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# Ameliorative effects of aqueous seed-extract of *Dacryodes edulis* on doxorubicin-induced cardiac tissue damage in albino rats

Matthias C. Ominyi[1], Augustine A. Uwakwe [2], Friday N. Nweke[3,*] , Fedrick U. Ali[1], Moses E. Ogbanshi [4], Lilian N. Ebenyi[1], Victor U. Nwankwo[1], Emmanuel O. Ogueji[5]

[1]*Dept. of Biotechnology, Ebonyi State University, P.M.B. 053 Abakaliki, Nigeria.*
[2]*Dept. of Biochemistry, Faculty of Chemical Sciences, University of Port Harcourt, Nigeria*
[3]*Dept. of Biotechnology, Alex Ekwueme Federal University Ndufu-Alike, Nigeria.*
[4]*Dept. of Biochemistry, Ebonyi State University, P.M.B. 053 Abakaliki Nigeria.*
[5]*Dept. of Fisheries and Aquaculture, Alex Ekwueme Federal University Ndufu-Alike, Nigeria*
[*]*Corresponding author: fridaynwalo@yahoo.com*

## Abstract

Ameliorative effects of aqueous seed extract of *D acryodes edulis* on doxorubicin-induced cardiac tissue damage in albino rats were investigated. The animals were divided into groups and fed for 14 days to acclimatize. A single dose of 15 mg/kg body weight doxorubicin was given intraperitoneally to each group except the control group. After 48 hours, two animals were randomly selected from each group and sacrificed under ethyl-ether anesthesia, to harvest their hearts for histological studies. Subsequently, groups 1 and 2 were given normal saline, while groups 3 and 4 were treated with 25 and 100 mg/kg body weight of aspirin and vitamin C respectively. Groups 5-8 received different doses of aqueous seed extract (200, 400, 600, and 800 mg/kg body weight). Treatments lasted for 21 days and the rats were sacrificed under anesthesia, and their hearts harvested and fixed in 10% formol saline for examination. The photomicrograph revealed severe and focal loss of cardiac fiber, intra-myocardial hemorrhages, fragmentation of cardiac muscle, and disorganization of histoarchitecture in the induced but untreated animal groups. Micrographs showed significant ameliorative effects of the extract in a dose-dependent way and healing effects of the standard drugs. These results showed that aqueous seed extract of *Dacryodes edulis* exhibits therapeutic potentials for cardiac disorders.

**Keywords:** Cardiac; *Dacryodes edulis*; damage; effects; tissue.

## 1. Introduction

*Dacryodes edulis* known as plum or bush butter tree in Africa has different names in Nigeria. The South-Eastern part of Nigeria (Igbo land) calls it *ube*, South-Western know it as eleme. Some countries like Gabon call it Atanga while people from Cameroon call it safouin. It is an evergreen tree and belongs to the family, *Burseraceae* (frankincense family). The plant is cultivated because of its edible fruit which is rich in nutrients like lipids, vitamins, proteins, and minerals (Ajibesin, 2011). In some African countries, most parts of the plant are used in traditional medicine for curing skin diseases, wounds, and dysentery.

The extracts of *D. edulis* seed have been found to show biological activities such as antioxidant and cardioprotective (Ominyi *et al*., 2018). In the cardioprotective activity of the *D. edulis*, the report revealed a decrease in the cardiac injury indices such as cardiac troponin I, creatine kinase, and lactate dehydrogenase on test groups of the animal model against their negative control (Ominyi *et al*., 2018). The report also recorded an increase in the activities of antioxidative parameters like catalase, superoxide dismutase, and reduced glutathione, and a decrease in the level of malondialdehyde with the administration of *D. edulis* aqueous

seed extract.

In medical care, the exudates are used as incense and antibacterial agent (Sofowora, 1993). When the plant is burnt, the smoke and fragrant odour can drive away evil spirits (Sofowora, 1993). These huge characteristics of African pear are due to the extensive array of bioactive compounds available in the seed viz; phytochemicals (polyphenols, saponins, terpenes, tannins, flavonoids, and alkaloids), minerals, carbohydrates, vitamins, lipids, and protein (Agbafor *et al*., 2017). Quality oil of different types has been produced from parts of the plant (Jirovetz *et al*., 2003).

Doxorubicin (also called Adriamycin) is an anti-cancer drug that belongs to the anthracycline antibiotics, which is a powerful, well-established, and highly effective antineoplastic agent used to cure malignancies and esophageal carcinomas (Abdalla *et al*., 2016). Nevertheless, the application in clinical medicine is limited due to its cardiotoxicity (Zhon *et al*., 2001). Congestive heart failure and electrocardiographic changes have been reported to occur after a cumulative doxorubicin administration (Lenaz & Page, 1976).

The mechanisms of cardiotoxic effects for doxorubicin are myocardial injury and lipid peroxidation (Myers *et al*., 1977), mitochondrial dysfunction (Bier & Jaenke, 1976), reduced activity of Na+–K+ adenosine triphosphatase (Geetha & Devi,1992), cellular toxicity, calcium overloading and peroxynitrite formation (Bristow *et al*.,1980; Loren,2005). Doxorubicin-induced cardiomyopathy and heart failure result from the availability of free radicals and a lack of endogenous antioxidants (Hanaa *et al*., 2005).

Cardiac-related disorders rank high among the major public health concern ailments and their prevalence is increasing at a geometrical rate especially in developing countries like Nigeria. To avert the impending menace, it is of utmost importance to search for alternative medicine which will be readily available, affordable, effective, and closer to nature to ensure minimal adverse effects. *Dacryodes edulis* is one of the plants used by traditional medicine practitioners in the southeastern States of Nigeria for the treatment of many ailments including heart diseases. These constituted our major motivating factor to embark on this research to evaluate the ameliorative influence of the *D. edulis* aqueous seed extract on histoarchitecture of induced heart tissue damage.

## 2. Materials and methods

### 2.1. Sample Collection

*Dacryodes edulis* seeds were collected from Izzi Local Government Area of Ebonyi State, Nigeria, while the albino rats were raised from the animal laboratory of the Biochemistry Department, Ebonyi State University, Abakaliki.

### 2.2. Sample preparation

The *Dacryodes edulis* seeds were air-dried, pulverized into fine powder. Two hundred (200) grams of the powdered seeds of *D. edulis* were soaked in 1000 mL of deionized water for 24 hours. Thereafter, the suspension was filtered using a muslin cloth. The filtrate was concentrated using a rotary evaporator. The extract was stored in an air-tight container at 4$^{o}$C.

### 2.3. Design of the study

Male albino rats (100-250g) numbering forty-eight (48) were divided into eight (8) groups comprising six (6) rats per group and were maintained on normal feed and free access to water and allowed to adapt for 2 weeks. After which, a sole dose of 15 mg per kg body weight doxorubicin was administered intraperitoneally to each rat in each group excluding the normal control (group 1). Forty-eight (48) hours post doxorubicin administration; two animals were randomly selected from each group

and sacrificed under ethyl-ether anesthesia, to harvest their hearts for histological study to establish successful induction of injury. Treatments were then randomly assigned to the groups as follows; groups 1 and 2 were given normal saline, while groups 3 and 4 were treated with 25 and 100 mg/kg body weight of aspirin and vitamin C respectively to serve as a control. Groups 5-8 received different doses of aqueous seed extract (200; 400; 600 and 800 mg per kg weight of the body respectively). The choice of the doses was based on the preliminary acute toxicity study on both seed and leaf extracts of the plant which revealed no sign of toxicity for the seed extract, while the leaf extract showed toxicity signs at dosages greater or equal to 3000 mg/kg body weight of the animals. Ajibesin (2011) of no toxicity in *Dacryodes edulis* plant remains significant because the plant seed or kernel with 3.3% protein, are commonly fed to domestic livestock such as sheep and goats (Orwa *et al*. 2009). However, toxicity at doses beyond 400mg/kg body weight could be a result of environmental contamination.

Treatments lasted for 21 days after which the rats were sacrificed under ethyl-ether anesthesia, and their hearts were harvested and fixed in 10% formol saline for tissue processing and examination.

2.4. Histological examination procedure

The histopathological investigation of the heart was done in line with (Talib & Khurana, 1999) metho*d.* The fixed tissues were then dehydrated to remove water that is not miscible with xylene and wax using graded concentrations of alcohol, 30, 50, 70, and 95% for 30 minutes each. The dehydrated tissues were cleared using xylene for 30 minutes and the tissues were impregnated and infiltrated to remove the xylene at 60$^{o}$C for 30 minutes. The infiltrated tissues were embedded with molten wax and allowed to solidify into blocks that were sectioned using a rotary microtome. They were trimmed to obtain the cutting surface of the tissues at 15 microns and was sectioned at 5 microns, and dried on a hot plate for staining. A bright-field microscope that has x10, x60, x150, and x600 magnifications were used to observe the structural alterations that occur after staining the five (5) micron parts with Harris hematoxylin and eosin (H/E). The dose/ dependent effect of the extract was quantified histologically by proper examination of tissue architecture on the photomicrograph in comparison to the normal control group.

## 3. Results

The photomicrographs of the heart tissues of rats induced toxicity with 15 mg/kg body weight doxorubicin before treatment are presented in plates (2a – 8a) of Figures 1-4, while those induced and treated with the extract, aspirin, and vitamin C for 21 days are shown in plates (2b – 8b) of the Figures. The plates (1a and 1b) were from normal control (groups 1). The negative control plates (2a - 8a) revealed abnormalities in the cardiac tissues ranging from severe loss of cardiac tissue, disorganization of cardiac muscle fiber, fragmentation of cardiac muscle fiber, focal intramyocardial hemorrhage, fragmentation and disorganization of the heart tissue architecture, focal loss of tissue and distortion of cardiac muscle tissue in the group's induced toxicity before treatment. However, the plates (Figures 2b – 8b) for the animals treated with the *D. edulis* aqueous seeds extract, aspirin, and vitamin C for 21 days showed significant restoration of the myocardium, well-perfused cardiac muscle, and rejuvenation of cardiac architecture. The healing effects of the extract are in dose-dependent manner (800 > 600 > 400 > 200 mg/kg body weight)

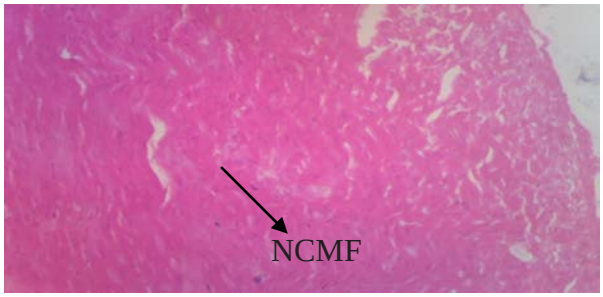


Plate 1a: Not induced toxicity showing (NCMF)

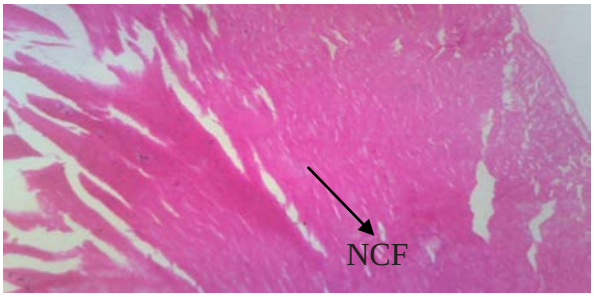


Plate 1b: Not induced and given normal saline

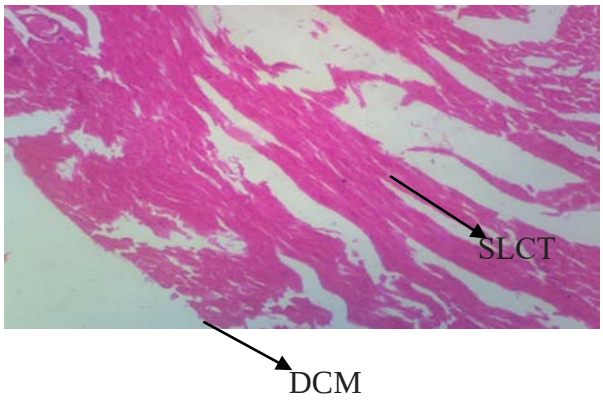


Plate 2a: Induced toxicity, before treatment

Group 2 Induced toxicity showing SLCT+(DCM

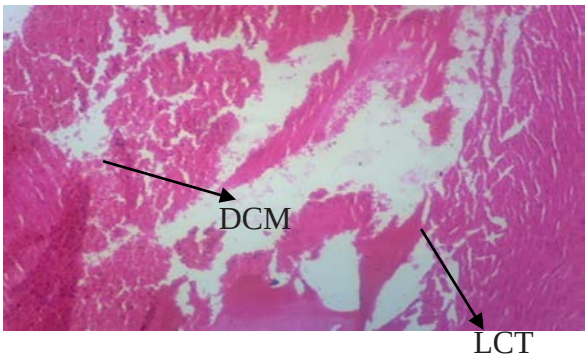


Plate 2b: Induced and treated with normal saline

Group 3: Induced toxicity showing IMCH +DCMF

**Fig.1.** Plate 1a and 1b of normal control (group 1), showing normal cardiac muscle fiber (NCMF) and normal cardiac fiber (NCF) respectively. Plate 2a and 2b of negative control (group 2), plate 2a, before treatment show severe loss of cardiac tissue (SLCT) and distorted cardiac muscle (DCM), while plate 2b after treatment with normal saline also shows disorganization of cardiac muscle (DCM) and loss of cardiac tissue (LCT).

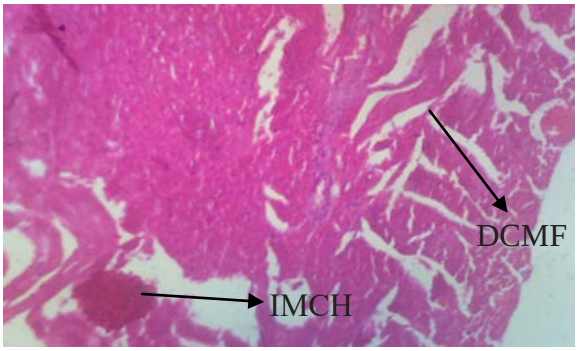


Plate 3a: Induced toxicity, before treatment

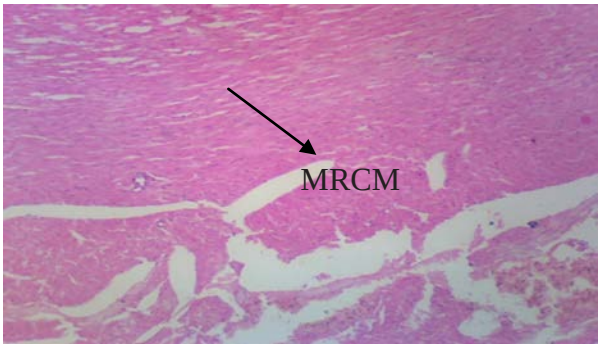


Plate 3b: Induced toxicity and treated with aspirin

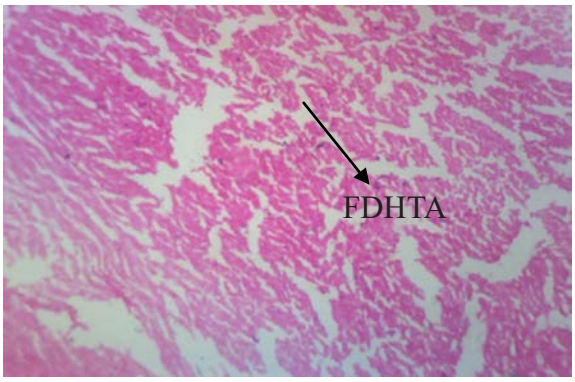


Plate 4a: Induced toxicity, before treatment

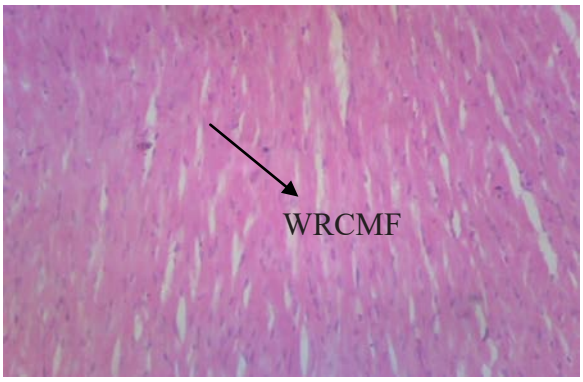


Plate 4b: Induced and treated with vitamin C

**Fig. 2.** Plates 3a and 3b are from animals in group 3; plate 3a, before treatment shows focal intramyocardial hemorrhage (IMCH) and disorganization of cardiac muscle fiber (DCMF), while plate 3b after treatment with aspirin shows mild restoration of cardiac muscle (MRCM). Plates 4a and 4b are of group 4; plate 4a before treatment shows fragmentation and disorganization of the heart tissue architecture (FDHTA), while plate 4b after treatment with vitamin C shows well-restored cardiac muscle fiber (WRCMF).

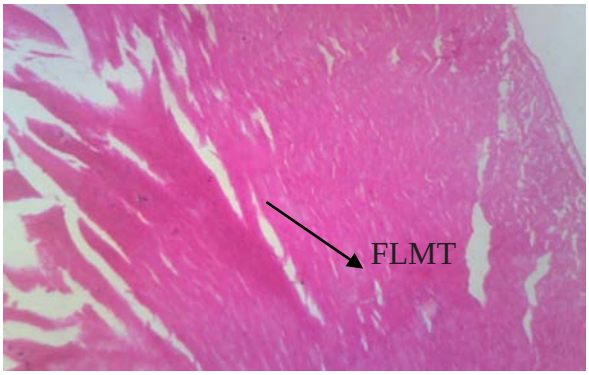


Plate 5a: Induced toxicity, before treatment

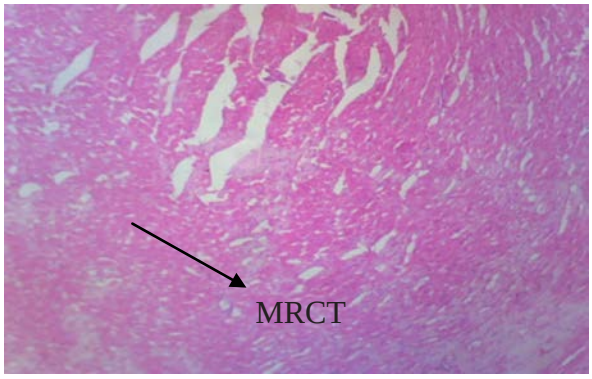


Plate 5b: Induced and treated with 200 mg/ kg, extract

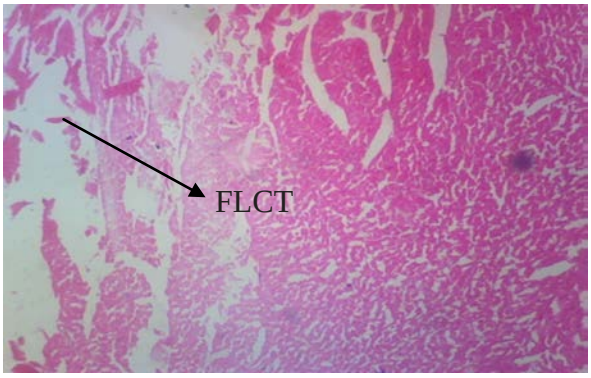


Plate 6a: Induced toxicity, before treatment

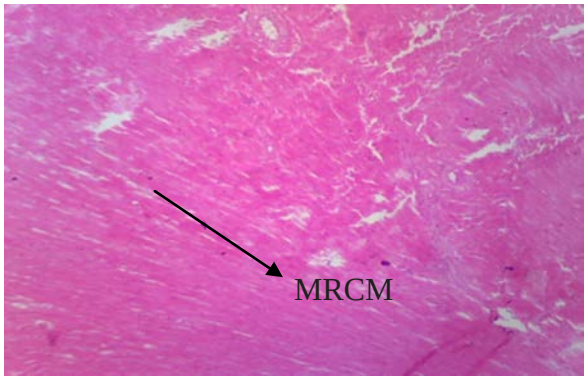


Plate 6b: Induced and treated with 400 mg/ kg, extract

**Fig. 3.** Plates 5a and 5b are from animals in group 5; plate 5a, before treatment shows focal loss of muscular tissue (FLMT), while plate 5b after treatment with 200 mg/kg body weight of the extract shows mild restoration of cardiac tissue (MRCT). Plates 6a and 6b are of group 6; plate 6a before treatment shows fragmentation and loss of cardiac tissues (FLCT), while plate 6b after treatment with 400 mg/kg body weight of the extract shows moderate restoration of cardiac muscle (MRCM).

## 4. Discussion

This research investigated the ameliorative effects of aqueous extract of *Dacryodes edulis* seeds on doxorubicin-induced cardiac tissue damage. In several animal species, doxorubicin has been reported to cause cardiomyopathy (Oliveira *et al*., 2004). Davis & Dorshow (1986) equally found that in cardiac tissue, doxorubicin can be transformed into unstable metabolites which are poisonous in their semiquinone form and interact with molecular oxygen to generate reactive oxygen species (ROS). Reactive oxygen species do interact with biomolecules such as protein and cellular components to cause damage in the cell membranes and mitochondria cells of the heart muscles (Singal *et al*., 2000). The results in Figures 1-4 (plates 2a-8a) showed extensive loss and distortion of cardiac tissues and fibers which could be as a result of reactive oxygen species.

The micrograph of normal control, Figure 1 (plates 1a and 1b) showed no changes morphologically, while their heart revealed normal architectural tissues appearance. The fibers of the cardiac muscle were established to be uniform in configuration, shape, and size without infiltration of inflammatory cells. Cardiomyopathy took place in every rat injected with doxorubicin as proved by the extensive loss of cardiac fiber and muscle Figures1-4 (plates 2a-8a). Doxorubicin caused an enormous change in the myocardium, thus displaying a different degree of cardiac muscle and fiber loss in form of myocardial tissue distortion, intramyocardial hemorrhage, fragmentation, and disorganization of the heart tissue architecture. Increased oxidative stress and depletion of antioxidants may be responsible for the damage of the cardiac tissue (Hardina *et al*., 2000). The aspirin-treated group Figure 2, (plate 3b) showed mild restoration of distorted cardiac tissue and fiber. Aspirin was able to have a mild effect on the heart tissues of the rat because it is an acetyl derivative of salicylic acid which has anti-inflammatory, antipyretic, and free radical scavenging properties (Mehmet *et al*., 2014).

In this study also, the vitamin C (ascorbic acid) treated group showed significant restoration of cardiac tissue and normal heart architecture. The ascorbic acid action is facilitated by the scavenging physiologically significant reactive oxygen species (ROS) and nitrogen species. In addition to ROS and reactive nitrogen molecules, ascorbic acid can regenerate antioxidants like β-carotene and α-tocopherol from their radical species (Carr & Frei, 1999). The lethal effect of ROS has been described to be mitigated indirectly by ascorbic acid through reducing oxidized form of vitamin E and directly via increasing antioxidant enzyme activities of cells. The oxidative stress effect of the antioxidant and free radical scavenger properties of ascorbic acid is perhaps reduced (Carnes *et al*., 2001). As obtained in Figure 2 (plate 4b), ascorbic acid protected the doxorubicin-induced histological changes in cardiac tissue of rats by restoring endogenous antioxidant activity. The findings of this research agreed with the work of Viswanatha *et al*. (2011) on the cardioprotective effects of vitamin C on doxorubicin-induced myocardial toxicity in mice.

Treatment using aqueous seed extract of *D. edulis* aided the restoration of cardiac tissues and cardiac fibers in the animals. The different doses of *D. edulis* extract ameliorated the effect of doxorubicin-induced cardiotoxicity in the heart tissue of the rats in a dose-dependent trend (800 > 600 > 400 > 200 mg/kg body weight). This finding is in line with the previous studies on the biochemical markers of cardiac injury such as cardiac troponin I, creatine kinase, and lactate dehydrogenase, and other oxidative stress indices which revealed that aqueous seed extract of *Dacryodes edulis* possesses protective activity on oxidative induced heart injury (Ominyi *et al*., 2018). The work also showed the antioxidative properties of aqueous seed extract of *D. edulis* against oxidative damage with increased activity of antioxidative enzymes such as catalase and

superoxide dismutase and increased level of an antioxidative compound like reduced glutathione. A decrease in the concentration of malondialdehyde was equally observed (Ominyi *et al*., 2018)

Similarly, the administration of aqueous seed extract of *D. edulis* enhanced the antioxidant level and thus restoring the damaged heart tissues, due to the antioxidant activities of the extract. The antioxidant process of the seed plant extract may comprise of neutralization of free radicals; inhibition of cytochrome P450 enzymes; reduction of oxygen thereby making it unavailable for oxidative reaction; interrelating with oxidative trends and avoiding its products respectively. Therefore, aqueous seed extract of *Dacryodes edulis* might have caused the amelioration of tissue injury by lessening the oxidative stress and repairing the antioxidant position. In addition, the restorative effects of the extract on the damaged heart tissue might be considered to result from the antioxidant vitamins and phytochemicals content of the plant-like flavonoids, alkaloids, saponins, tannins phenols, and glycosides (Agbafor *et al*., 2017). The restoration effects of *Dacryodes edulis* recorded in this work agreed with the lipid-lowering effects of aqueous root extract of *Eurycoma longifolia* jack in the hepatocytes of humans as evidence of therapeutic effects of plants bioactive compounds (Pei *et al*., 2019).

## 5. Conclusion

The micrographs of the study showed that aqueous seed extracts of *Dacryodes edulis* possess ameliorative effects against the doxorubicin-induced cardiac damages. These findings could explain the reasons for using plant seeds as a remedy for heart-related ailments by traditional medical practitioners. These effects might also be a result of biologically active compounds present in the plant.

## Ethics approval

The experimental protocols and procedures used in this study were approved by the Ethical Committee for the care and use of Laboratory Animals of Ebonyi State University, Directorate of Research, Innovation and Commercialization (EBSU-DRIC) Abakaliki, Ebonyi State, Nigeria with approval no: EBSU-DRIC/EA/18/176.

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