World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Evaluation of ethanol extract of *Allium cepa* and fractions on haematological parameters in doxorubicin-induced Cardiotoxicity using Wistar rats

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World Journal of Advanced Research and Reviews, 2024, 21(02), 1551–1564

Publication history: Received on 07 January 2024; revised on 18 February 2024; accepted on 20 February 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.21.2.0376

Abstract

This research study evaluated the effects of Ethanol extract of *Allium cepa* (*A. cepa*) popularly known as Onions and its fractions on haematological parameters in doxorubicin-induced cardiotoxicity using Wistar rats. A total of 45 Wistar rats weighing between 150-200 g were recruited for this study. They were randomly divided into 9 groups of 5 animals each; as follows: Group 1: served as control and received 10 ml /kg/ body weight (b.w) per oral (p.o) of normal saline. Group II: received 3.5 mg / kg b.w intraperitoneal (i.p) of Doxorubicin (Dox). Group III: received 500 mg/kg/ b.w p.o of vitamin E plus Dox. Group IV: received 1000 mg/kg/ b.w of crude extract (C.E) of *A. cepa* plus Dox. Group V: received 1000 mg/kg b.w of n-Hexane fraction o*f A. cepa* plus Dox. Group VI: received 1000 mg/kg b.w of dichloromethane (DCM) fraction o*f A. cepa* plus Dox. Group VII received 1000 mg/kg b.w of ethyl acetate (E.A) fraction of *A. cepa* plus Dox. Group VIII: received 1000 mg/kg b.w of methanol (METH) fraction, and Group IX received Combinations of C.E o*f A. cepa* + vitamin E and Dox respectively for 14days. Groups I and II treatments lasted for 14 days, while treatments for groups III-IX lasted for 16 days (making 14 days for respective treatments and additional 2 days for doxorubicin administered once 48 hourly before sacrificing. All substances in this study were administered orally except doxorubicin that was administered intraperitoneally. Haematology results obtained from this study showed Dox administration negatively altered full blood count; Red blood cell (RBC), White blood cell (WBC) and Platelets levels significantly ($p<0.05$). However, pre-treatment administration with *Allium cepa* showed significant ($p<0.05$) improvement of full blood count levels from the effects of singular administration of Dox. Injury to the myocardium was also observed on histological examination in the Dox treated group. Our findings indicate that haematological parameters RBC, WBC, Platelets could be useful diagnostic tools in prediction and evaluation of Cardiotoxicity. Haematology results obtained from this study showed Doxorubicin administration significantly altered ($p<0.05$) full blood count; Red blood cell (RBC), White blood cell (WBC) and Platelets levels. However, pre-treatment administration with *Allium cepa* showed significant ($p<0.05$) improvement of full blood count levels from the effects of singular administration of Doxorubicin. Injury to the myocardium was also observed on histological examination in the Doxorubicin treated group. Our findings indicate that haematological parameters RBC, WBC, Platelets could be useful diagnostic tools in prediction and evaluation of Cardiotoxicity.

Keywords: Ethanol extract; *Allium cepa;* Haematological parameters; Doxorubicin; Cardiotoxicity.

1. Introduction

The full potential of the clinical use of Dox for its therapeutic purposes in the management of cancer is hampered by its cumulative and irreversible cardiotoxicity, which leads to myocardial dysfunction manifesting as aberrant arrhythmias,

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ventricular dysfunction, and congestive heart failure, even years after chemotherapy cessation (Dolci *et al*., 2006; Christopher *et al*., 2020). Clinically used drugs and treatments first recognized to cause cardiovascular toxicity were anthracyclines and radiation therapy (Kanu *et al*., 2010). Research studies demonstrated the estimated cumulative incidence of congestive heart failure to be 5% at a cumulative dose of 400 mg/m^2, 26% at a dose of 550mg/m^2, and 48% at a dose of 700 mg/m^2 (Goodman *et al*., 2006). Dox is believed to cause cardiotoxicity generally by myocardial cell loss, apoptosis, and necrosis, mediated by oxidative stress, although the exact mechanism of anthracycline-induced cardiotoxicity is yet to be uncovered. Some proposed hypotheses are; the iron and free radical theory, in which oxidative stress is involved due to depletion of endogenous antioxidants. A metabolic hypothesis; in which an alcoholic anthracycline metabolite interferes with the myocardial energy pathway and intracellular calcium concentration; The unifying hypothesis, in which an alcoholic anthracycline metabolite also causes increased calcium concentration in the myocardial fibre and damages it and an apoptosis hypothesis, in which there is an up-regulation of pro-apoptotic markers (Cai *et al*.., 2010). Doxorubicin cardiotoxicity can be acute, occurring during and within 2-3 days of its administration (Takemura *et al.*, 2007). The incidence of chronic doxorubicin cardiotoxicity is usually evident within 30 days of administration of its last dose, but may occur even after 6-10 years after its administration. Antioxidants are reported as molecules, which interact with free radicals terminating the chain reaction before vital molecules are damaged. Antioxidants donate an electron to stabilize a free radical and they have long been known to reduce the free radical mediated oxidative stress caused by elements and compounds in the environment (George et al., 2012). Vitamin E is an antioxidant synthesized by plants protecting all membranes and other fat-soluble parts of the body, such as low-density lipoprotein cholesterol, from damage. Some food sources of vitamin E are; carrot, garlic, leafy greens, mushrooms, lemon grass, mash mellows, bee pollen, alfalfa sprouts, avocado bee pollen, carrot, chickweed, cumfrey root, dandelion root. Some others are sunflower seeds and sunlight. Vitamin E is absorbed from the intestine through via the lymph. Circulates through the body plasma in associations with beta-lipoprotein, Vitamin E has been used in connection with the following conditions like immune functions, rheumatoid arthritis, angina, atherosclerosis, cold sores, heart attack, leucoplakia, skin ulcers, infertility, Alzheimer's disease, bronchitis, osteoarthritis, stroke, age related cognitive decline etc. (Ding *et al*., 2019). Medicinal plants have been reported to be valuable therapeutic agents, used in the treatment or prevention of many diseases (Aletan *et al*., 2019). Recent studies suggest that in developing countries, a large proportion of the population relies mostly on traditional practitioners and medicinal plants to meet primary health care needs (Wu *et al.*, 2013)). Medicinal plants continue to provide valuable therapeutic agents, in both modern medicine and in traditional system to treat or prevent many diseases (Meraiyebu *et al*., 2013). *Allium cepa* (*A. cepa*) popularly known as Onion is used as food additive or supplement for many centuries. The onion plant is a bulbous vegetable 1.2m in height, with 4 to 6 hollow, cylindrical leaves. It is botanically referred to as *Allium cepa*. *Allium cepa* known for its nutritional and medicinal uses (Saxena et al., 2013), is one of the oldest vegetables eaten all over the world with several economic benefits. The common name of the species *Allium cepa* is garden onion or bulb onion and shallot (Grubben et al., 2004). Further Classification of Onion can be seen among the species; the fresh market *Allium cepa L* is classified to the *Liliaceae* and belongs to the genus *Allium*. The Liliaceae family includes a variety of species (Nari et al., 2012; Akash et al., 2014; Bisen and Emerald 2016). Central Asia is said to be the place of origin of the entire family of onions. Botanists place *Allium cepa* as part of the lily family and it is currently cultivated all over the world particularly in zones with moderate climate (Yu et al., 2021). Studies report *A. cepa* has anticancer and similar biological properties, which are thought to be attributed to the presence of different organosulfur derivatives; flavonoids, polyphenols, quercetin and its glycosides (Asemani et al., 2019). The prevalence of cytotoxic-induced heart failure in Nigeria and Africa is not known (Anakwue et al., 2020). The first and foremost organ affected by Dox is the heart, due to its less developed antioxidant defence mechanisms (Sathvik et al., 2023). There is also the prevalence of breast cancer increase across Africa, and cytotoxics are some of the most common and best drugs used during their management (Anakwue et al., 2020). Studies of the side effects of such cytotoxic drugs are imperative (Reuter et al., 2010). Antioxidants are considered to be the solution to many diseases, some of which are life (Ahmed et al., 2020). Due to its high energetic demand, the heart has the highest rate of production of ROS, yet in comparison to other mammalian organs, the heart has a significantly lower level of antioxidants and total antioxidant enzyme activities (Ahmed *et al.,* 2020). It is widely agreed that ROS and oxidative damage are pathological components of cardiovascular diseases (CVDs). Historically, free radicals were merely considered toxins that induce oxidative stress and concomitant cellular damage, and for decades, considerable research has focused on approaches to eliminate excess free radicals generated in the body. Yet, experimental and clinical studies focusing on the use of antioxidant therapy to mitigate myocardial damage have yielded mixed results. Mechanisms which maintain the balance between ROS generation and antioxidant production and consumption in CVD require fine tuning for optimal therapeutic outcomes (Koss-Mikolajczyk *et al.,* 2021). In this present study *Allium cepa* was used as a medicinal herb, rich in antioxidants and also one of the most consumed vegetables amongst the people of Rivers State, Nigeria, used traditionally for its medicinal virtues in a plethora of indigenous cultures for its several ameliorative effects in the prevention and treatment of diseases like common cold, respiratory infection, etc. was used to assess its ameliorative effects in cardiotoxicity and diagnostic tools like circulating haematological parameters were used to investigate the ameliorative effects of *A. cepa* in the treatment of drug-induced cardiotoxicity. Studies reporting links between the risk of possible outcomes in patients diagnosed with cardiovascular

disease and their haematological parameters could lead to circulatory failure; thus, showing hematological and coagulation markers play a role in the diagnosis of cardiovascular diseases (CVD) (Kong *et al.,* 2022). Studies also reported patients with chronic heart failure, increased red distribution width (RDW) which is a significant independent marker of increased morbidity and mortality (Jenca *et al.,* 2021). Alterations in the red cell distribution width are also associated with inflammation in rheumatoid arthritis, cancer, autoimmune hepatitis, COVID-19 infection, and autoimmune diseases (Chinko *et al.,* 2023). The mean platelet volume (MPV) is an activation measure commonly used for routine screening and has been shown to be a prognostic predictor in CVD patients (Dahlen *et al.,* 2021); measurement of MPV has been associated with a diverse range of inflammatory diseases. As inflammation and cardiovascular conditions are closely related, investigation of haematological parameters in cardiac conditions has been reported as important (Jenca *et al.*, 2021). There is additional evidence that RDW, white blood cells (WBC), and MPV, when combined with the coagulation profile, contribute to the diagnosis of acute coronary syndrome (ACS) in individuals presenting with chest pain (Dahlen *et al*., 2021). Thus, haematological biomarkers can be utilized to monitor CVDs and evaluate patient's prognosis (Kong *et al.,* 2022). However, the importance of these haematological markers in predicting in-hospital and short-term outcomes in patients with acute coronary syndrome (ACS) and other cardiovascular diseases are important (Barale *et al.*, 2020). Early and accurate detection of cardiotoxicity is essential for disease control and management. Diagnosis of cardiac toxicities generally requires clinical evaluation utilizing costly procedures. Physicians have been reported to frequently use the complete blood count (CBC) and other routine tests to monitor the health of both sick and healthy people. The accessibility and affordability of these tests make them ideal for investigations and diagnosis (Hoglund *et al.,* 2014). The haematological parameters including red and white blood cell counts, haemoglobin concentration and platelet estimation have been widely used clinical indicators of health and disease (Lin *et al*., 2007). The effects of *A. cepa* crude extract and its fractions on haematological parameters of doxorubicin-induced cardiotoxicity is the focus of this study. There is paucity of information on the cardiotoxic potential of *A. cepa* on haematological parameters, hence its use as an interventionist agent.

2. Materials and Method

2.1. Material and methods

2.1.1. Study Design and Site

The study was an experimental-based study and a laboratory animal model was used. The study was conducted between July and December, 2021 following protocol conditions in the Animal House of the Department of Medical Physiology, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Akwa Ibom State, Nigeria.

2.2. Preparation of Plant Extract and Material

The extract was prepared separately from outer scales of the edible portion of onion bulbs. *A. cepa* leaves was collected, dried under shade and coarsely powdered and sent to the Department of Pharmacology, Uyo, for extraction and evaluation of Pharmacological action on Allium cepa. The powdered samples were stored in a clean glassware container until needed for analysis.

2.3. Partitioning of extract

Fraction of plant extract was done via the separation funnel method (Rimando et al., 2001). The four different solvents (n-hexane, dichloromethane, ethyl acetate and methanol) are selected, moistened and complete dissolution of crude extract was done with 250 mL of water followed by transfer into a separating funnel. 250 mL of n-hexane, the least polar solvent was added and shaken and contents were allowed to settle. The bottom of the separating funnel was opened to remove the aqueous layer. The remaining content was then poured into a clean container to get n-hexane fraction. Equal volume of n-hexane was added again, shaken and then separated. This addition continued until after adding n-hexane and shaking until no reasonable quantity of extract appeared to be more into the n-hexane portion. The procedure was repeated for Dichloromethane (DCM), Ethyl acetate (E.A) and Methanol (Meth) solvents to get their fractions.

2.4. Experimental Design

Forty-five (45) male wistar rats (150-250g) were randomly divided into nine (9) groups of five (5) animals each. And treated as follows for sixteen (16) days: Group 1 served as the control and received distilled water, while experimental group 2 received 3.0mg/kg body weight/i.p of doxorubicin (dox) on alternate days for fourteen (14) days. Group 3 received standard drug, Vit. E for 4 days followed by dox administration forty-eight (48) hours before sacrifice. Group $ animals received crude extract of *A. cepa* for 14 days followed by dox administration 48 hours before sacrifice. Groups

5,6, 7, and 8 received n-hexane, dichloromethane (DCM), Ethyl Acetate (EA), methanol fractions of *A. cepa* respectively for 14 days followed by dox administration 48 hours before sacrifice. Group 9 received a combination of crude extract of *A. cepa*, Standard drug, for 14 days followed by dox administration 48 hours before sacrifice.

2.5. Blood collection and Assay

Following the 14 days administration of doxorubicin to treatment groups and normal saline for the control group, the animals were anesthetized via cervical dislocation and blood was collected by cardiac puncture. Blood samples were transferred into EDTA bottles containing ethylenediaminetetraacetic acid for haematological assay (Auto-haematology Analyzer, Mindray, China).

2.6. Histological Study

After sacrificing rats from each group, the Heart was excised and fixed in Bouin's fluid for 6 h and transferred into 10% formalin. Afterwards, the tissues were rinsed with distilled water and dehydrated with varying percentage of ethanol. Sections were cleared in xylene and embedded in oltene wax. Thin sections were cut with a rotary microtone and stained with hematoxylin and eosin and were examined under a microscope (Fischer *et al.*, 2008). Heart tissue was assessed for any distortion from normal cytotexture. Data was collected to find the injury associated with each group.

2.7. Statistical Analysis

All results were expressed as mean ± SEM. One way analysis of variance (ANOVA) test was used to compare between groups. Values of $P<0.05$ were considered statistically significant (McCormick *et al.*, 2017). All data was recorded as it was represented.

2.8. Ethical Consideration

Ethical approval for the study was sort for from the University of Uyo, Institutional Health Research Committee (IHERC), with approval No. UU/CHS/IHERC/VOL.1/030. The study was carried out in accordance with international guidelines of the National Institute of Health (NIH) on the care and use of laboratory animals.

3. Results

3.1. Red blood cell count

Table 1 Effects of Treatment groups on red blood cells in Doxorubicin treated Wistar rats.

Groups	RBC g/dL	HGB g/dL	PCV %	MCV %	MCH g/c	MCHC g/dL
I (Control)	8.09 ± 0.18	14.18 ± 0.35	52.38 ± 1.54	64.70 ± 1.07	17.55 ± 0.18	27.18 ± 0.20
II (DOX)	7.03 ± 0.36*	12.78 ± 0.67*	42.45 ± 2.17*	60.48 ± 0.91*	18.10 ± 0.06*	30.05 ± 0.36*
III (VIT.E + DOX)	7.74 ± 0.66*	13.43 ± 0.13*	48.13± 0.18*	62.20± 0.59*	17.30± 0.0*	27.88 ± 0.19*
IV (C.E + DOX)	7.52 ± 0.29	12.98 ± 0.46	43.60 ± 1.33*	58.25 ± 0.94*	17.25 ± 0.08*	29.73 ± 0.49*
V (n-Hex + DOX)	5.91 ± 0.38*	10.50 ± 0.66*	40.20 1.49*	70.25 ± 3.70*	17.75 ± 0.08*	26.05 ± 1.31*
VI (DCM + DOX)	5.80 ± 0.33*	10.40 ± 0.54*	33.88 ± 1.08*	59.23 ± 1.40*	17.98 ± 0.10*	30.35 ± 0.67*
VII (E.A + DOX)	6.26 ± 0.12*	11.23 ± 0.20*	41.90 ± 2.31*	66.98 ± 3.60*	17.93 ± 0.24*	27.53 ± 1.2*
VIII (Meth + DOX)	6.92 ± 0.23*	12.45 ± 0.33*	43.23 ± 1.38*	62.55 ± 1.11*	18.07 ± 0.17*	28.86 ± 0.32*
IX (C.E+Vit.E+DOX)	5.74 ± 0.36*	10.00 ± 0.62*	43.25 ± 1.86*	76.93 ± 2.22*	17.50 ± 0.06*	22.90 ± 0.64*

*Indicates significance at ($p<0.05$), difference compared to a = versus group 1, b = versus group 11, c = versus group III, d= versus group IV, e = versus group V, f = versus group VI, g = versus group VII, h = versus group VII, i = versus group IX

The results obtained following treatment with C. E of *A. cepa* and its fractions on red blood cell parameters presented in Table 1. The results showed that Doxorubicin treatment to group II animals significantly ($p<0.05$) reduced RBC, HGB, PCV, and MCV but significantly ($p<0.05$) increased MCH and MCHC compared with group I. Administration of vitamin E and Dox to group III animals did not alter erythrocyte parameters significantly except PCV and MCH that were significantly ($p<0.05$) elevated with significant ($p<0.05$) reduction in MCV and MCH. C.E and Dox administration

significantly ($p<0.05$) increased RBC, HGB, PCV and MCV compared with groups I, II and III. The administration of different fractions of *A. cepa* plus doxorubicin significantly ($p<0.05$) reduced the erythrocyte parameters RBC, HGB, PCV and MCHC except DCM and Dox treated group compared with groups IV. Also, fraction groups significantly ($p<0.05$) increased MCV compared with the crude extract plus Dox group. A similar pattern of significant ($p<0.05$) reduction was recorded with group IX administered crude extract of *A. cepa* plus vitamin E plus Doxorubicin for RBC, HGB, PCV and MCHC, whilst significantly ($p<0.05$) increasing MCV and MCH compared with groups III and IV. (Table I).

3.2. Erythrocyte parameters

The results obtained following treatment groups on White blood cell count are as presented in Table 2. The results showed that Dox treatment to group II animals significantly ($p<0.05$) reduced WBC, Eosinophil and lymphocyte counts compared to the control whilst significantly ($p<0.05$) increasing Neutrophil, Basophil and Monocytes. Administration of vitamin E plus Dox to group III animals significantly ($p<0.05$) reduced WBC, Basophil, monocyte, lymphocyte but increased Neutrophil compared with group I, whilst significantly ($p<0.05$) increasing WBC, Neutrophil, lymphocytes and reducing Basophil, and monocyte compared with group II. All fraction groups significantly ($p<0.05$) reduced WBC, Neu, Eos except Meth plus Dox fraction that increased WBC, Neut compared with C.E plus Dox group whilst an increase in Basophil, lymphocyte and monocyte except E.A and Meth fractions of *A. cepa* plus Dox group was also recorded compared with the C.E plus Dox group. Combination group significantly ($p<0.05$) reduced WBC, Neu, Eos, Lymph but increased Basophil, Monocyte compared with groups III and IV.

3.3. Platelet Count

Results from Table 3 showed Dox treated group compared to Control group significantly ($p<0.05$) reduced Platelet count, MPV and, PDW size. Results also revealed that C.E of *A. cepa* plus Dox treated group compared with Dox treated group significantly ($p<0.05$) increased Platelet count showing an insignificant reduction in MPV and a not so significant increase in PDW. Comparing fractions of *A. cepa* plus Dox treated groups to C.E. of *A. cepa* plus Dox treated groups revealed that all fractions of *A. cepa* plus Dox significantly ($p<0.05$) reduced Platelet levels whilst significantly ($p<0.05$) increasing MPV concentrations except for n-Hexane fraction. A significant ($p<0.05$) reduction in PDW size for all fractions of *A. cepa* compared with C.E of *A. cepa* plus Dox except n-Hexane was recorded, where PDW size significantly ($p<0.05$) increased. Results from Table 3 also revealed that, Vit. E plus Dox compared to C.E of *A. cepa* plus Dox showed a significantly ($p<0.05$) increased Platelet count, MPV concentration but reduced PDW size significantly ($p<0.05$). Combinations group IX results showed a significantly ($p<0.05$) reduced Platelet count compared to C.E of *A. cepa* plus Dox group and Vit. E plus Dox, but significant ($p<0.05$) increase in MPV levels of combination group IX and PDW size (Table 3).

Table 2 Effect of Treatment groups on White blood cell (WBC) parameters in Doxorubicin treated Wistar rats

Groups	WBCs /mm^3	Neutrophils cells/mcL	Eosinophils/mcL	Basophils/mcL	Lymphocytes/mcL	Monocyte %/mcL
I (Control)	14.82 ± 1.22	31.25 ± 3.95	3.95 ± 0.28	0.98 ± 0.13	62.98 ± 1.90	0.85 ± 0.10
II (Dox)	6.89 ± 0.31*	42.65 ± 4.41*	2.80 ± 0.70	1.05 ± 0.18	37.35 ± 5.26*	16.15 ± 6.8*
III (C. E+ Dox)	14.20 ± 1.08	48.88 ± 3.24*	2.80 ± 0.11	0.73 ± 0.05*	46.85 ± 3.24*	0.78 ± 0.11*
IV (Hexane+ Dox)	10.09 ± 1.03*	49.95 ± 3.95*	2.63 ± 0.15	1.20 ± 0.14	45.05 ± 3.97*	1.18 ± 0.7*
V (DCM + Dox)	5.23 ± 0.58*	24.98 ± 3.50*	1.18 ± 0.24*	1.70 ± 0.18*	65.65 ± 2.24*	6.50 ± 1.85*
VI (E.A + Dox)	6.93 ± 1.01*	39.30 ± 5.73*	2.03 ± 0.46	1.53 ± 0.25	52.13 ± 4.84*	5.03 ± 1.55*
VII (METH + Dox)	6.20 ± 0.49*	36.58 ± 0.35*	1.55 ± 0.88	0.98 ± 0.09	57.10 ± 1.36*	3.80 ± 1.22*

VIII (Vit.E+ Dox)	10.61 ± 1.24*	50.74 ± 1.29*	1.83 ± 0.51	1.15 ± 0.06	43.05 ± 1.46*	3.24 ± 0.08*
(C.E IX+ Vit.E + Dox)	7.03 ± 0.67	34.53 ± 5.29*	0.70 ± 0.12	1.78 ± 0.34*	42.00 ± 1.38*	21.00 ± 6.23*

*Indicates significance at ($p<0.05$), difference compared to a = versus group 1, b = versus group 11, c = versus group III, d= versus group IV, e = versus group V, f = versus group VI, g = versus group VII, h = versus group VII, i = versus group IX.

Table 3 Effect of Treatment groups on Platelet and Platelet parameters in Doxorubicin treated Wistar rats

Groups	Platelet (mcL)	MPV (FL)	PDW (FL)	PCT (P/mcL)
I (Control)	710.75 ± 13.86	6.93 ± 0.07	15.53 ± 0.06	0.49 ± 0.17
II (Dox)	660.25 ± 72.35	5.93 ± 0.05	15.23 ± 0.07	0.39 ± 0.42
III (C.E + Dox)	1050.00 ± 179.16*	5.85 ± 0.05	15.25 ± 0.12*	0.61 ± 0.94*
IV (Hexane + Dox)	543.50 ± 111.46*	5.65 ± 0.07*	15.33 ± 0.13*	0.31 ± 0.63*
V (DCM + Dox)	752.00 ± 150.70*	5.98 ± 0.03*	15.18 ± 0.03*	0.45 ± 0.13*
VI (E.A + Dox)	741.75 ± 91.35*	6.10 ± 0.05*	15.15 ± 0.07*	0.59 ± 0.56*
VII (Meth. + Dox)	964.55 ± 33.87*	6.16 ± 0.04*	15.20 ± 0.00*	0.95 ± 0.62*
VIII (Vit.E +Dox)	1580 ± 14.21*	6.03 ± 0.05*	15.10 ± 0.04*	0.45 ± 0.01*
IX (C.E + Vit.E + Dox)	722.00 ± 75.90*	6.20 ± 0.06*	15.30 ± 0.04	4.47 ± 0.43*

*Indicates significance at ($p<0.05$), difference compared to a = versus group 1, b = versus group 11, c = versus group III, d= versus group IV, e = versus group V, f = versus group VI, g = versus group VII, h = versus group VII, i = versus group IX.

3.4. Histopathology

The histological assessment for the control group showed a histologically normal heart muscle with congested blood vessel (BV), cardiac myofibrils that are branched (MF), A centrally placed nuclei (Nu). The DOX treated group showed a histologically distorted heart muscle with hypertrophied myocardial fibre (HYP), centrally placed nuclei (Nu), patchy areas of fibrosis (FIB), cardiac myofibrils (MF). Capillaries congested with RBC between MF and blotchy areas of haemorrhage due to stagnation of blood (HEA). Histology of the standard drug group (Vit.E), showed a histologically normal heart muscle, with cardiac myofibrils (MF), branched, weaved and re-united, nuclei (Nu) centrally placed, cavity and capillaries (CAP) congested with red blood cells.

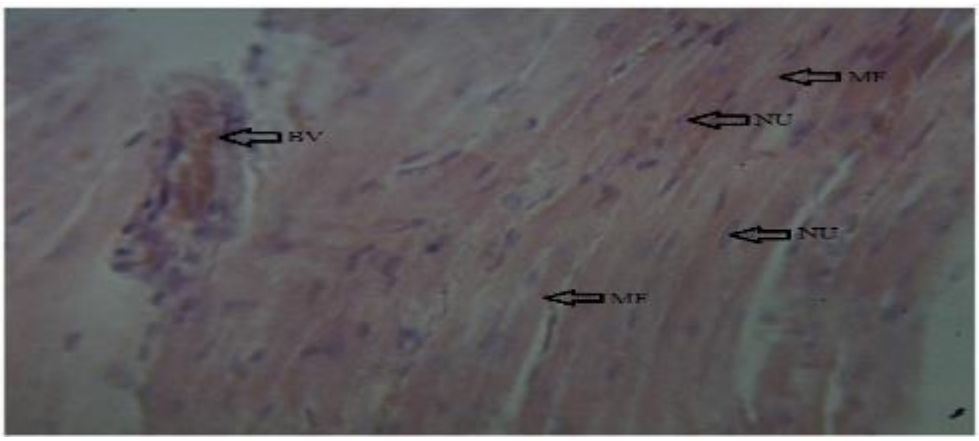

Figure 1 Heart Section of rat showing normal heart muscle, blood vessels (BV), With centrally placed nucleus (NU) and cardiac myofibrils (MF). H & E X400

The crude extract as well as the Vit.E treated group, fractions of *A. cepa* groups and combination group showed a histological normal heart muscle with congested blood vessel (BV), cardiac myofibrils (MF), and a centrally placed nuclei (Nu) when compared with the control group. This finding indicates that *A. cepa* was able to protect against the

induced heart damage. Further assessment of heart muscle of DOX treated group revealed blotchy areas of haemorrhage due to stagnation of blood (HEA)compared with the control group is indicative of myocardial insult and damage in the group.

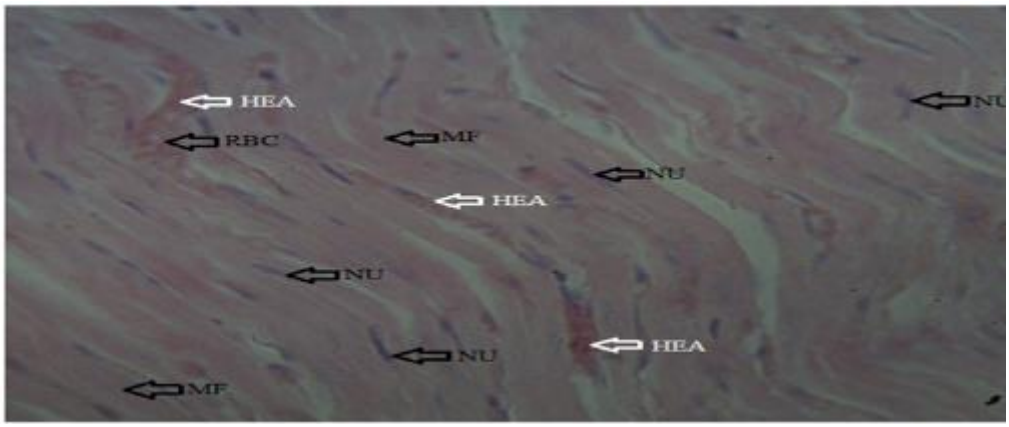

Figure 2 Heart Section of rat showing capillaries congested with RBC between myofibrils (MF), Blotchy areas of haemorrhage due to stagnation of blood (HEA). H&E X 400.

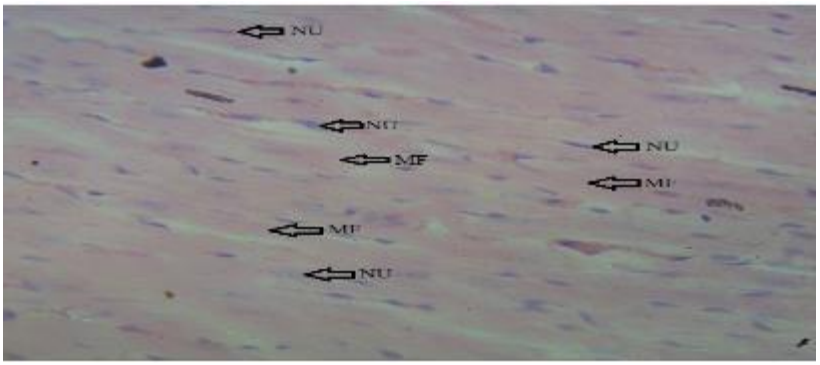

Figure 3 Heart section of rat showing cardiac myofibrils (MF), centrally placed nucleus (NU), H & E X400**.**

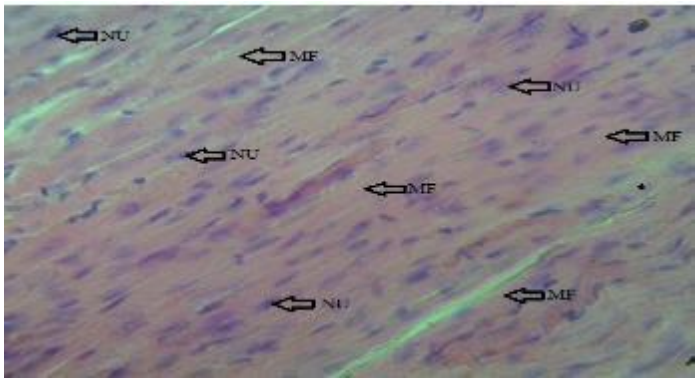

Figure 4 Heart section of rat showing cardiac myofibrils (MF), Capillaries and centrally placed nucleus (NU), H & E X400

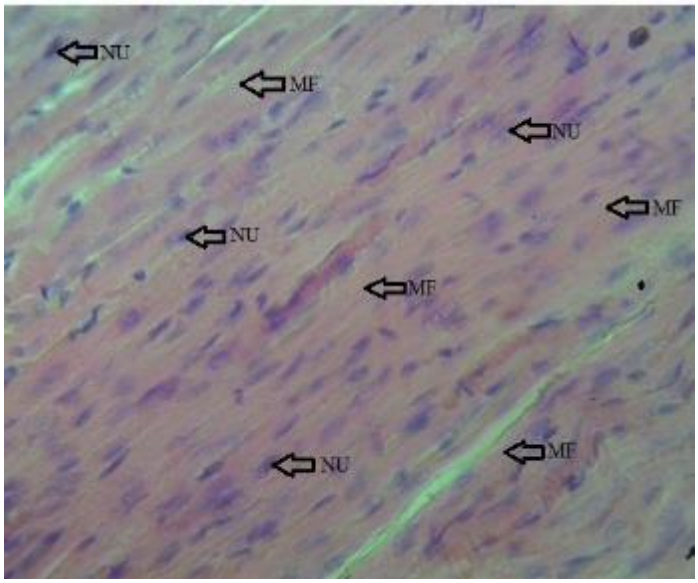

Figure 5 Heart section of rat showing normal heart muscle, centrally placed nucleus (NU). H & E X400

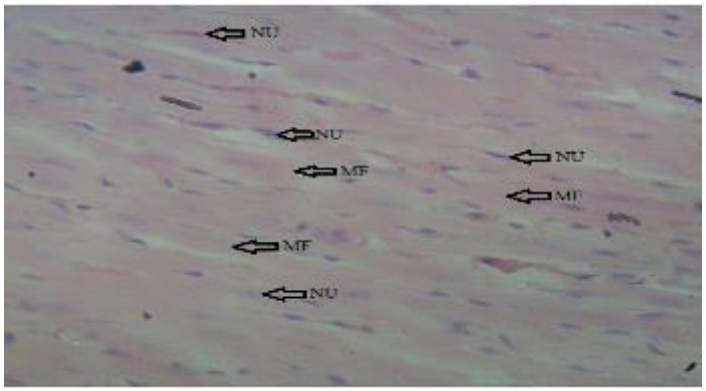

Figure 6 Heart section of rat showing cardiac myofibrils (MF), centrally placed nucleus (NU). H & E X400

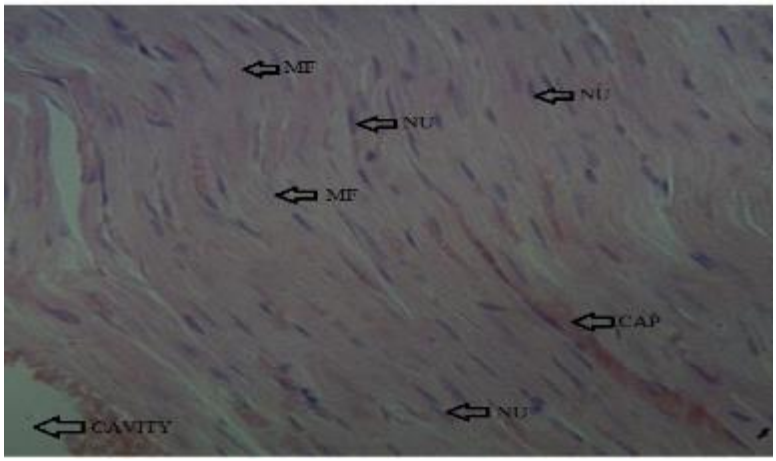

Figure 7 Heart section of rat showing cardiac myofibrils (MF), Blood vessels (BV), centrally placed nucleus (NU). H & E X400

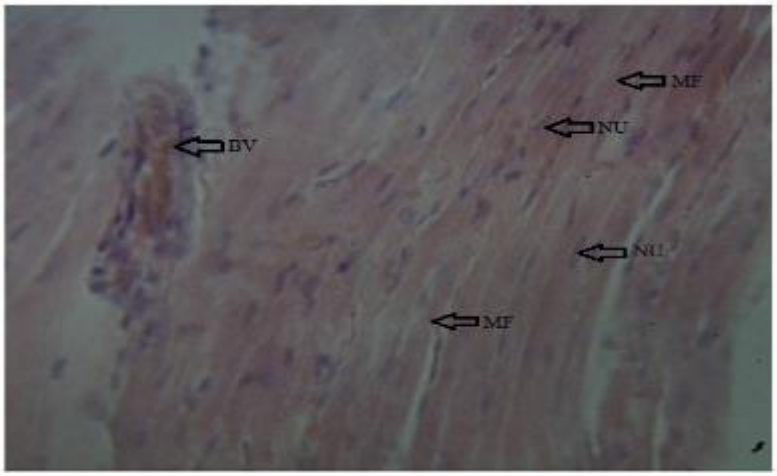

Figure 8 Heart section of rat showing cardiac myofibrils (MF), Blood vessels (BV), centrally placed nucleus (NU). H & E X400

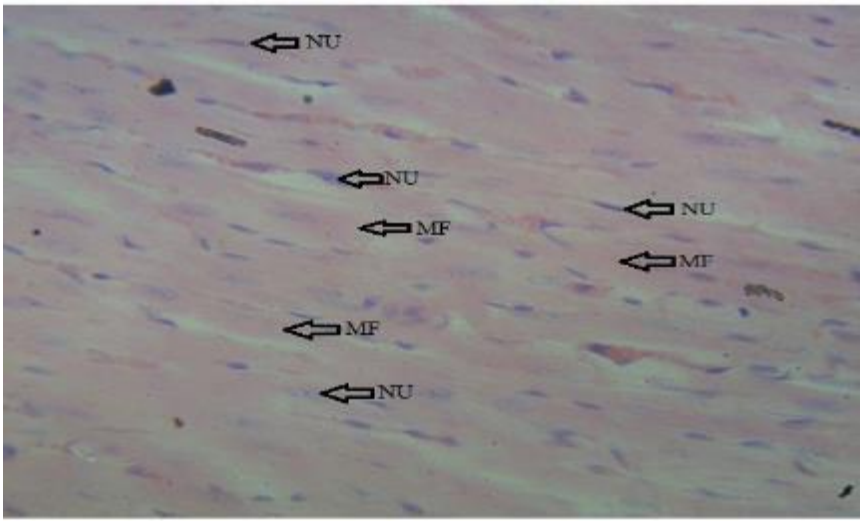

Figure 9 Heart section of rat showing cardiac myofibrils (MF), Blood vessels (BV), centrally placed nucleus (NU). H & E X400

4. Discussion

The principal finding in this study shows that Dox administration had effects on erythrocyte parameters, WBC and Platelet counts. Table I showed Dox administration reduced RBC, HGB, PCV and MCV. HGB concentration has been reported to be the most common haematological assessment method used to define anemia (Barale et al., 2020). Several diseases (such as diabetes, cancer, and neurodegenerative disorders) have been reported to affect the morpho-functional aspects of red blood cells, sometimes altering their normal metabolism (Cai *et al*., 2010). In addition to volatile substances in *alliums*, there are non-volatile sulfur-containing peptides and proteins which have been shown to have potential health benefits (Villela et al., 2009)). Pre-treatment with *A. cepa* as shown in Table I increased concentrations of RBC, HGB, PCV, MCV. This corroborates results by Meraiyetu et al., 2013 that juice extracts of A. cepa at various doses significantly increased the total white blood cell count, Lymphocyte count, Neutrophil count, also there was significant {$p<0.05$} increase in the red blood cell count of rats pretreated with *A. cepa*. *Allium* contains substances that has antibiotic effects and antibiotics enable the proliferation of circulating white blood cells considering that white blood cells function to protect the body from teratogens (Lucas et al., 2019). *Allium* species, have been implicated in the induction of haemolytic anaemia. They contain toxic components that may damage red blood cells and provoke haemolytic anaemia accompanied by Heinz bodies in erythrocytes of animals such as cattle, water buffalos, sheep, horses, dogs and cats (Yakout et al., 2021). *Allium cepa* juice has been found to exhibit antioxidant effect in alloxan

induced diabetic rats; it also repaired hepatic and renal damage caused by the administered alloxan (Saxena et al., 2013). Epidemiological, clinical and laboratory studies have demonstrated the role of onion in cancer prevention (Lucas et al., 2019) especially in relation to digestive tract cancers, including esophageal and stomach cancers (Meraiyebu et al., 2013). In the case of GI-related cancers, proposed mechanisms of action for *Allium* species include an inhibition of Helicobacter pylori and other bacterial activity, as well as a general decrease in the endogenous production of carcinogenic N-nitroso compounds (Aletan et al., 2019). The effect of *A. cepa* crude extract and its fractions on WBC level was reported in Table 2. Reports show that Dox treatment reduced white blood cell count but pre-treatment with Ethanol extract of A. cepa increased WBC, Neutrophil and Neutrophil. The count of white blood cells (WBC) has gained attention as an inexpensive test and various studies have consistently shown associations with increased risk of incident cardiovascular outcomes (Lassale et al.,2018). Also, n-hexane fraction significantly elevated WBC, Basophil and lymphocyte levels compared to the Dox treated group and significantly reduced levels of Neutrophil, Eosinophil, and monocyte. Ethyl acetate fraction significantly increased WBC, Neutrophil, Basophil, and lymphocyte levels compared to Dox treated group, whilst reducing levels of Eosinophil, and monocyte. Significantly elevated levels of WBC, Neutrophil, and lymphocyte were shown in the methanol fraction compared to Dox treated group however levels of Basophil and monocyte were reduced. Standard drug intervention group (Vit.E) compared to Dox treated group significantly elevated levels of WBC, Neutrophil, basophil and lymphocyte but significant reduction was shown in Eosinophil and monocyte levels. The combination group compared to the Dox treated group showed significantly elevated levels of WBC, Basophil, lymphocyte, and monocyte levels whilst significant reduction was shown in Neutrophil, and Eosinophil levels. All blood cells (white blood cells (WBC), red blood cells (RBC) and platelets) can play a role in atherosclerosis. Complete blood count (CBC) is widely available in clinical practice but utility as potential risk factors for cardiovascular disease (CVD) is uncertain (Lassele et al., 2018). Total and differential WBC count, MCV, RDW and platelet count likely play a role in the aetiology of CVD but only WBC provide a modest improvement for the prediction of 10-year CVD risk over traditional CVD risk factors in a general population (Lassele et al., 2018). Total WBC counts were related with the severity of coronary artery disease, and higher WBC counts increased the risk of CVDs in asymptomatic Koreans mainly by virtue of monocytes (Kim et al., 2017). Scientific reports by Lee et al., (2001), concluded that the WBC count is associated with several cardiovascular disease risk factors. Elevated WBC count **is** directly associated with an increased risk of coronary heart disease, ischemic stroke with mortality recorded from cardiovascular disease in African-American white men and women (Lee et al., 2001). A more recent study however encouraged medical center biobanks to collect, isolate and store circulating white blood cells as a rich source of biomarkers to facilitate the discovery of novel diagnostic tools for heart failure (Meier et al., 2021**).** The effect of *A. cepa* crude extract and its fractions on Platelet level was reported in Table **3.** There was no significant increase in the platelet count, monocyte count, eusonophil count, basophil count of the rats in all groups. Research study by Dahlen et al., (2021) emphasized the important value of Platelet and Platelet parameters in providing additional information on the pathophysiology and risk stratification in HF syndrome. Also, another research study by Barale et al., (2020), concluded that a number of clinical platelet markers are elevated in obese and type 2 diabetes (T2DM) patients, including the mean platelet volume, circulating levels of platelet microparticles, oxidation products, platelet-derived soluble P-selectin and CD40L, thus contributing to an intersection between obesity, inflammation, and thrombosis. The current study evaluated haematological parameters with particular focus on effects of treatment groups on serum erythrocytes, WBC and platelet count. Changes in their functionality have been reported to provide important information on disease severity and progression (Meier et al., 2021). Lower concentrations of HGB and anemia have been reported in several studies and an alteration in concentration of antioxidant enzymes has been shown to promote a hazardous state of oxidative stress in red blood cells. In accordance with findings in this study, Ding et al., (2019), reported haematological parameters valuable in distinguishing between acute myocardial infarction (AMI) and stable coronary artery disease (SCAD). Results from the present study suggest that fractions of *A. cepa* were shown to perform synergistic effects with the crude fraction of *A. cepa* except Methanol fraction of *A. cepa* that was shown to reduce erythrocyte count as shown in Table 1. Single treatment of Dox significantly reduced erythrocyte, WBC counts but pretreatment with *A. cepa* was shown to increase erythrocyte count, WBC count significantly, Also, results from the standard drug group (Vit.E) corroborated the effects of antioxidants being essential in ameliorating disease conditions. Our findings indicate that haematological parameters RBC, WBC, Platelets could be useful diagnostic tools in prediction and evaluation of Cardiotoxicity.

5. Conclusion

From the data presented in this study, haematological parameters have been shown to have significant effects in the diagnosis of Cardiotoxicity induced by doxorubicin in Wistar rats. It is strongly suggested that functional abnormalities in RBCs, WBCs and Platelet concentrations could be a useful diagnostic tool in the diagnosis of Cardiotoxicity also research studies on more purer forms of *A. cepa* extracts should be exploited. The study considers haematological parameters, as useful diagnostic tools in the assessment of Cardiotoxicity.

Compliance with ethical standards

Disclosure of conflict of interest

The Authors declare that they are responsible for the article's scientific content including the study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Statement of ethical approval

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national re-search committee and in line with guidelines for animal experimentation. The research design and protocol were approved by the institutional research ethics committee.

The research design and protocol were approved by the institutional research ethics committee. Ethical approval for the study was sort from the University of Uyo, Institutional Health Research Committee (IHERC), UU/CHS/IHERC/VOL.1/030.

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national re-search committee and with the 1964 Helsinki declaration and its later amendments or

Funding

The Authors did not receive any type of financial assistance that could be considered as a potential conflict of interest regarding the manuscript or its submission.

Data Availability

All data generated or analyzed during the study are included in this article.

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