOX was administered subcutaneously weekly while extract was given orally for three weeks.

2.6 Biochemical Analysis

2.6.1 Cardiac biochemical marker assessment

Methods described by Wurzburg et al. [9] and Szasz et al. [10] were used to determine the activity of creatine kinase (CK). Lactate dehydrogenase (LDH) activity was determined using the Witt and Trendelenburg method.

2.6.2 Assessment of Oxidative stress marker

The activity of superoxide dismutase (SOD) in the heart was measured using Misra and Fridovich's [11] method, which was later modified by Kakkar et al. The activity of catalase was determined using the Aebi method [12].] The level of lipid peroxidation as malondialdehyde (MDA) was determined using the Varshey and Kale principle [13].

2.6.3 Determination of lipids profile

Determination of lipids profile (HDL, LDL. TG and Cholesterol) was strictly by established laboratory methods.

2.6 Preparation of serum and tissues

Rats were sacrificed under anaesthesia by cervical dislocation. The blood sample was aseptically collected through cardiac puncture and transferred into sample labeled bottles, while the heart was still beating. Whole blood was used for enzyme assays while other part was allowed to stand for 2 hours to perfect clotting and centrifuged (model SM800B, Surgifriend Medicals, Essex, England) at 1000 rpm. Sera were removed with Pasteur pipette for determination of other parameters. Rats were quickly dissected and their heart were excised and transferred into ice-cold 0.25 M sucrose solution for histopathological examination.

2.7 Histopathological evaluation

Heart tissues were fixed in buffered 10% formalin and processed for histopathological examination as described by Abdel-Raheem [14]. Briefly, four micrometer-thick paraffin sections were prepared and stained with haematoxylin and eosin for light microscope examination by pathologist.

2.8 Statistical Analysis

Data collected were analysed using one-way analysis of variance (ANOVA). Level of significance was used to assess significant difference between the control and treated group at $p<0.05$. Results were expressed as mean ±SEM.

III. RESULT

Cardiac markers; Troponin T, Creatinekinase and Lactate dehydrogenase were determine.

Doxorubicin caused significant myocardial damage, resulting in a significant ($p<0.05$) increase in TroponinT level and increased activities of Creatine Kinase and Lactate dehydrogenase in group 2 as against the test groups and normal control. (Fig.1, 2 and 3). Significant ($p<0.05$) decrease of creatine kinase and LDH concen- tration were observed in groups received 400 mg/Kg of the extract (Fig. 2 and Fig.3) compared with other groups administered 100 and 200 mg/ Kg b.wt of the extract respectively. Similar observation was made in CAT and GPx activities where SOD activity demonstrated nonsignificant ($p>0.05$) decreased. Increase activities of these antioxidant enzymes were recorded across the test groups against group 2(DOX –induced only). Elevation of MDA was recorded (Fig.7) in group2 compared with other groups. Dose dependent decrease in MDA was observed in group 3, 4 and 5 which received 200, 300 and 400 mg/Kg b.wt of A.digitata respectively. Lipid profile was determined. Significant ($p<0.05$) reduction in cholesterol, TG, and LDL as shown in Fig. 8, 9 and 11 levels were obtained in group fed 400 mg/Kg b.wt of the extract in comparison with group 2 (DOX–induced only) and Group1 (Normal control). Significant variations of Chol, TG and LDL levels were observed within the text groups in dose dependent manner. Marked increase of HDL concentration was obtained in all the text groups which received treatment as against the rats induced with doxorubicin only (Fig. 7). Further more, 400 mg/Kg treatment recorded significant ($p<0.05$) increase of HDL level compared with other test groups.

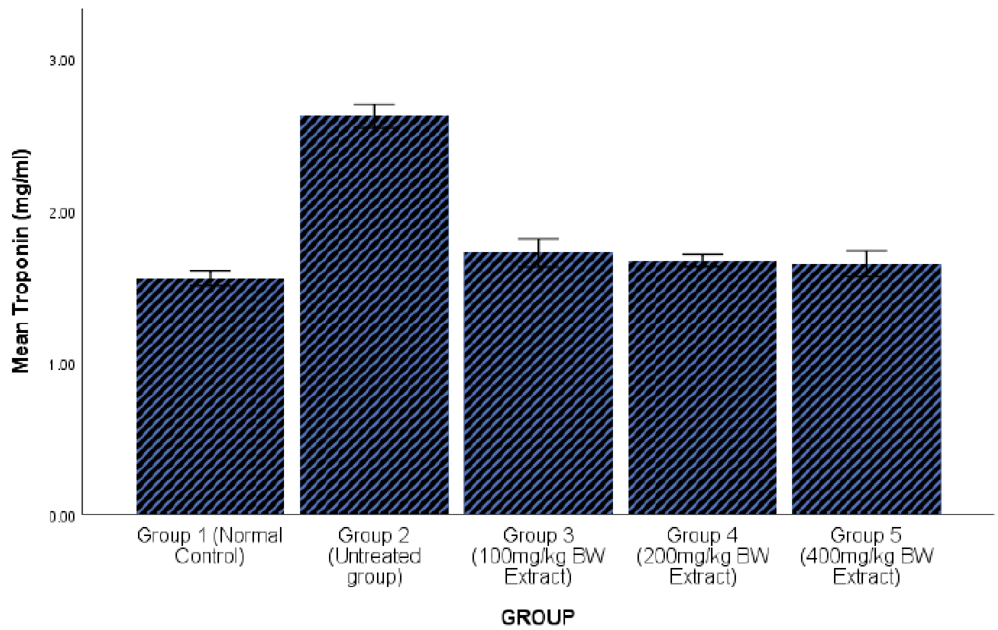

Fig. 1: Troponin level (mmol/L) of DOX- induced male wistar rats administered methanol leaf extracts of A.digitata. Values are mean of 6 determination (n=6)

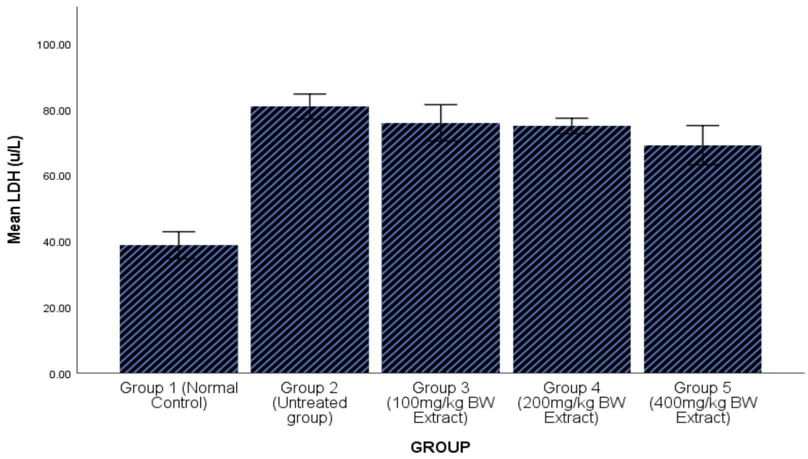

Fig.2. LDH (u/L) concentration of DOX- induced male wistar rats administered methanol leaf extracts of A. digitata. Values are mean of 6 determination (n=6)

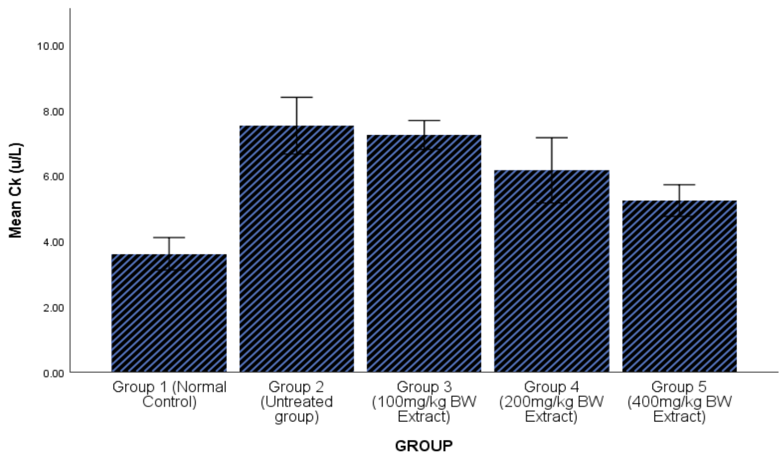

Fig. 3: Ck (u/L) level of DOX- induced male wistar rats administered methanol leaf extracts of A. digitata. Values are mean of 6 determination (n=6)

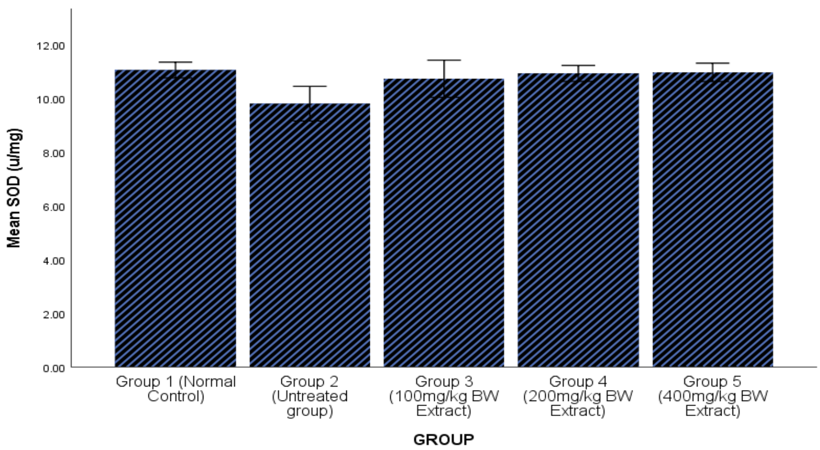

Fig. 4: SOD (u/mg) activity of DOX- induced male wistar rats administered methanol leaf extracts of A. digitata. Values are mean of 6 determination (n=6)

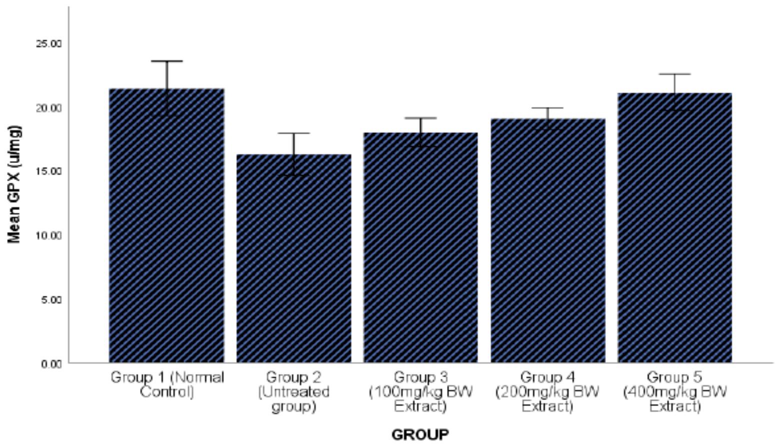

Fig. 5: GPX (u/mg) activity of DOX- induced male wistar rats administered methanol leaf extracts of A. digitata. Values are mean of 6 determination (n=6)

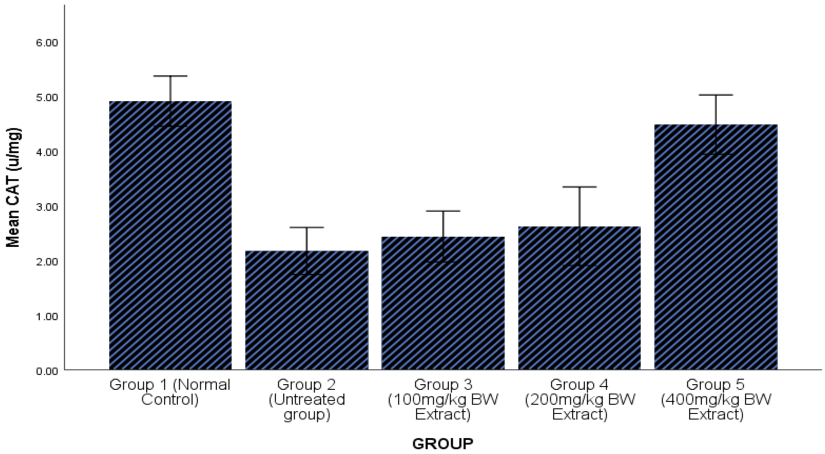

Fig. 6: CAT (u/mg) activity DOX- induced male wistar rats administered methanol leaf extracts of A. digitata. Values are mean of 6 determination (n=6)

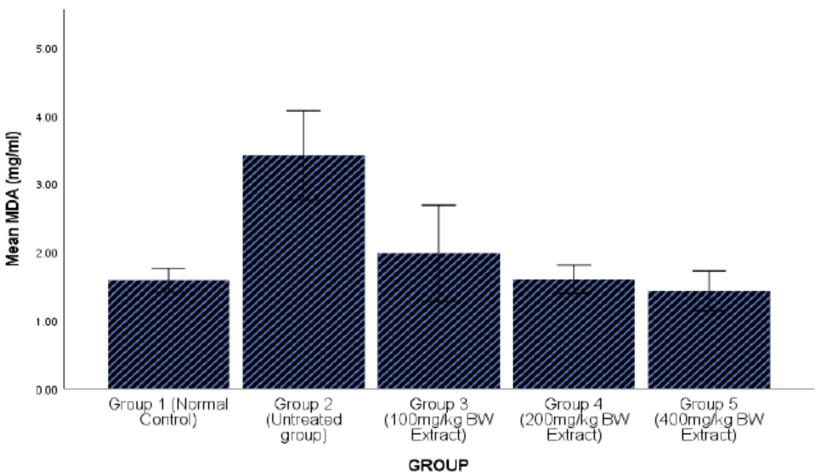

Fig. 7: MDA (mg/ml) concentration of DOX- induced male wistar rats administered methanol leaf extracts of A.digitata. Values are mean of 6 determination (n=6)

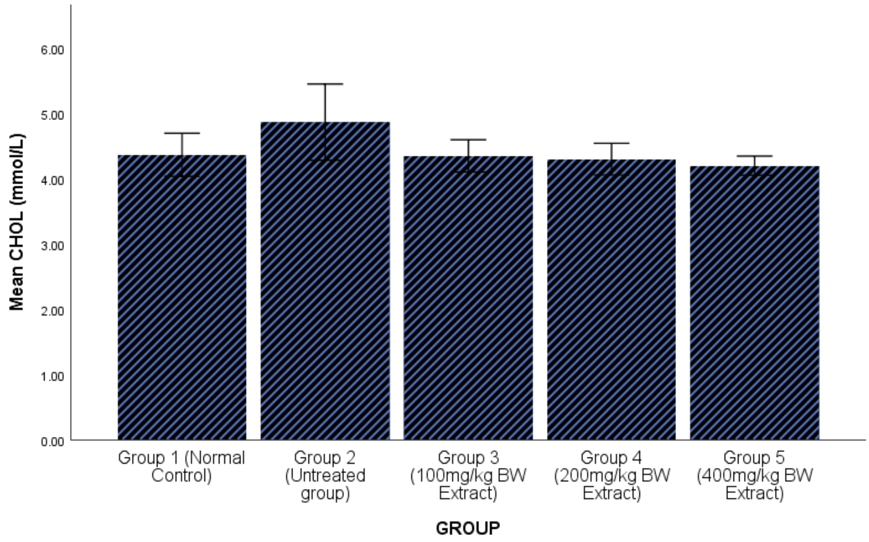

Fig. 8: Cholesterol (mmol/L) concentration of DOX- induced male wistar rats administered methanol leaf extracts of A.digitata. Values are mean of 6 determination (n=6)

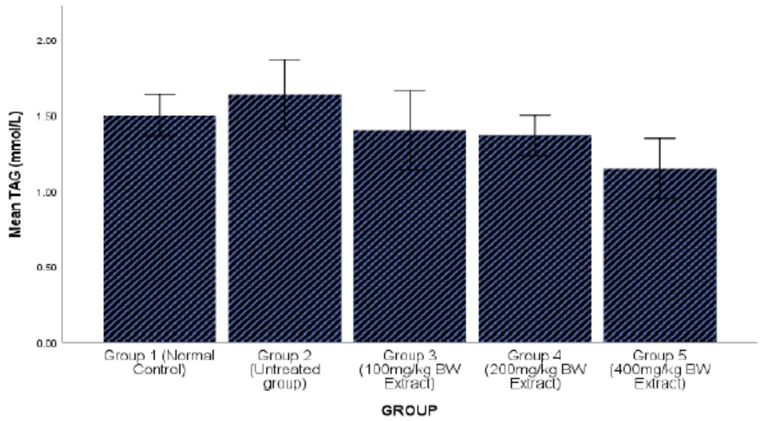

Fig. 9: TAG (mmol/L) Concentration of DOX- induced male wistar rats administered methanol leaf extracts of A. digitata. Values are mean of 6 determination (n=6)

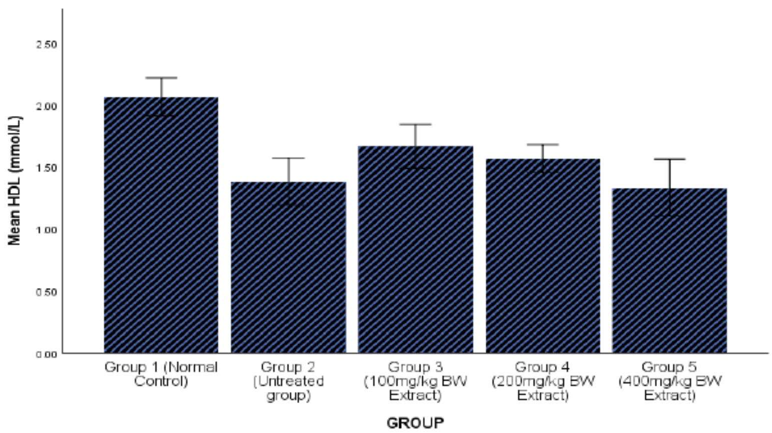

Fig. 9: HDL (mmol/L) concentration of DOX- induced male wistar rats administered methanol leaf extracts of A. digitata. Values are mean of 6 determination (n=6)

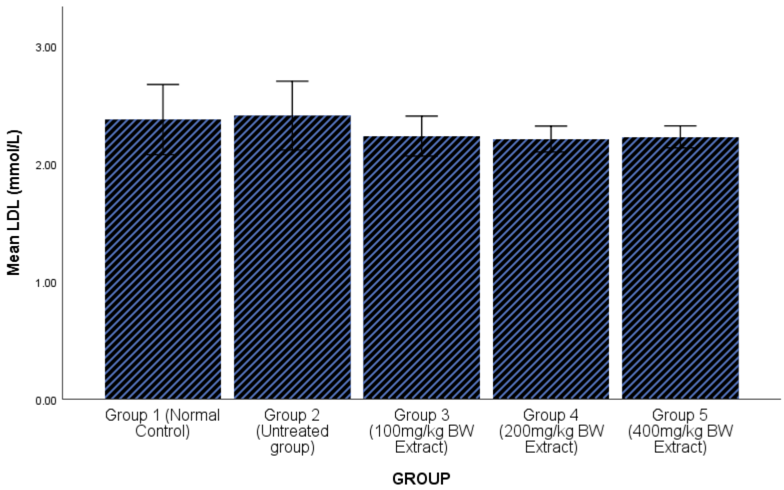

Fig. 10: LDL (mmol/L) concentration of DOX- induced male wistar rats administered methanol leaf extracts of A. digitata. Values are mean of 6 determination (n=6)

IV. DISCUSSION

The heart is distinctively proper to oxidative damage. Depletion of endogenous antioxidants facilitates oxidative stress in DOX–induced cardiomyopathy. Accumulation of free radicals in the myocardium increases the chance of DOX-induced cardiomyopathy [3]. Heart uses large amounts of fatty acids (FAs) as energy-providing substrate. Oxidation of lipid plays an important role in the onset of cardiovascular diseases (CVD), especially in the atherosclerosis-based diseases in which oxidized lipids and their adducts have been extensively characterized and associated with several processes responsible for the onset and development of atherosclerosis, such as endothelial dysfunction and inflammation.

Cardiac markers; Troponin T, Creatine kinase and Lactate dehydrogenase were determined. Result revealed a significant ($p<0.05$) increase in TroponinT level and increase activities of creatinekinase and Lactate dehydrogenase in group 2 as against the test groups and normal control. However, administration of A.digitata extract reduced the concentration of these markers indicating repair mechanism offered by the extract. This could be attributed to viable bioactive constituents present in the plant extract.

The bioactive agents from natural sources have gained fundamental importance in modern system of medicines, reducing the risks of cardiac ailments by scavenging the free radicals formation [15]. These natural medicinal plants exert protective therapeutic effect through a series of processes, including the inhibition, modulation, and regulation of the expression of various proteins such as contractile and structural proteins, and glycoproteins, regulating the calcium levels and improvement in the functioning of mitochondria [16].

Oxidative stress plays major role in DOX-induced cardiotoxicity by generation of lipid peroxidation. Myocardial tissue is prone to free radical damage due to fewer amounts of antioxidants like SOD and CAT present in the heart [18, 19]. Administration of DOX at cumulative dose (20 mg/kg) increases the lipid peroxidation and depletion of the endogenous antioxidants in the myocardium. Similar biochemical changes have been reported by several other studies [17, 18].

High levels of serum triglycerides help mark conditions that are associated with increased risk for CHD and peripheral atherosclerosis. High triglycerides are associated with increased risk for Coronary Artery Disease (CAD) in patients with other risk factors, such as low HDL-cholesterol.

Consequently, elevated levels of cholesterol increase the risk for coronary heart disease (CHD). Cholesterol is measured to help assess the patient's risk status and to follow the progress of patient's treatment to lower serum cholesterol concentrations. In this study, statistical significant decrease in some lipids parameters was obtained followed with increase in HDL especially at 400 mg/Kg b.wt administration of A. digitata, The ability of the extract to ameliorate the lipids abnormality is due to, partly phytoconstituents (phenolic compound, flavonoids, saponin, and many more) and inhibitory activity to lipid synthesis. Stimulation of HMGCoA-reductase activity by insulin was proposed to be blocked by the extracts, although other insulin-dependent phenomena may not be influenced. These results suggest an indirect modulation of hydroxy-methylglutaryl-CoA-reductase activity as the most likely inhibitory mechanism of the A. digitata extracts.

Decrease in MDA in all the text groups was recorded when compared with the DOX-induced only. A.digitata, based on this finding could delay or inhibits lipidoxidation. The extent of the extract to counteract the free radicals that could abstract electron (s) from the unsaturated lipids molecule by donating its electron is the most predictable mechanism. Pre-treatment with A. digitata extract significantly ($p>0.05$) reduced the elevated level of malondialdehyde across the test groups and much effect was observed in group4 administered with 400 mg/kg b. wt. The result was in agreement with the report Ogunleye et al., [19], Ebaid et al., [20] and Olayemi et al., [21].

V. CONCLUSION

Methanol extract of Adansonia digitata alleviated lipids abnormalities and oxidative stress induced by doxorubicin as shown by improved oxidative stress biomarkers. Finally, it was observed that leaf extract of Adansonia digitata shielded rats from doxorubicin-induced myocardial injury in dose dependent manner.

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Conflict of interest

Authors declare non existing conflict of interest

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