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CARDIOPROTECTIVE ACTIVITY OF AQUEOUS AND ETHYLACETATE SEED-EXTRACTS OF *DACRYODES EDULIS* ON DOXORUBICIN INDUCED HEART DAMAGE IN ALBINO RATS

OMINYI M.C[1*], AGBAFOR K. N[2], ALI F. U[3], OGBANSHI M. E[4], EBENYI L. N[5] AND ORINYA O. F[6]

1: Department of Biotechnology, Ebonyi State University, P.M.B. 053 Abakaliki, Nigeria

2: Department of Biochemistry, Ebonyi State University, P.M.B. 053 Abakaliki, Nigeria

3: Department of Biotechnology, Ebonyi State University, P.M.B. 053 Abakalik,i Nigeria

4: Department of Biochemistry, Ebonyi State University, P.M.B. 053 Abakaliki, Nigeria

5: Department of Biotechnology, Ebonyi State University, P.M.B. 053 Abakaliki, Nigeria

6: Medical Biochemistry Department, Ebonyi State University, P.M.B. 053 Abakaliki, Nigeria

***Corresponding author: Ominyi, M. C.**

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ABSTRACT

The evaluation of cardioprotective activity of aqueous and ethylacetate seed extracts of *Dacryodes edulis* on doxorubicin induced cardiac toxicity in albino rats was carried out with seventy two (72) albino rats. The animals were divided into twelve (12) groups of six rats per group. Group 1 was negative control (NS), 2 was positive control (DOX), 3 and 4 were standard drugs control groups, 25 mg/kg body weight aspirin (Asp) and 100 mg/kg body weight vitamin C (Vit C), respectively. While 5, 6, 7 and 8 were test groups for aqueous seed-extract of *D. edulis* at different doses (200, 400, 600, and 800 mg/kg body weight respectively), whereas 9, 10, 11 and 12 were test groups for ethylacetate seed-extract of *D. edulis at different dosses (200, 400, 600, and 800 mg/kg body weight respectively). Each group was kept in* separate cage, and the animals were given normal feed and allowed for one week to be acclimatized before extracts administration commenced for 21 days. After which, the animals were induced with a single dose of 15 mg/kg body weight doxorubicin, excluding the negative control group. The animals

were sacrificed 24 hours after doxorubicin administration under diethyl ether anesthesia to collect their blood and heart for biochemical analyses. There was a significant ($P<0.05$) increase in the activities of serum CK and LDH, and levels of CTnI and MDA, in addition to a significant ($P<0.05$) decrease in the activities of cardiac tissue SOD, CAT and GR in the positive control group indicating cardiac tissue injuries, lipid peroxidation and oxidative stress. Conversely, there was a significant ($P<0.05$) decrease in the activities of serum CK and LDH and levels of CTnI and MDA, as well as significant ($P<0.05$) increase in the activities of SOD, CAT and GR in the groups administered the *D. edulis* aqueous and ethylacetate seed-extracts and the standard drugs comparable to the negative control group. The results of this study suggest that the extracts are potential protective agents to cardiac damage and could also be recommended as antioxidants against heart tissues' oxidative injuries.

Keywords: Cardioprotective, antioxidant, oxidative-stress, *Dacryodes edulis*, extracts, doxorubicin

INTRODUCTION

In most developing countries, plants have shown to be very useful in the treatment of diseases, and provide important sources of most of the world's pharmaceuticals. Plants at all level have served as valuable starting material for drug development (Ajibesin, 2011). Medicinal plants contain physiologically active components which over the years have been exploited in the traditional medical practices for the treatment of various ailments (Ajibesin, 2011). Polyphenols, saponins, glycosides, flavonoids, tannins and alkaloids among others are bioactive components which endowed the plants with antimicrobials, antioxidants, anticancer, antilipidemic, analgesic and antimalarial potential among others (Mamta *et al*., 2013).

Dacryodes edulis also known as African native pear, bush butter fruit plant, *ube* in Southeast of Nigeria, is a dioecious, shade loving, evergreen tree, indigenous to the Gulf of Guinea and widely cultivated in other tropical parts of Africa for its fruit. It belongs to the family, *Burseraceae* (frankincense family). The fruits are edible and are rich source of lipids, vitamins, proteins, minerals and other nutrients. The fruit yields a high content of essential oil (Ajibesin, 2011). The plant has long been used in the traditional medicine in some African countries to treat various ailments such as wound and skin diseases, dysentery and fever. The extracts have been found to show biological activities such as antimicrobial, antioxidant and anti-sickle cell anaemia. The seeds contain oil

with considerable nutritional value that can be harnessed to supplement feed for household ruminants (Omoti and Okiy, 1987; Obasi and Okoli, 1993; Ajiwe *et al*., 1997; Leakey, 1999).

Cardiac related diseases are class of diseases that affect the heart (Mendis *et al.*, 2011). These diseases include ischemic heart disease (IHD), stroke, hypertensive heart disease, rheumatic heart disease (RHD), cardiomyopathy, congenital heart disease, coronary heart disease, and endocarditis, among others (Mendis *et al.*, 2011). Cardiac diseases have emerged one of major health problems of public concern and are predicted that by the year 2020 these diseases will persist as major and the most common threat to human life (Capewell, 2008). Obstructions of the coronary arteries cause more deaths than any other factors (Gebreselema and Mebrahtu, 2013). When the arteries which supply the heart with blood and oxygen become narrowed and the blood supply becomes restricted, resulting to oxygen deprivation from certain portions of the heart and cause heart attack. This can lead to abnormal levels of serum cardiac troponin I and increased in the activities of creatine kinase and lactate dehydrogenase (Rice and MacDonald, 1999).

Doxorubicin (DOX), an anthracycline antibiotic, is an excellent drug for the treatment of a wide variety of human solid tumors and leukemias. However, its clinical uses are limited by high incidence of toxicity (Eman *et al.*, 2011). An initial acute effect includes hypotension and transient electrocardiographic abnormalities. Doxorubicin is converted in the tissues into its semiquinone form, which is a toxic, short lived metabolite that interacts with molecular oxygen and initiates a cascade of reaction leading to generation of free radicals (Vikas *et al*., 2015).

In an effort to find alternative medicine for cardiac disorders, owing to the toxic side effects of most synthetic drugs and their high costs which made them not readily accessible to many patients in developing countries, many herbal formulations have been recommended for the treatment of the diseases. In some rural communities in Ebonyi State of Nigeria, seed-extracts of *Dacryodes edulis* have been used for herbal preparations for the treatment of patients with heart. This has increased the number of people in the area that use the plant for traditional medicine. However, less of scientific investigations have been focused on the use of the plant extracts for the treatment of cardiac disorders. To cover up

the lacuna, it becomes imperative to perform scientific investigations on the potentials of seed-extracts of *D. edulis* for the management and treatments of cardiac ailments in order to encourage their proper usage for health benefits.

MATERIALS AND METHODS

Sample collection

Dacryodes edulis seeds were collected from Amachi community in Abakaliki Local Government Area of Ebonyi State, Nigeria, while the albino rats were purchased from the Department of Animal Science, University of Nigeria Nsukka, Enugu State of Nigeria.

Sample preparation

The *Dacryodes edulis* seeds were air dried at room temperature. The dried samples were pulverized into fine powder and stored in air-tight containers at 4 ^{o}C.

Preparation of extracts

Two hundred (200) grams of the powdered seeds of *D. edulis* were soaked in 1000 mL of deionized water and ethylacetate separately for 24 hours. Thereafter, the suspensions were filtered using muslin cloth. The filtrates were concentrated using rotary evaporator. The extracts were stored in air-tight containers at 4^{o}C.

Design of the study

Male albino rats numbering seventy two (72) were maintained on normal feed and free access to water and allowed to acclimatize for one week. After which, the rats were grouped into twelve (12) with each group comprising six (6) rats. Groups 1 and 2 (negative and positive controls respectively) were given normal saline; groups 3 and 4 were given 25 mg/kg body weight aspirin and 100 mg/kg body weight vitamin C respectively. Groups 5-8 were given 200, 400, 600 and 800 mg/kg body weight of aqueous seed-extract of *Dacryodes edulis* orally respectively. Groups 9-12 were given 200, 400, 600 and 800 mg/kg body weight of ethylacetate seed-extract respectively. This was carried out daily for 21 days after which they were administered with a single dose of 15 mg/kg body weight doxorubicin intraperitoneally (i.p) on the 22nd day to groups 2-12. Animals were sacrificed 24 hours after doxorubicin administration under diethyl ether anesthesia to collect their blood and heart tissues for biochemical analyses.

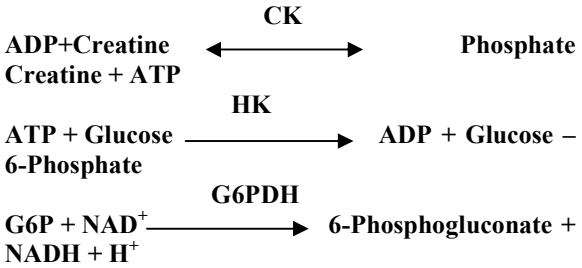
Preparation of tissue homogenate (Ottu *et al*., 2013)

The heart tissues from each animal were removed after sacrificing the animals and were rinsed in normal saline and immediately stored in deep freezer. Tissues were homogenized in ice-cold potassium phosphate buffer (pH 7.4) using mortar and pestle. The homogenate was centrifuged at

3000 ×g for 15 minutes and the supernatant collected for the analysis of oxidative stress indices in the organ tissue. Protein concentration of the samples was determined by Biuret method, using bovine serum albumin as standard.

Determination of creatine kinase activity (Steen *et al.*, 2010)

Principle



Creatine kinase catalyzes the reversible phosphorylation of ADP, in the presence of creatine phosphate, to form ATP and creatine. The auxiliary enzyme hexokinase (HK) catalyzes the phosphorylation of glucose by the ATP formed, to produce ADP and glucose-6-phosphate (G6P). The G6P is oxidizes to 6-phosphogluconate with the concomitant production of NADH. The rate of NADH formation, measured at 340 nm, is directly proportional to serum CK activity.

Procedure

Reagents were prepared according to laboratory instructions. Exactly 1.0 mL of each of the reagents were collected and dispensed into appropriate tubes and pre-warmed at 37 °C for five minutes. The spectrophotometer was zeroed with water at 340 nm, and exactly 0.025 mL of the serum was transferred to the reagent tube, mixed and incubated at 37 °C for two minutes. After which, the reading and recording of the absorbance were taken. The reading was repeated for two minutes. The average absorbance was calculated per minute ΔAbs/min.

Determination of lactate dehydrogenase activity (Tietz, 1987)

Principle

Lactate dehydrogenase (LDH) catalyzes the conversion of lactate to pyruvate, the forward reaction and the conversion of pyruvate to lactate, the reverse reaction. The enzyme can be assayed using either material as a substrate; however, the enzyme activities obtained by the two methods are not directly comparable. The forward reaction does not require pre-incubation to exhaust endogenous α-keto acids and displays linearity over a wider range of activity in patient samples. This method utilizes the forward reaction. Lactate and NAD^+ are converted to pyruvate and NADH by the action of lactate dehydrogenase. The NADH absorbs light strongly at 340 nm, whereas NAD^+ does not. The rate of increase in absorbance at 340 nm is directly proportional to the LDH activity in the sample.

Procedure

The working reagent was prepared as directed. Exactly 1.0 mL of each of the reagent was collected and transferred into the appropriate tubes and pre-warmed at 37 ^{o}C for five minutes. The spectrophotometer was zeroed with water at 340 nm. Exactly 0.050 mL (50 µL) of serum sample was transferred into the reagent, mixed and returned to the heating block. The reading and recording of the absorbance were taken after 30 seconds (1st reading). The tube was returned to 37 ^{o}C for one minute. After exactly one minute, the reading and recording of absorbance were again taken (2nd reading). The change in absorbance (2nd – 1st reading) was multiplied by the factor 3376 yields results min u/L.

Determination of serum cardiac troponin I level (Etievent *et al.*, 1995)

Enzyme-linked immunosorbant assay (ELISA) method for CTnI determination was used.

Principle

Antibodies specific for human cardiac troponin I (CTnI) are located on an electrochemical sensor fabricated on a silicon chip. Also deposited in another location on the sensor silicon chip are an antibody/alkaline phosphatase enzyme conjugate specific to a separate portion of the CTnI molecule. The whole serum sample was brought into contact with the sensors allowing the enzyme conjugate to dissolve into the sample. The CTnI within the sample becomes labeled with alkaline phosphatase and is captured onto the surface of the electrochemical sensor during an incubation period of approximately seven minutes. The sample, as well as excess enzyme conjugate, was washed off the sensors. Within the wash fluid was a substrate for the alkaline phosphatase enzyme. The enzyme bound to the antibody/antigen/antibody sandwich cleaves the substrate releasing an electrochemically detectable product. The electrochemical (amperometric) sensor measures this enzyme product which is proportional to the concentration of CTnI within the sample.

Procedure:

The desired number of coated wells was secured in the holder and the standards, sera and controls were dispensed into the appropriate wells at 100 µL each and were gently mixed for 10 seconds. Enzyme conjugate reagents (100 µL) were dispensed into each well and were thoroughly mixed for 30 seconds. They were incubated at room temperature (18-25 °C) for 90 minutes. The incubation mixture was removed by flicking plate contents into a waste container. The microtiter wells were rinsed and flicked 5

times with deionized water. All residual water droplets were removed using paper towels and were sharply striked onto absorbent paper. The reagent (100 µL) each was dispensed into each well gently mixed for 5 seconds and incubated at room temperature for 20 minutes. The reaction was stopped by adding 100 µL of stop solution into each well and gently mixed for 30 seconds. Measures were taken to ensure that all the blue color changes to yellow color completely. The absorbance was read at 450 nm with a microtiter reader within 15 minutes.

Estimation of superoxide dismutase activity

Principle

Superoxide dismutase activity was assayed by the method of Kakkar *et al.* (1984). The assay was based on the inhibition of the formation of NADH-phenazine methosulphate-nitroblue tetrazolium formazan. The reaction is initiated by the addition of NADH. After incubation for 90 seconds, the reaction is stopped by adding glacial acetic acid. The colour developed at the end of reaction is extracted into n-butanol layer and measured at 520 nm in spectrophotometer.

Procedure

Exactly 0.5 mL of the heart tissue homogenate was diluted to 1.0 mL with ice cold water followed by 2.5 mL ethanol and 1.5 mL chloroform (chilled reagent). The mixture was shaken for 60 seconds at 4 °C and then centrifuged. The enzyme activity in the supernatant was determined as follows. The assay mixture contained 1.2 mL of sodium pyrophosphate buffer, 0.1 mL of phenazine methosulphate (PMS) and 0.3 mL of nitroblue tetrazoliumchloride (NBT) and approximately diluted enzyme preparation in a total volume of 3 mL. The reaction was started by the addition of 0.2 mL NADH. After incubation at 30 °C for 90 seconds, the reaction was stopped by the addition of 1 mL glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 mL n-butanol. The mixture was allows to stand for 10 minutes, centrifuged and butanol layer was separated. The colour intensity of the chromogen in the butanol layer was measured in a spectrophotometer at 520 nm. A system devoid of enzyme served as control.

Estimation of catalase activity

Principle

To assay for the activity of catalase in the heart tissue homogenates, the method of Aebi (1983) was employed. Dichromate in acetic acid was reduced to chromic acetate, when

heated in presence of hydrogen peroxide with the formation of per chromic acid as an unstable intermediate. The chromic acetate formed was measured at 590 nm. Catalase was allowed to split H_2O_2 for different periods of time. The reaction was stopped at different time intervals by the addition of dichromate acetic acid mixture and the remaining H_2O_2 was determined by measuring chromic acetate colorimetrically after heating the reaction mixture.

Procedure

To 0.9 mL of phosphate, 0.1 mL of the heart tissue homogenate and 0.4 mL of H_2O_2 added. The reaction was after 15, 30, 45 and 60 seconds by adding 2 mL of dichromate acetic acid mixture. The tubes were kept in a boiling water-bath for 10 minutes, cooled and the colour developed was read at 530 nm. Standards in the concentration range of 20 to 100 µmoles were processed for the test.

Glutathione reductase activity assay (Anderson, 1996)

Principle

Glutathione reductase reduces oxidized glutathione (GSSG) to reduced glutathione (GSH) in the presence of NADPH. Subsequently, the chromogen reacts with the thiol group of GSH to produce a colored compound that absorbs at 405 nm. The glutathione reductase content in the tissue homogenate is determined by comparison with the predetermined glutathione reductase standard curve. The rate of chromophore production is proportional to the concentration of glutathione reductase activity within the heart tissue homogenate. The rate can be determined from the absorbance change over time.

Procedure

All the reagents were thoroughly prepared and mixed before use. Glutathione reductase standards were prepared simultaneously with the samples so they may be assayed together. To each well tested of a 96-well plate was added 25 µL of 1X NADPH solution. Exactly 100 µL of the prepared glutathione reductase standards or the tissue homogenate was added to each tested well. Also exactly 50 µL of the 1X Chromogen was added and mixed briefly. Plate reader was prepared for a kinetic assay and was set to read at 405 nm. Exactly 25 µL of the glutathione disulfide

(GSSG) solution was added and mixed briefly. Immediately the absorbance was recorded at 405 nm at 1 minute intervals for 10 minutes. The concentration of standards and the homogenates was then calculated as below.

Calculation

The absorbance for glutathione reductase standard, heart tissue homogenate, and negative control for every time point taken was first determined. Graph of the standard, tissue homogenate and blank absorbance at 405 nm against incubation time was plotted. The slope for each value from the linear portion of each curve was determined. Then, the blank slope was subtracted from the slope of the standards and homogenate. The net slopes of the glutathione reductase standards against the microunits/mL concentration of glutathione reductase were plotted. The net slopes of the samples with the standard curve were compared and the microunits/mL concentration of glutathione reductase for each sample was determined.

Estimation of malondialdehyde (MDA) concentration

The method of Buege and Aust (1978) was adopted for the quantification of MDA present in the heart tissue homogenates.

Principle

Lipid peroxidation in the heart tissue homogenates was determined spectrophotometrically by measuring thiobarbituric acid reactive substance (TBARS). Results are expressed in malondialdehyde (MDA) formed relative to an extinction coefficient of 1.56×10^6 nmol/g protein. Small quantities of MDA formed during lipid peroxidation combine with thiobarbituric acid to produce a coloured pink complex that in acid solution will absorb light at 532 nm and fluorescence at 532 nm and is readily extractable into organic solvents such as butan-1-ol.

Procedure

Exactly, one milliliter of the heart tissue homogenate sample was introduced into two milliliter of (1:1:1) TCA-TBA-HCl reagent (thiobarbituric acid 0.37 %, 0.24 M HCl and 15 % TCA) trichloroacetic acid-thiobabituric-hydrochloric acid reagent and allowed to boil at 100 ^{o}C for 15 minutes and left to cool. A centrifuge was used to remove flocculent materials at three thousand revolutions per minute for ten minutes. The upper layer was decanted and the absorbance taken at five hundred and thirty-two nanometer against the blank with a spectrophotometer. The calculation of MDA was carried out by employing molar

extinction coefficient for MDA-TBA complex of 1.56 x 10^6 nmol/g protein.

RESULTS

Cardiac Parameters of Rats Administered with the Extracts for 21 Days and Then Induced Toxicity with Doxorubicin

Results of cardiac parameters in the serum of albino rats administered with aqueous and ethylacetate seed-extracts of *Dacryodes edulis* at different doses (200, 400, 600, and 800 mg/kg body weight) including groups administered with standard drugs (aspirin and vitamin C) for 21 days and then induced toxicity with 15 mg/kg doxorubicin are shown in Figures 1, 2 and 3 for creatine kinase (CK), lactate dehydrogenase (LDH) and cardiac troponin I (CTnI) respectively. The cardiac indices showed significantly ($P<0.05$) lower activities of CK and LDH and level of CTnI in the groups administered the extracts. However, the groups not administered the extracts but was also induced toxicity with doxorubicin (DOX group) showed significantly ($P<0.05$) higher activities of LDH, CK and level of CTnI. The protective effect from alterations in the activity of LDH and CK and level of CTnI was higher in the group that received ethylacetate seed-extract than aqueous seed-extract.

Oxidative Stress Indices in the Heart Tissue Homogenates of Rats Administered with the Extracts for 21 Days and then Induced Toxicity with Doxorubicin

Results of oxidative stress indices in the heart tissue homogenates of albino rats administered *Dacryodes edulis* aqueous and ethylacetate seed at different doses (200, 400, 600 and 800 mg/kg body weight) including groups administered with standard drugs (aspirin and vitamin C) for 21 days and then induced toxicity with doxorubicin are shown in Figures 4, 5, 6 and 7 for superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and malondialdehyde (MDA) respectively. The results indicated a significant ($P<0.05$) higher activities of antioxidants (SOD, CAT and GR) and significant ($P<0.05$) lower levels of MDA in the groups administered the extracts. However, the group not administered the extracts but was induced with doxorubicin (DOX group) showed significantly ($P<0.05$) lower activities of the antioxidative enzymes, SOD, CAT and GR and significantly ($P<0.05$) higher level of MDA as a result of oxidative injuries. The protective activity of the extracts to oxidative stress in the heart tissue homogenate was most effective in the ethylacetate seed-extracts than aqueous seed-extract of *Dacryodes edulis.*

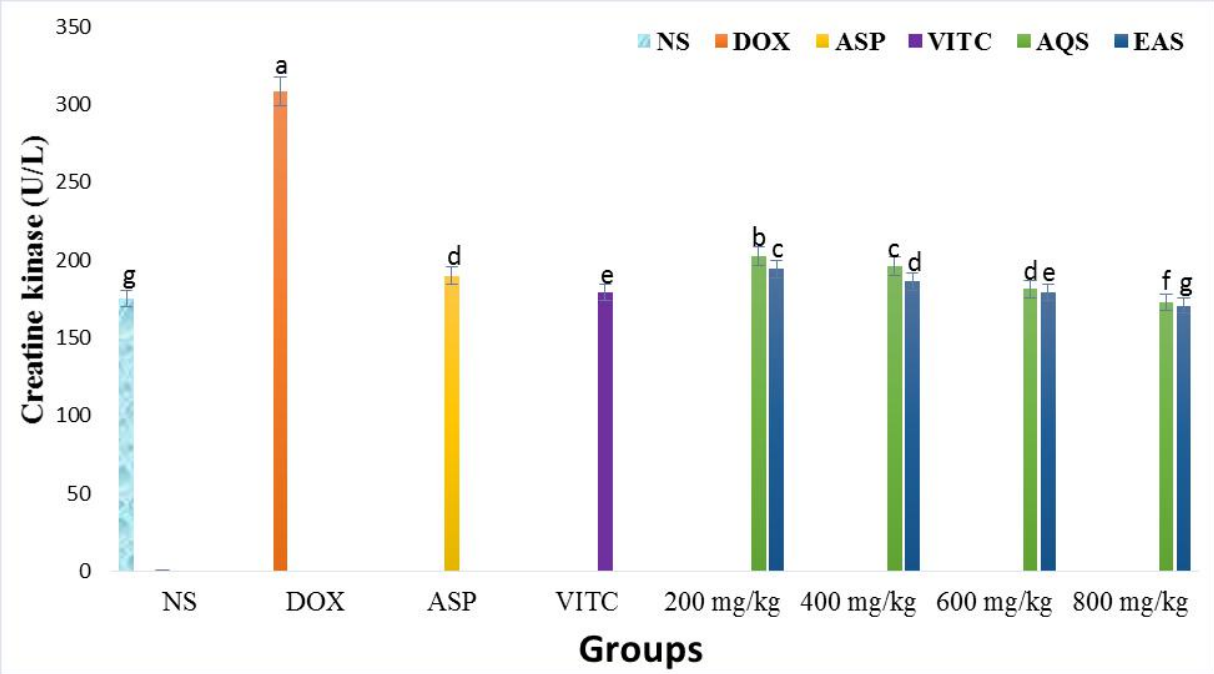


Figure 1: Creatine kinase (CK) activity in the serum of albino rats administered *Dacryodes edulis* aqueous and ethylacetate seed-extracts for 21 days and induced toxicity with 15 mg/kg doxorubicin. The data are presented as mean ± SD (n=6) and significant difference at P<0.05. NS= negative control, DOX= positive control, ASP= aspirin, VITC= vitamin C, AQS= aqueous seed and EAS= ethylacetate seed-extracts at different doses (200, 400, 600 and 800 mg/kg). * Bars with the same letter are not significantly different.

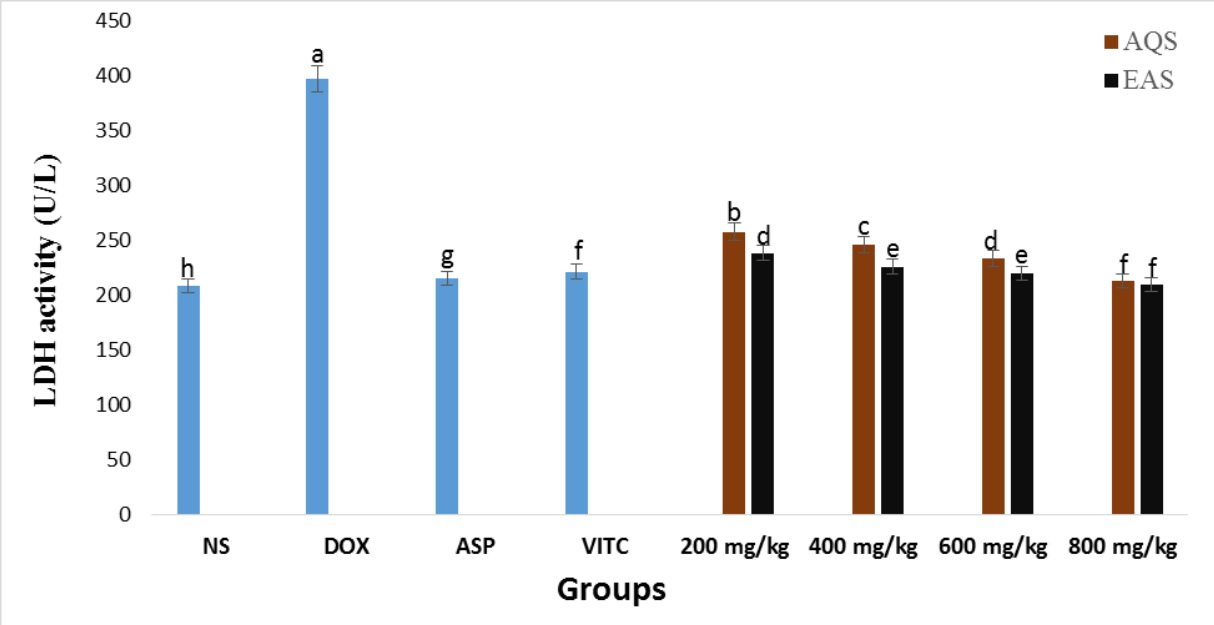


Figure 2: Lactate dehydrogenase (LDH) activity in the serum of albino rats administered *Dacryodes edulis* aqueous and ethylacetate seed-extracts for 21days and induced toxicity with 15 mg/kg doxorubicin. The data are presented as mean ± SD (n=6) and significant difference at P<0.05. NS= negative control, DOX= positive control, ASP= aspirin, VITC= vitamin C, AQS= aqueous seed and EAS= ethylacetate seed-extracts at different doses (200, 400, 600 and 800 mg/kg). * Bars with the same letter are not significantly different.

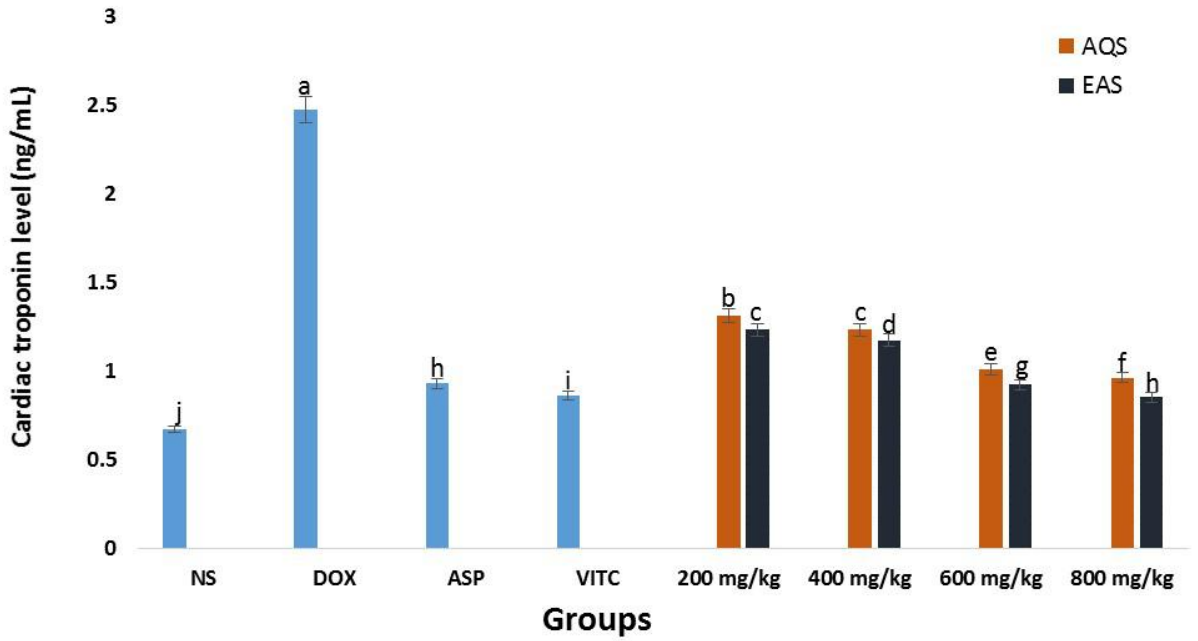


Figure 3: Cardiac troponin I (CTnI) level in the serum of albino rats administered *Dacryodes edulis* aqueous and ethylacetate seed-extracts for 21 days and induced toxicity with 15 mg/kg doxorubicin. The data are presented as mean ± SD (n=6) and significant difference at P<0.05. NS= negative control, DOX= positive control, ASP= aspirin, VITC= vitamin C, AQS= aqueous seed and EAS= ethylacetate seed-extracts at different doses (200, 400, 600 and 800 mg/kg). * Bars with the same letter are not significantly different

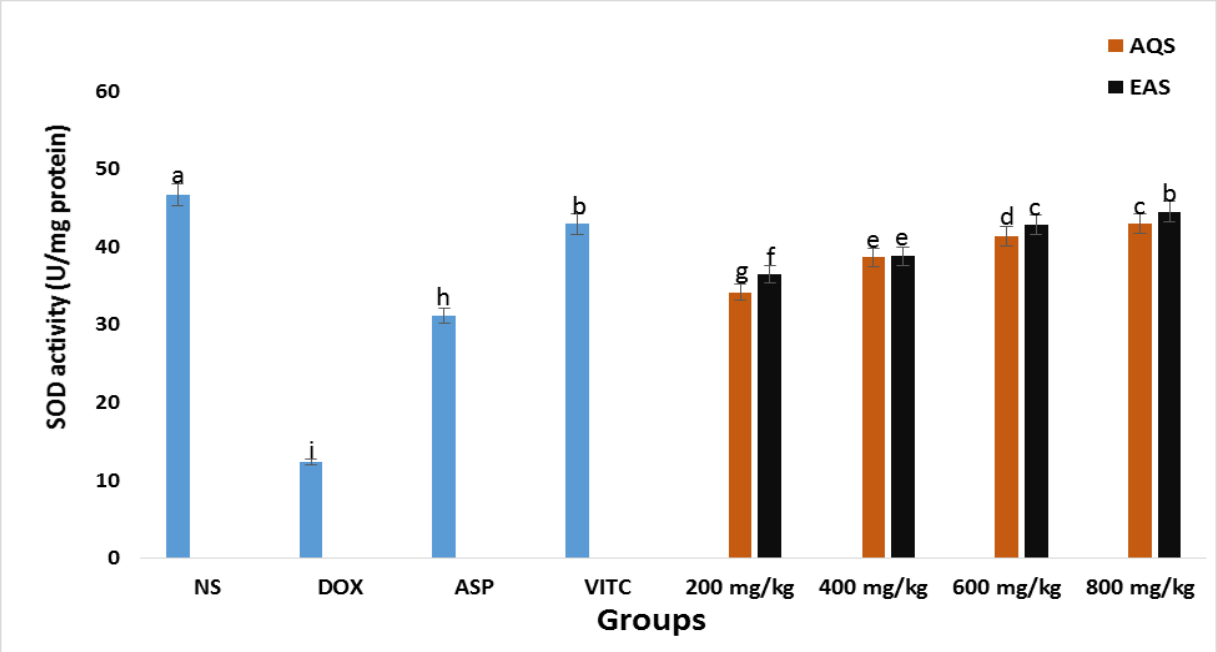


Figure 4: Superoxide dismutase (SOD) activity in the heart tissue homogenates of albino rats administered *Dacryodes edulis* aqueous and ethylacetate seed-extracts for 21 days and induced toxicity with 15 mg/kg doxorubicin. The data are presented as mean ± SD (n=6) and significant difference at P<0.05. NS= negative control, DOX= positive control, ASP= aspirin, VITC= vitamin C, AQS= aqueous seed and EAS= ethylacetate seed-extracts at different doses (200, 400, 600 and 800 mg/kg). * Bars with the same letter are not significantly different.

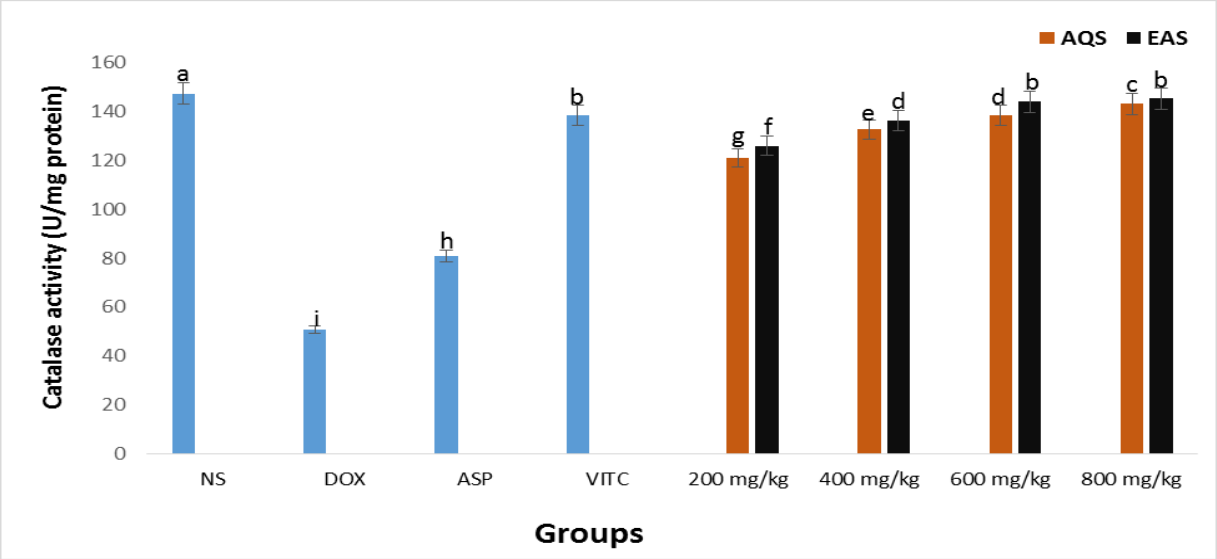


Figure 5: Catalase (CAT) activity in the heart tissue homogenates of albino rats administered *Dacryodes edulis* aqueous and ethylacetate seed-extracts for 21 days and induced toxicity with 15 mg/kg doxorubicin. The data are presented as mean ± SD (n=6) and significant difference at P<0.05. NS= negative control, DOX= positive control, ASP= aspirin, VITC= vitamin C, AQS= aqueous seed and EAS= ethylacetate seed-extracts at different doses (200, 400, 600 and 800 mg/kg). * Bars with the same letter are not significantly different.

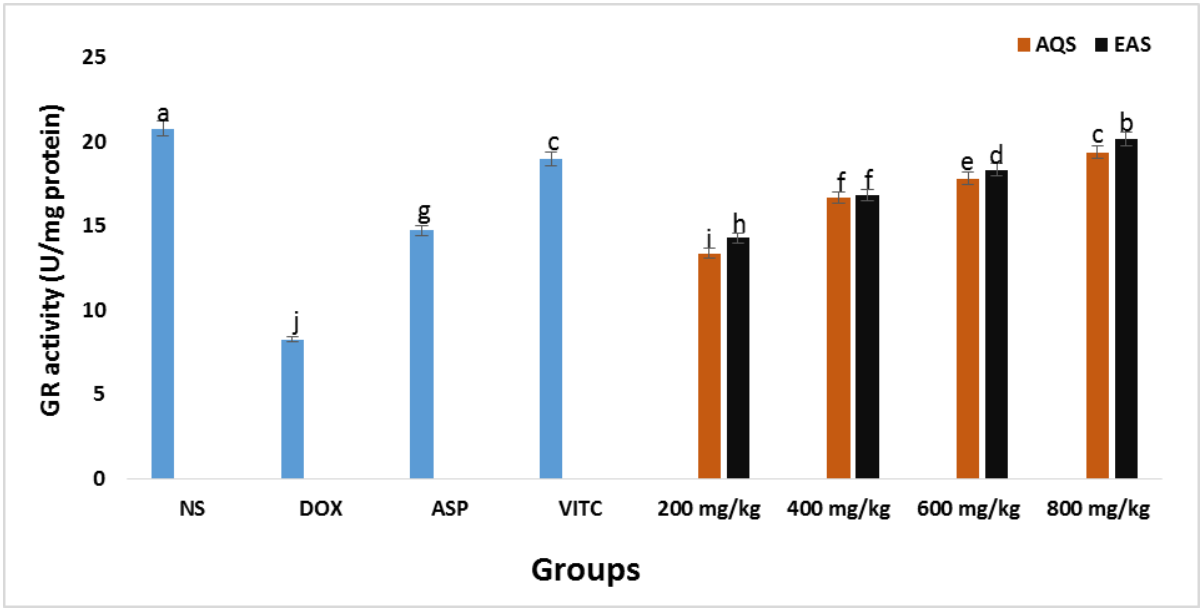


Figure 6: Glutathione reductase (GR) activity in the heart tissue homogenates of albino rats administered *Dacryodes edulis* aqueous and ethylacetate seed-extracts for 21 days and induced toxicity with 15 mg/kg doxorubicin. The data are presented as mean ± SD (n=6) and significant difference at P<0.05. NS= negative control, DOX= positive control, ASP= aspirin, VITC= vitamin C, AQS= aqueous seed and EAS= ethylacetate seed-extracts at different doses (200, 400, 600 and 800 mg/kg). * Bars with the same letter are not significantly different.

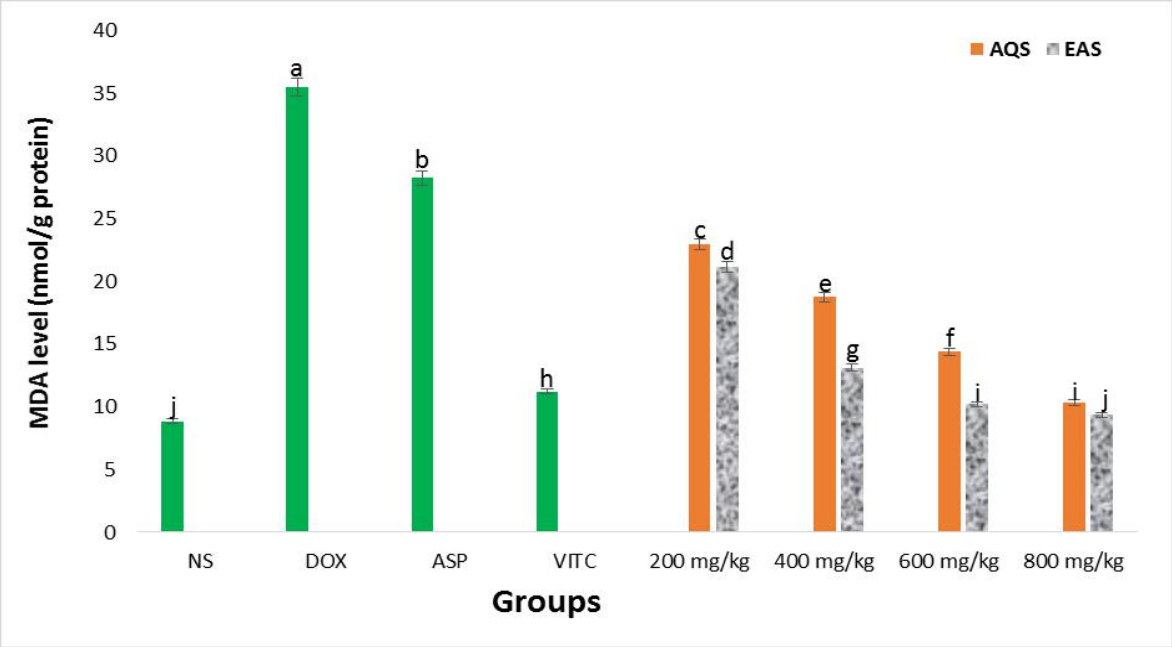


Figure 7: Malondialdehyde level in the heart tissue homogenate of albino rats administered *Dacryodes edulis* aqueous and ethylacetate seed-extracts for 21 days and induced toxicity with 15 mg/kg doxorubicin. The data are presented as mean ± SD (n=6) and significant difference at P<0.05. NS= negative control, DOX= positive control, ASP= aspirin, VITC= vitamin C, AQS= aqueous seed and EAS= ethylacetate seed-extracts at different doses (200, 400, 600 and 800 mg/kg). * Bars with the same letter are not significantly different.

DISCUSSION

The primary function of the heart is to pump blood through the circulatory system in order to generate and sustain an arterial blood pressure necessary to provide adequate perfusion of the body organs (Klabunde, 2013). The heart achieves this by contracting its muscular walls around a closed chamber to generate sufficient pressure to propel blood from the left ventricle, through the aortic valve and into the aorta (Klabunde, 2013). Simultaneously, the right ventricle contracts and propels blood through the pulmonic valve and into the pulmonary artery to perfuse the lungs (Klabunde, 2013). When the heart or the blood vessels are in their abnormal conditions, these functions are impaired. The parameters which indicate the health status of the heart are regarded as the cardiac function specific indices. These parameters include the activities of creatine kinase (CK), lactate dehydrogenase (LDH) and level of cardiac troponin I (CTnI) in the peripheral blood.

The present study deals with the effect of aqueous and ethylacetate seed-extracts of *Dacryodes edulis* on doxorubicin-induced cardiotoxicity. Doxorubicin has been reported to cause cardiomyopathy in various animal species (Oliveira *et al*., 2004). Doxorubicin is converted in the tissue into its semiquinone form, which is a toxic, short

lived metabolite that interacts with molecular oxygen and initiates a cascade of reaction leading to ROS generation (Vikas *et al*., 2015). Reactive oxygen species react with lipids, proteins and other cellular constituents causing oxidative damage to mitochondria and cell membranes of the heart muscle cells (Singal *et al*., 2000). The results in Figures 1-3 show significant ($p< 0.05$) higher activities of cardiac enzymes (CK and LDH) and level of CTnI in the serum of the positive control group (DOX). These are indications of cardiac tissue damage due to doxorubicin administration, and contrary to the results obtained from the negative control groups (NS). In a similar manner, the results presented in Figures 4-7 for oxidative stress indices in the cardiac tissues show significantly ($p< 0.05$) higher activities of antioxidant enzymes (SOD, CAT and GR) and significantly ($p< 0.05$) higher level of lipid peroxidation (MDA) of the positive control groups. The reduction in the antioxidants activities with increase in lipid peroxidation and the resultant elevation in the activities and level of the cardiac indices were strong indications of increased oxidative stress in the positive control group. These findings are in support of an earlier report of Hardina *et al.* (2000).

The aspirin pretreated group showed significant ($p< 0.05$) cardioprotection as observed with the lower activities of serum CK and LDH and level of CTnI with increased activities of SOD, CAT and GR and reduced level of MDA in the aspirin group when compared to the positive control group. This is in line with the earlier report of Mehmet *et al.* (2014), who observed that low-dose aspirin increased the serum total antioxidants in a short time period which may be considered as another beneficial effect of aspirin.

Also in this study, vitamin C (ascorbic acid) pretreated groups showed significant ($p< 0.05$) protective activity of cardiac tissues. The results of the groups pretreated with vitamin C (Figures 1-7) show that the activities of the cardiac enzymes (CK and LDH), activities of antioxidant enzymes (SOD, CAT and GR) and levels of CTnI and MDA were comparable to the negative control group (NS). This is an indication of a significant ($p< 0.05$) protective activity of vitamin C against the doxorubicin induced cardiac tissue toxicity. The possible action of ascorbic acid may be attributed to its scavenging action of relevant reactive oxygen species (ROS) and nitrogen species. In addition to scavenging of ROS and reactive nitrogen species, ascorbic acid can

regenerate other antioxidant molecules, such as α-tocopherol, urate and β-carotene from their respective radical species as reported by Carr and Frei (1999). The antioxidant and free radical scavenging properties of ascorbic acid possibly reduced the effects of oxidative stress (Carnes *et al*., 2001). This also supports the report of Viswanatha *et al*. (2011) on the cardioprotective effects of ascorbic acid on doxorubicin induced myocardial toxicity in rats.

Results of the cardiac parameters of the groups administered aqueous and ethylacetate seed-extracts for 21 days before induced with 15 mg/kg body weight doxorubicin as presented in Figures 1-3 show significantly ($P<0.05$) lower activities of serum CK and LDH, and lower level of CTnI in the groups that received the extracts. Pretreatment with aqueous and ethylacetate seed-extracts indicated significant ($p< 0.05$) protection of the myocardium against the doxorubicin-induced cardiac toxicity. In the same manner, the administration of aqueous and ethylacetate seed-extracts improved the antioxidant status in the studied animal groups and thereby prevented oxidative injuries of the heart tissues as a result of doxorubicin intraperitoneal injection, in contrary to what that was observed in positive control group (Figures 4-7). The results indicated significant ($P<0.05$) higher activities of SOD, CAT and GR, and reduced MDA level in the groups pretreated with the extracts, depicting the antioxidant sparing action on the doxorubicin induced reactive substances. The antioxidant mechanism of the seed-extracts could involve scavenging or neutralization of free radicals, inhibition of oxidative enzymes like cytochrome P_{450}, interaction with oxidative cascade and preventing the progression and disarming oxidative properties of metal ions such as iron among others. These observations were different in the group not administered the extracts but was also injected doxorubicin which showed significantly ($P<0.05$) higher activities of LDH, CK and level of serum CTnI. In this group, records show significantly ($P<0.05$) lower activities of SOD, CAT and GR and higher level of MDA. The protective activity of the extracts was more efficacious in the groups that received ethylacetate seed-extract than aqueous seed-extract and in dose dependent manner. This report is in support of earlier report of Atawodi *et al*. (2014) which observed that antioxidants can be effective in the prevention of oxidative stress and they act as scavengers of free radicals.

CONCLUSION

The results of the study showed that aqueous and ethylacetate seed extracts of *Dacryodes edulis* are potential antioxidative agents and exhibited protective properties on the cardiac toxicity. And relatively could serve as protective agents to cardiac related disorders. These activities which may be due to the bioactive constituents of the extracts could explain why the herbal practitioners apply the seed extracts of the plant in the management and treatments of patients with cardiac related diseases.

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