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# Curative Effects of Aqueous and Ethanol Stem Bark Extracts of *Vitex doniana* on Doxorubicin-Induced Cardiotoxicity in Rats

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors MAS and AIH performed experiments, provided equipment and re-agents. Author MAS conducted the statistical analysis and wrote the manuscript. Authors DD and MSJ made manuscript revisions. All authors read and approved the final manuscript.*

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## ABSTRACT

**Background:** Cardiovascular diseases (CVDs) constitute the number one cause of mortality at the global level, representing 30% of all global deaths. Therefore, finding ways to reduce deaths due to CVDs remain an important public health goal. Traditional healers in northern Nigeria use the stem bark of *Vitex doniana* to treat hypertensive patients. This study was aimed to investigate the cardiocurative potential of *Vitex doniana* on doxorubicin-induced Cardiotoxicity in rats.
**Methods:** Thirty five (35) adult Albino rats weighing 175 ± 25 g were used, of which 30 were induced with cardiotoxicity by intraperitoneal injection of doxorubicin (10 mg/kg) for three consecutive days. Rats were treated by oral administration of Silymarin (100 mg/kg) and *Vitex doniana* aqueous or ethanol extract (100 mg/kg and 200 mg/kg) for 14 consecutive days and thereafter were sacrificed on the 15th day. Blood, plasma and serum were analyzed for lipid profile and serum markers for cardiotoxicity.

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**Results:** Phytochemical analysis of the extracts showed the presence of alkaloids, tannins, flavonoids, steroids, phenols, saponins, terpenoids and glycosides. Oral treatment with *Vitex doniana* extracts significantly ($p<0.05$) lowered the elevated levels of total cholesterol, triglycerides and LDL but significantly ($p<0.05$) increased the level of HDL (18.61 ± 0.55 mg/dl to 57.98 ± 0.78 mg/dl). The extracts also significantly ($p<0.05$) decreased the levels of serum marker enzymes for cardiotoxicity ALT, AST, CK – mb and LDH.

**Conclusion:** The prophylactic cardiocurative use of *Vitex doniana* stem bark has been confirmed in this study as the extracts exhibited hypolipidemic and cardiocurative effects in dose dependent manner in doxorubicin-induced cardiotoxicity rat model.

*Keywords: Cardiocurative; cardiotoxicity; Vitex doniana; doxorubicin; hypolipidemic.*

## ABBREVIATIONS

*ALT : Alanine transaminase.*
*AST : Aspartate transaminase.*
*CK-mb : Creatine kinase-mb.*
*CVDs : Cardiovascular diseases.*
*DNA : Deoxyribonucleic acid.*
*DOX : Doxorubicin.*
*GLP : Good laboratory practice.*
*HDL : High density lipoprotein.*
*LDH : Lactate dehydrogenase.*
*LDL : Low density lipoprotein.*
*ROS : Reactive oxygen species.*
*TC : Total cholesterol.*
*TG : Triglycerides.*

## 1. INTRODUCTION

Cardiotoxicity is defined by the National Cancer Institute of United States as the 'toxicity that affects the heart'. This definition includes a direct effect of drug on the heart but also an indirect effect due to enhancement of hemodynamic flow alterations or due to thrombotic events [1]. Cardiotoxicity includes a wide range of cardiac effects from small changes in blood pressure and arrhythmias to cardiomyopathy. It occurs during therapy with several cytotoxic drugs and may be the dose-limiting factor in cancer treatment. Cardiotoxicity can be acute, occurring during or within days of the drug infusion, or can occur years after chemotherapy [2]. It usually begins with myocyte injury, progresses to silent left ventricular systolic dysfunction, and eventually becomes symptomatic and irreversible. Cardiotoxicity can also be responsible for long-term side effects and may cause severe morbidity in surviving cancer patients [3]. Different mechanisms of chemotherapy-induced cardiotoxicity have been postulated, including cellular damage due to the formation of free oxygen radicals and the induction of immunogenic reactions with the presence of antigen presenting cells in the heart [4].

Doxorubicin (DOX) anthracycline is an anti-cancer drug used against solid tumors, leukemia, breast cancer, small cell carcinoma of lung and esophageal carcinoma [5]. However, it has specific toxicity to cardiac tissue [6]. The mechanisms proposed are that DOX bound with ferric ion induces the production of reactive oxygen species (ROS) that causes the impairment of cell function and cytolysis and also binds to glycoprotein to induce the production of caspase and apoptosome that cause deoxyribonucleic acid (DNA) damage [7,8]. DNA damage in proliferative cells activates a pathway that arrest cell division to allow either DNA repair or the induction of cell death by apoptosis [9]. Although research methodology and investigations have greatly improved over the years, the specific mechanism underlying DOX-induced cardiotoxicity remains unclear. The mechanisms responsible for DOX-induced cardiotoxicity appears to be multifactorial, involving increased lipid peroxidation, oxidative stress, DNA/RNA damage, inhibition of autophagy, endoplasmic reticulum mediated apoptosis, and disturbance of calcium homeostasis [10].

*Vitex doniana* sweet, (Verbanaceae) is a perennial shrub widely distributed in tropical West Africa, and some East African countries including Uganda, Kenya and Tanzania; and high rainfall areas. It is found in the middle belt of Nigeria particularly Kogi, Benue, and parts of the savannah regions of Kaduna, Sokoto and Kano states [11]. It is variously called dinya (Hausa), ngalbihi (Fulfulde) ori nla (Yoruba) ejiji (Igala) and ucha koro (Igbo) [12]. In Nigeria, from information available from the indigenous traditional healers, a decoction of the chopped stem bark of *Vitex doniana* is prepared and taken orally for treatment of gastroenteritis. It is administered for ailments including diarrhea and dysentery. It is also consumed to improve fertility and the juice may be squeezed into the eyes to treat eye troubles. It is also used in the treatment

of liver disease [13]. The anti-hypertensive effect of the stem bark of *Vitex doniana* has been reported. The extract exhibited a marked dose related hypotensive effect in both normotensive and hypertensive albino rats [14].

There is a large and increasing global burden of cardiovascular diseases (CVDs). CVDs constitute the number one cause of mortality at the global level, an estimated 17.9 million people died from CVDs in 2016, representing 31% of all global deaths, it was projected that about 23.6 million people will die from CVDs, mainly from heart disease and stroke by 2030 (WHO, 2017) [15]. Cardiocurative treatments are few and those that have been examined include renin angiotensin system blockade, beta-blockers, or the iron chelator, dexrazoxane. New treatments exploiting the ErbB or other novel pro-survival pathways, such as conditioning, are on the cardioprotection horizon. Even in the forthcoming era of targeted cancer therapies, the substantial proportion of today's anthracycline-treated cancer patients may become tomorrow's cardiac patients [16]. Therefore, finding ways to reduce morbidity and mortality due to CVDs remain an important public health goal. This study was aimed to investigate the cardiocurative potential of *Vitex doniana* as a preliminary basis for developing cheaper and readily available drugs for the treatment of CVDs.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Animals

Thirty five young Albino rats of both sexes were purchased from the National Veterinary Research Institute Vom, Plateau State, Nigeria with an initial mean body weight of 105.45 ± 10.74 g. The animals were housed and maintained in plastic laboratory rat cages in temperature and humidity controlled room (temperature: 25 ± 2°C, humidity: 60 ± 5%, 12-hour light/dark cycle). Moreover, all the animals were fed with a commercial rat diet (Vital Feeds, Jos, Nigeria) and drinking water *ad libitum,* were allowed to acclimatize for two weeks, and attained a weight of 175 ± 25 g before they were used for the experiment. A standard protocol according to the guidelines of the Good Laboratory Practice (GLP) regulations of world health organization (WHO) as well as the rules and regulations of experimental animal ethics committee of Modibbo Adama University of Technology (M. A. U. Tech), Yola were strictly followed.

### 2.2 Chemicals

Doxorubicin was purchased from Zuvius Life Sciences Pvt Ltd., (India). Silymarin was purchased from Micro Labs Ltd., (India), Creatine kinase (CK – mb), and Lactate dehydrogenase (LDH) kits were purchased from Fortress Diagnostics Ltd. (U.K). Total cholesterol (TC), Triglyceride (TG), High-density lipoprotein (HDL), Aspartate transaminase (AST) and Alanine aminotransferase (ALT) kits were purchased from Randox Laboratories (U.K). All other chemicals used were of analytical grade.

### 2.3 Preparation of Plant Sample

The matured stem bark of *Vitex doniana* was collected in the dry season from Yolde pate (9°12'0" N and 12°27'0" E) in Yola South Local Government area of Adamawa State. The stem bark was identified and authenticated at the Department of Plant Science M. A. U. Tech. Yola, Adamawa State, Nigeria. The stem bark of *Vitex doniana* was washed and air-dried under a shade for a period of two weeks after which they were cut into small pieces and pulverized into fine powder using an electric grinding machine.

### 2.4 Preparation of Aqueous Extract

Aqueous extract of the stem bark of *Vitex doniana* was prepared as described by Oluduro and Aderiye [17]. Exactly 100 g of the powdered stem bark of *Vitex doniana* was soaked in 600 ml distilled water at ambient temperature for three days and filtered using Whatman filter paper No. 1. The bulk filtrate was reduced in vacuum at 14˚C. The solid residue was stored at low temperature until it is needed.

### 2.5 Preparation of Ethanol Extract

The ethanol extract of the stem bark of *Vitex doniana* was prepared according to the method described by Chivapat et al., [18]. Exactly 100 g of the pulverized stem bark powder was macerated in 600 ml of 70 % ethanol at 40˚C for 48 hours. The extract solution was filtered using Whatman filter paper No.1 and evaporated using rotary evaporator under reduced pressure and then the concentrated extract was dried and stored at low temperature until it is required.

### 2.6 Qualitative Phytochemical Analysis

The aqueous and ethanol stem bark extracts of *Vitex doniana* were subjected to qualitative

analysis for various phytoconstituents by observing characteristics color change using standard procedures described by Trease and Evans and Sofowora [19, 20].

## 2.7 Animal Grouping and Induction of Cardiotoxicity

Animals were randomly divided into seven groups of five rats each, namely: Normal Control (NC), Cardiotoxic Control (CC), Cardiotoxic + 100 mg/kg BW of Silymarin (Micro Labs Ltd., India) (CSC), Cardiotoxic + low dose (100 mg/kg BW) of VDAE (CVAL), Cardiotoxic + high dose (200 mg/kg BW) of VDAE (CVAH), Cardiotoxic + low dose (100 mg/kg BW) of VDEE (CVEL), Cardiotoxic + high dose (200 mg/kg BW) of VDEE (CVEH). After two weeks acclimatization period, animals in CC, CSC, CVAL, CVAH, CVEL and CVEH groups were given intraperitoneal injection of 10 mg/kg BW doxorubicin (Zuvius Life Sciences Pvt, Ltd., India) for three consecutive days to induce cardiotoxicity. While animals in NC were only supplied with feed (Vital Feeds, Jos, Nigeria) and water only throughout the fourteen days experimental period.

At the end of the 14 days experimental period, animals were euthanized by chloroform anesthesia and the whole blood of each animal was collected via cardiac puncture and immediately preserved in a refrigerator until further processing. The blood samples were centrifuged at 3000 rpm for 15 minutes, serum and plasma was separated and preserved for further analysis. The serum, plasma and whole blood were used for various biochemical analysis. The biochemical parameters determined were alanine aminotransferase (ALT) and aspartate transaminase (AST), creatine kinase – mb (CK- mb), lactate dehydrogenase (LDH), total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL).

## 2.8 Determination of Alanine Aminotransferase (ALT)

Serum ALT was determined using colorimetric method described by Reitman and Frankel [21].

Into test tubes labelled Sample blank and Sample, 500 µl each of solution 1 was pipetted and into the test tube, labelled Sample, 100 µl of serum was added, mixed and incubated in water bath at 37°C for exactly 30 minutes. Then 500 µl of solution 2 was added to both test tubes. Exactly 100 µl of distilled water was then added to the test tube labelled Sample blank, mixed and allowed to stand for 20 minutes at 20 - 25°C and finally 5000 µl of Sodium hydroxide was added to both test tubes labelled mixed and poured into cuvette. The absorbance of the sample was measured against sample blank after 5 minutes. Enzyme activity and average absorbance difference per minute (∆Abs/min) was also calculated using the following formula:

ALT (IU/L) = ∆Abs/min x TV x 1000/18.75 x LP x SV

Where ∆Abs/min = Average absorbance change per minute

1000 = Conversion of IU/ml to IU/L
TV = 1.025 = Total reaction volume (ml)
18.75 = Millimolar absorptivity of ρ-Nitrophenol
SV = 0.025 = Sample volume (ml)
LP = 1 = Light path in cm.

## 2.9 Determination of Aspartate Transaminase (AST)

Serum AST was determined using colorimetric method described by Reitman and Frankel [21].

## 2.10 Determination of Creatine Kinase – mb

Creatine kinase was determined by enzymatic method as described by Bablok [22]. Creatine kinase was determined by pipetting into test tubes labelled macro, semi macro and micro, 2.5 ml, 1.0 ml and 0.5 ml of working reagent respectively. This was followed by addition of sample 100 µl, 40 µl and 20 µl to the test tubes labelled macro, semi macro and micro respectively. The test tubes were mixed and incubated for 3 minutes at 37°C. The initial absorbance was taken and repeated simultaneously after 1, 2 and 3 minutes.

Creatine kinase activity was calculated using the following formula

∆A 340nm/mm x 4130 – macro,
∆A 340nm/mm x 4130 – semi macro
∆A 340nm/mm x 4130 – micro.

## 2.11 Determination of Lactate Dehydrogenase (LDH)

Lactate dehydrogenase was determined by method described by Amador et al. [23].

Lactate dehydrogenase was determined by pipetting into a test tube labelled sample, 20 µl of sample serum and into the test tube labelled blank 1000 µl of distilled water. To the test tube labelled sample, 20 µl of serum was added and the test tubes were mixed and incubated at 37°C for 1 min and the initial absorbance was taken and subsequently taken after 1, 2 and 3 minutes.

Lactate dehydrogenase activity was calculated using

U/l = 8095 x ∆A 340nm/min.

### 2.12 Determination of Serum Total Cholesterol (TC)

Exactly 10 µl each of the sample and standard was pipetted into cuvettes containing 1,000 µl each of reagent 1. The contents was then mixed and incubated at 25˚C for 10 minutes in a water bath. The absorbance of the sample ($A_{sample}$) and the standard ($A_{standard}$) was then read at 546 nm against the reagent blank containing 10 µl of distilled water and 1,000 µl of reagent 1.

The concentration of cholesterol (in mmol/L) in the serum was calculated using the formula below:

Concentration of Cholesterol = $\frac{A_{sample}}{A_{standard}}$ × Concentration of standard

### 2.13 Determination of Serum Triglycerides (TG)

Serum triglycerides was determined by method described by Bucolo and David [24]. Exactly 10 µl each of the standard and sample was pipetted into test tubes containing 1,000 µl each of reagent 1 (Buffer). The contents was then mixed and incubated at 25˚C for 10 minutes in a water bath. The absorbance of the sample ($A_{sample}$) and the standard ($A_{standard}$) was read at 546 nm against the reagent blank containing 10 µl of distilled water and 1,000 µl of reagent 1.

The concentration of triglycerides (in mmol/L) in the serum was calculated using the formula below:

Concentration of Triglycerides = $\frac{A_{sample}}{A_{standard}}$ × Concentration of standard

### 2.14 Determination of High Density Lipoprotein Cholesterol (HDL - c)

Exactly 100 µl each of the standard and sample was pipetted into test tubes containing 1,000 µl each of reagent 1. The contents was then mixed and incubated at 25˚C for 10 minutes in a water bath. The absorbance of the sample ($A_{sample}$) and the standard ($A_{standard}$) was read at 546 nm against the reagent blank containing 100 µl of distilled water and 1,000 µl of reagent 1.

The concentration of HDL – c (in mmol/L) in the serum was calculated using the formula below:

Concentration of HDL-c = $\frac{A_{sample}}{A_{standard}}$ × Concentration of standard

### 2.15 Determination of Low Density Lipoprotein Cholesterol (LDL-c)

Low density lipoprotein cholesterol (LDL-c) was calculated using the formula described by Mendes de Cordova and Mendes de Cordova [25].

LDL-c = $\frac{3}{4}$ × (TC – HDL-c)

### 2.16 Statistical Analysis

The experimental results are expressed as mean ± SEM. The statistical analysis of data was done using one – way ANOVA (Analysis of variance) and the difference between two means determined using student 't' - test with level of statistical significance taken as $p<0.05$, with the aid of SPSS software, version 24. (SPSS Inc., Chicago, USA).

## 3. RESULTS

The qualitative phytochemical analysis of aqueous and ethanol stem bark extracts of *Vitex doniana* showed the presence of tannins, flavonoids, alkaloids, steroids and total phenols in both extracts. Additionally, the aqueous extract possessed saponins, terpenoids and glycosides, which were found to be absent in the ethanol extract (Table 1).

Animals induced with doxorubicin (CC, CSC, CVAL, CVAH, CVEL and CVEH groups) showed a significant ($P<0.05$) increase in serum levels of triglycerides, total cholesterol and LDL, with significant ($P<0.05$) but, decrease in level of HDL when compared to those in NC group. Oral

treatment with aqueous and ethanol stem bark extracts of *Vitex doniana* (100 mg/kg and 200 mg/kg BW) to doxorubicin induced cardiotoxicity rats (CVAL, CVAH, CVEL and CVEH groups) significantly (P<0.05) decreased the levels of total cholesterol, LDL and triglycerides and significantly (P<0.05) but, increased HDL level when compared with the CC group (Table 2) .

A significant (P < 0.05) increase in the activities of serum marker enzymes of cardiac function was observed in doxorubicin induced animals (CC, CSC, CVAL, CVAH, CVEL and CVEH groups) when compared with animals in NC group. However, oral treatment with *Vitex doniana* aqueous and ethanol stem bark extracts significantly (P < 0.05) decreased the levels of AST, ALT, CK and LDH compared to values of the untreated group (Table 3).

## 4. DISCUSSION

This study was able to detect some important phytochemical compounds in stem bark extracts of *Vitex doniana* that are of relevance in phytomedicine. They include alkaloids, flavonoids, phenols and tannins, which have biological and medicinal values such as anti–inflammatory, anti– diabetic, anti–microbial and anti–atherosclerotic properties [26]. Plants are generally well known to contain phytoconstituents some of which have been shown to be highly biologically active and as well as exhibiting physiological activity [27,28]. Saponins cause hypocholesterolemia by binding cholesterol, making it unavailable for absorption [29]. Flavonoids have been shown to exert potent antioxidant activity against the superoxide radical [30]. Its consumption has been documented not to be associated with mortality due to coronary heart disease. This may be as a result of its antioxidant activity and subsequent inhibitions of LDL oxidation known to have been attributed to the dietary and supplemental intake of flavonoids and other micronutrients. Tannins hasten the healing of wounds and inflamed mucous membrane [31]. The presence of these phytochemical thus supports the medicinal uses of *Vitex doniana*.

Doxorubicin induced mitochondrial injury is critical to the heart because it would presumably have extremely adverse effects on the contractile functioning of the cardiac myocyte by alterations in energy metabolism [32]. Increase in lipid profile like cholesterol, triglycerides, LDL, and decreased HDL in doxorubicin treated groups indicates that doxorubicin may be interfering with metabolism or biosynthesis of lipids. The daily oral administration of aqueous and ethanol stem bark extracts of *Vitex doniana* showed significant (p<0.05) reduction in total cholesterol, triglycerides, LDL, and significant (p<0.05) increase in HDL. The presence of saponins in the aqueous stem bark extract of *Vitex doniana* may explain the anti-hyperlipidemic effect observed in this study and this finding is in agreement with that of James and colleagues [13].

The lowering level of serum cholesterol using diet or drugs decreases the incidence of coronary heart disease [33,34]. Increased LDL with decreased HDL usually increases the serum total cholesterol; this is because the plasma clearance of cholesterol is often impaired in the presence of low HDL, triacylglycerol levels have also been found to increase with increase in plasma cholesterol. Atherogenicity therefore develops when LDL, triacylglycerol and total cholesterol are elevated relative to plasma HDL. Elevated HDL improves the transportation of cholesterol from the plasma to the liver for biotransformation and excretion, thereby preventing atheroma formation and blood vessel occlusion [35]. LDL on the other hand transports cholesterol to the arteries where they can be retained in arteria proteoglycans starting the formation of plaques, which possess a risk of cardiovascular disease. Thus, increase of LDL is associated with atherosclerosis, heart attack, stroke and peripheral vascular disease [36]. The importance of this LDL lowering effect observed in the present study is that the extracts may aid in the prevention or reduction of morbidity of cardiovascular diseases.

Cardiotoxicity induced by doxorubicin in rats was further indicated by elevated levels of serum biomarker enzymes such as AST, ALT, LDH and CK-mb. LDH is usually abundant in red blood cells and functions as a marker of hemolysis. LDH level is elevated due to tissue damage. AST is a biochemical marker used for diagnosis of acute myocardial infarction [37]. The increased levels of these marker enzymes in serum suggests an increased leakage of these enzymes from mitochondria as a result of toxicity induced by treatment with doxorubicin. These indices has been recently used in other studies to test for cardiotoxicity [38]. Daily oral administration of aqueous and ethanol stem bark extracts of *Vitex doniana* for 14 days significantly (P<0.05) lowered the release of AST, ALT LDH and CK-mb into serum.

**Table 1. Phytochemical composition of aqueous and ethanol stem bark extracts of *Vitex doniana***

| Phytochemical constituent | Aqueous | Ethanol |
|---|---|---|
| Alkaloids | + | + |
| Saponins | + | - |
| Tannins | + | + |
| Terpenoids | + | - |
| Flavonoids | + | + |
| Glycosides | + | - |
| Steroids | + | + |
| Phenols | + | + |

*Key: +: Present -: Absent*

**Table 2. Effects of aqueous and ethanol stem bark extracts of *Vitex doniana* on doxorubicin – induced alterations in serum lipid profile of rats**

| Treatment | LDL (mg/dl) | HDL (mg/dl) | Total Cholesterol (mg/dl) | Triglycerides (mg/dl) |
|---|---|---|---|---|
| NC | 54.99 ± 2.64 | 42.28 ± 1.05 | 118.72 ± 1.82 | 107.63 ± 3.08 |
| CC | 221.63 ± 3.77$^{a}$ | 18.61 ± 0.55$^{d}$ | 278.03 ± 3.28$^{a}$ | 188.95 ± 0.67$^{a}$ |
| CSC | 122.04 ± 1.36$^{ab}$ | 47.87 ± 1.31$^{a}$ | 199.32 ± 0.71$^{ab}$ | 147.03 ± 1.41$^{ab}$ |
| CVAL | 115.87 ± 1.92$^{abc}$ | 48.98 ± 0.35$^{a}$ | 195.08 ± 1.99$^{abc}$ | 151.18 ± 0.72ab |
| CVAH | 122.89 ± 1.96ab | 54.38 ± 0.73$^{a}$ | 209.84 ± 1.81$^{ab}$ | 162.87 ± 0.44$^{ab}$ |
| CVEL | 111.83 ± 2.24$^{abc}$ | 57.98 ± 0.78$^{a}$ | 204.55 ± 1.86$^{ab}$ | 173.74 ± 2.25$^{ab}$ |
| CVEH | 92.58 ± 20.36$^{abc}$ | 54.44 ± 0.57$^{a}$ | 203.91 ± 1.86$^{ab}$ | 174.44 ± 1.52$^{ab}$ |

*Values are expressed as mean ± SEM; (n = 5), a = significantly (P<0.05) higher compared to normal control, b = significantly (P<0.05) lower compared to negative control, c = significantly (P<0.05) lower compared to positive control, d = significantly (P<0.05) lower compared to normal control*

**Table 3. Effects of *Vitex doniana* stem bark extracts on levels of serum marker enzymes for cardiotoxicity**

| Treatment | AST (IU/L) | ALT (IU/L) | LDH (U/L) | CK – mb (U/L) |
|---|---|---|---|---|
| NC | 87.84 ± 0.97 | 62.62 ± 1.32 | 244.37 ± 1.55 | 87.28 ± 2.27 |
| CC | 250.23 ± 1.14$^{a}$ | 178.90 ± 2.41$^{a}$ | 591.54 ± 3.47$^{a}$ | 231.01 ± 0.64$^{a}$ |
| CSC | 92.13 ± 2.38$^{bd}$ | 66.50 ± 0.73$^{b}$ | 320.27 ± 0.71$^{ab}$ | 130.07 ± 0.53$^{ab}$ |
| CVAL | 96.59 ± 2.04$^{bd}$ | 98.36 ± 0.56$^{ab}$ | 342.15 ± 1.50$^{abc}$ | 89.62 ± 0.69$^{bc}$ |
| CVAH | 114.19 ± 5.38$^{b}$ | 71.26 ± 0.74$^{ab}$ | 479.54 ± 1.61$^{ab}$ | 92.62 ± 0.35$^{abc}$ |
| CVEL | 127.53 ± 0.93$^{ab}$ | 109.41 ± 0.62$^{ab}$ | 488.04 ± 2.00$^{ab}$ | 100.11 ± 0.52$^{abc}$ |
| CVEH | 125.22 ± 1.26$^{ab}$ | 82.48 ± 1.26$^{ab}$ | 488.76 ± 3.83$^{ab}$ | 108.53 ± 2.13$^{abc}$ |

*Values are expressed as mean ± SEM; (n = 5), a = significantly (P<0.05) higher compared to normal control, b = significantly (P<0.05) lower compared to negative control, c = significantly (P<0.05) lower compared to positive control, d = significantly (P<0.05) lower compared to normal control*

The results of this study showed that CK-mb and LDH activities, the most specific and highly sensitive markers for myocardial cell damage [39] were extremely elevated in the untreated doxorubicin – induced cardiotoxicity group indicating severely damaged heart tissue. Serum Creatine kinase activity is a more sensitive indicator of early stage myocardial ischemia whereas, LDH levels give a rough estimate of the extent of injury to myocardial tissues. The levels of these cellular enzymes present in blood are directly related to the intactness of the plasma membrane of the cardiac cells, therefore, the inhibition of doxorubicin-induced elevation and dose-dependent reduction in serum levels of CK-mb and LDH by *Vitex doniana* stem bark extracts could be due to their action on maintaining cardiac membrane integrity and restricting the leakage of these enzymes [40]. The results of this study are consistent with those of Abd El-Gawad and El-Sawalhi and Emam et al. [41,42]. However, significant ($p<0.05$) decrease of CK-mb and LDH levels by aqueous and ethanol stem bark extracts of *Vitex doniana* confirms the cardiocurative effects of the stem bark of *Vitex doniana*.

## 5. CONCLUSION

The prophylactic cardiocurative use of *Vitex doniana* stem bark has been confirmed as the extracts exhibit hypolipidemic and cardiocurative effects on doxorubicin-induced rats. The present study demonstrates the curative effect of *Vitex doniana* against doxorubicin-induced cardiotoxicity and lends credence to the ethno pharmacological use of the plant in the treatment of cardiovascular related diseases and disorders. The observed cardiocurative potential of the plant might be due to its anti - hyperlipidemic activities. The findings of this research agree with the current use of *Vitex doniana* extract by folk medicine practitioners as antihypertensive agent and these findings may be useful to scientists and patents in the field of pharmacology to develop evidence-based alternative medicine in the treatment of cardiovascular related diseases and disorders.

## DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no competing interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. The research was also not funded by the producing company rather, it was funded by personal efforts of the authors.

## AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## CONSENT

It is not applicable.

## ETHICS APPROVAL

The animal study was conducted in strict compliance with the Animal Research Ethical Committee guide of the Modibbo Adama University of Technology Yola, Nigeria. Ethical clearance for the use of experimental animals for all procedures was obtained from the Ethics Committee, M. A. U. Tech. Yola, Nigeria.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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