NJBMB (2023) 38(1) 33-42

Nigerian Journal of Biochemistry and Molecular Biology

The Official Publication of the Nigerian Society of Biochemistry & Molecular Biology (NSBMB). Journal homepage: https://www.nsbmb.org.ng/journals

e-ISSN: 2659-0042

Research Article

Hypolipidemic effect of *Clerodendrum violaceum* methanol leaf extract in mice

Ahmed H. Zailani[1,*], Elizabeth A. Balogun[2], Joseph O. Adebayo[2]

[1]*Department of Biochemistry, Modibbo Adama University, Yola, Adamawa State, Nigeria*
[2]*Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria*

OPEN ACCESS

CORRESPONDENCE
Zailani, A.H.
howwrkoulou@yahoo.com
+234-803-493-9586

ARTICLE HISTORY
Received: 06/06/2022
Reviewed: 17/12/2022
Revised: 17/01/2023
Accepted: 26/01/2023
Published: 30/03/2023

CITATION
Zailani A.H., Balogun E.A. and Adebayo J.O. (2023). Hypolipidemic effect of *Clerodendrum violaceum* methanol leaf extract in mice. *Nigerian Journal of Biochemistry and Molecular Biology*. 38(1), 33-42

ABSTRACT

Effects of methanol leaf extract of *Clerodendrum violaceum* on selected indices of cardiovascular disease in mice were evaluated. Six groups of ten mice each were used. Groups B-F received orally 31.25, 62.5, 125, 250 and 500 mg/kg body weight of methanol leaf extract of *Clerodendrum violaceum* respectively while group A received 5% DMSO (control). Half of the animals in each group were sacrificed after 14 days of administration while the remaining half were sacrificed after 28 days of administration. Selected indices of cardiovascular disease were then determined. At the end of 14 days, serum concentrations of total cholesterol, triglyceride and LDL-c, with the atherogenic index were all significantly decreased ($p<0.05$) at higher doses compared to control; however, serum HDL-c concentration was significantly increased ($p<0.05$). A similar pattern was observed for these parameters after 28 days of administration. There was significant decrease ($p<0.05$) in AST activity at the highest dose of the extract in heart and serum and significant increase ($p<0.05$) in serum AST at other doses on days 14 and 28. Creatine kinase activity was significantly increased ($p<0.05$) at all doses of the extract in heart and serum on day 14 compared to control but was reversed back to the range of control on day 28. The extract at all doses did not cause any significant change ($p>0.05$) in serum lipoprotein (a) concentration on days 14 and 28 compared to controls. The results suggest that methanol leaf extract of *Clerodendrum violaceum* may not predispose subjects to cardiovascular diseases.

Keywords: *Clerodendrum violaceum, Cardiovascular, Risk factors, Leaf extract*

INTRODUCTION

Cardiovascular disease (CVD) is a general term for a group of disorders that lead to conditions affecting the heart and/or blood vessels. These include peripheral arterial disease, stroke, atherosclerosis, hypertension, coronary heart disease (CHD), cerebrovascular disease and rheumatic cardiac disease. It is one of the most prominent groups of non-communicable diseases (NCDs), accounting for the most NCD deaths in the world (Razmpoosh *et al*., 2022). Annual deaths have been reported to reach about 17.9 million people worldwide, primarily due to CVDs including cerebrovascular disease and CHD (Tsao *et al*., 2022). Leading risk factors for CVDs include high blood pressure, diabetes, high alcohol use, smoking and secondhand smoke exposure, obesity, unhealthy diet, physical inactivity, and high low-density lipoprotein cholesterol (LDL-c) (Roth *et al*., 2020). Elevated LDL-c has persisted as a leading modifiable risk factor and is one of the most closely linked markers of atherosclerotic CVD. In 2021, 3.81 million cardiovascular deaths and 3.81 million deaths overall were attributed to elevated LDL-c levels worldwide (Zhang *et al*., 2021). High LDL cholesterol can double the risk of heart disease because it can cause a buildup in the walls of arteries and limit blood flow to the heart, brain, kidneys, and other organs. The levels of plasma lipids including cholesterol, triglycerides, LDL-c, and HDL-c have been listed as part of the modifiable risk factors for cardiovascular diseases and maintaining them within normal limits is one of the strategies for the control of the progressivity of

cardiovascular diseases; thus, they serve as useful indices to assess cardiovascular disease risk (Vaduganathan *et al*., 2022).

Plant-derived medicines are used in most parts of the world and have always been an important part of health care systems worldwide. This is especially true in most developing countries including Africa where herbal treatment forms a part of the culture and is sometimes the only available source of healing and therapy (Porwal *et al*., 2017). The development of resistance against synthetic drugs, cost and their unavailability have also made the use of medicinal plants popular. One of such plants used in traditional herbal treatment of some ailments is *Clerodendrum violaceum*. *Clerodendrum violaceum* (Verbenaceae) is commonly called Clerodendrum in English and "Ewe isedun" in Yoruba (Nigeria); a decoction of the leaves is used in the traditional treatment of fever/malaria. *Clerodendrum* species generally contain similar phytochemical components (Shrivastava and Patel, 2007) and have been reported to have hepatoprotective effects (Remya *et al*., 2021) and anti-inflammatory activity (Tiwari *et al*., 2021). They have also been shown to inhibit key enzymes linked to type 2 diabetes (Erukainure *et al*., 2018) and exhibit significant anticancer activity against breast carcinoma (Shendge *et al*., 2021). The acclaimed antimalarial activity of the leaf extract of *Clerodendrum violaceum* in folk medicine has been authenticated in our previous studies (Balogun *et al*., 2010; Adebayo *et al*., 2022).We also reported the contribution of its antioxidant activity in augmenting the antimalarial activity (Balogun *et al*., 2014). Although medicinal plants play a critical role in the maintenance of health worldwide and some of them have been shown to be effective, they may elicit adverse side-effects (Nath and Yadav, 2015). Therefore, evaluating the effects of any medicinal plant extract is an important step to determine its suitability for use in complementary and alternative medicine. The present study, therefore, was set out to evaluate the effects of methanol leaf extract of *Clerodendrum violaceum* on selected cardiovascular disease indices in mice.

MATERIALS AND METHODS

Chemicals and reagents

Methanol was obtained from British Drug House Laboratory Supplies, Poole Dorset BH15 UK. Assay kits for enzyme assays were obtained from Randox Laboratories Ltd., Co. Antrim, U.K. All other reagents used were of analytical grade and were prepared in all glass distilled water.

Animals

Adult Swiss albino mice with an average weight of 20 ± 2.0 g were obtained from the animal breeding unit of the Department of Biochemistry, University of Jos, Plateau State. The mice were maintained at room temperature and 12 h light/dark cycle with free access to mouse pellets (Vital Feed; Grand Cereals, KM 17, Zawan Round About. P.O. Box13462, Jos, Plateau State) and portable water ad libitum. The research adhered to the Principles of Laboratory Animal Care (NIH publication #85−23, revised in 1985).

Plant material

Fresh leaf samples of *Clerodendrum violaceum* were collected in Oyo town, Oyo State, Nigeria and botanically authenticated at Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. A specimen with voucher number FHI 109857 was deposited.

Plant extract preparation

Fresh leaf samples of the plant were dried under shade at room temperature (25±2 °C) and pulverized to powder using an electric blender (Mazeda Mill, MT 4100, Japan). Four hundred and fifty gram (450 g) of the powder was exhaustively extracted with 4 L n-hexane, 4 L ethyl acetate and 4 L absolute methanol for 72 h each successively. The extracts were filtered using Whatman filter paper No 1 and concentrated after each extraction period under pressure at 45˚C using a rotary evaporator (Scilogex SCI 100-s 5L, 1275 Cromwell Avenue, C-6 Rocky Hill, Hampshire, USA). The concentrates were then allowed to evaporate at room temperature to dryness (Adebayo *et al*., 2003). Only the methanol extract was used in this study because it was found to have the highest antioxidant activity (Balogun *et al*., 2014) and the best antimalarial activity (Adebayo *et al*., 2022).

Experimental design

Sixty Swiss laboratory mice were randomly divided into six groups (A-F) of ten mice each and given 0.2 ml of the methanol extract of *Clerodendrum violaceum* leaf orally as follows:

Animals in group A were administered 5% DMSO and served as control while those in groups B, C, D, E and F were administered 31.25, 62.5, 125, 250 and 500 mg/kg body weight of the methanol leaf extract of *Clerodendrum violaceum*, respectively. After fourteen days of administration, five animals from each group were sacrificed and blood and heart were collected for analysis. Extract administration continued for another fourteen days and thereafter, the remaining animals in all the groups were sacrificed and treated similarly to the first half.

Collection and preparation of samples

The mice were sacrificed after they were slightly anesthetized with diethyl ether. Blood was collected by cardiac puncture from the unconscious mice into clean, dry test tubes. This was allowed to stand for fifteen minutes at room temperature in order to clot. It was then centrifuged at 1000 rpm (Gallenkamp Centrifuge 200) for fifteen minutes and the clear serum supernatant was carefully collected using a Pasteur pipette. The animals were then dissected and the hearts were removed, cleaned of blood, weighed and then homogenized separately in ice-cold 0.25 M sucrose solution (1:5 w/v). The homogenates were stored frozen overnight before centrifuging at 1000 rpm for fifteen minutes. The supernatants obtained after centrifuging were used for the analyses. Some of the weighed heart tissues were also transferred into specimen bottles containing 10% formalin for histopathological examination.

Organ-body weight ratio

The organ-body weight ratio was calculated using the formula:

$$\text{Organ} - \text{body weight ratio} = \frac{\text{Weight of the organ}}{\text{Weight of the animal}}$$

Analysis of biochemical parameters

To determine the serum concentrations of total cholesterol and high density lipoprotein-cholesterol (HDL-c), the methods of Allain *et al*. (1974) and Bachorik *et al*. (1980) were used respectively while low density lipoprotein-cholesterol (LDL-c) concentration was determined by the method of Friedwald *et al*. (l972). The method of Jacobs and Van-Demark (1960) was used to determine the triglyceride concentration. For the concentration of lipoprotein a, the method of Marcovina *et al*. (1996) was used. Protein concentrations in serum and heart homogenate were determined using the Biuret method as reported by Gornall *et al*. (1949). Alkaline phosphatase (ALP) activity was determined as described by Wright *et al*. (1972) while the activities of alanine and aspartate aminotransferases (ALT and AST) were assayed by the method of Reitman and Frankel (1957). The method of Di Witt and Trandelenburg (1982) was used to determine the activity of creatine kinase (CK). To assess the histopathological effect(s) of the methanol leaf extract of *Clerodendrum violaceum* on the heart of mice, the method described by Krause (2001) was used.

Statistical analysis

The group means ± SD for each parameter was calculated and significant differences were determined by Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at 95% confidence level using SPSS-PC programme package (Version 24.0, SPSS Inc. Chicago).

RESULTS AND DISCUSSION

Organ-body weight ratio

The effects of methanol leaf extract of *Clerodendrum violaceum* on organ-body weight ratios of mice after 14 and 28 days of extract administration are shown in Table 1. There was no significant alteration ($p>0.05$) in the organ-body weight ratios at all doses administered throughout the study period.

Table 1. Effects of Methanol Leaf Extract of *Clerodendrum violaceum* on Organ-Body Weight Ratios of Heart after 14 and 28 days of Administration

Treatment	Day 14 ($x10^{-3}$)	Day 28 ($x10^{-3}$)
Control	2.6 ± 0.05^{a}	3.0 ± 0.05^{a}
31.25 mg/kg b.wt	2.8 ± 0.07^{a}	2.8 ± 0.02^{a}
62.5 mg/kg b.wt	2.9 ± 0.05^{a}	2.5 ± 0.04^{a}
125 mg/kg b.wt	3.3 ± 0.06^{a}	2.7 ± 0.07^{a}
250 mg/kg b.wt	3.5 ± 0.05^{a}	2.9 ± 0.06^{a}
500 mg/kg b.wt	3.7 ± 0.02^{a}	2.3 ± 0.04^{a}

Values are means of 5 replicates ± SD. Means in the same column with different superscripts are significantly different ($p<0.05$).

Cardiovascular disease indices

Extract administration for 14 days did not cause significant alterations ($p>0.05$) in all the serum lipid parameters except at the doses of 250 and 500 mg/kg body weight which significantly reduced ($p<0.05$) concentration of total cholesterol, triglycerides, and low density lipoproteins cholesterol and the atherogenic index and significantly increased ($p<0.05$) high density lipoprotein cholesterol concentration compared to controls (Table 2).

After 28 days of extract administration, there was significant decrease in the concentrations of total cholesterol, triglycerides, low density lipoprotein cholesterol and the computed atherogenic index ($p<0.05$) at all doses of the extract. On the other hand, a significant increase ($p<0.05$) in the concentration of high density lipoprotein cholesterol was observed at all doses compared to control (Table 3)

Table 4 shows the effects of methanol leaf extract of *Clerodendrum violaceum* on serum lipoprotein (a) [Lp(a)] concentration in mice after 14 and 28 days of administration. Extract administration did not significantly alter ($p>0.05$) the concentration of serum lipoprotein (a) at all doses throughout the study period compared to control.

Table 2. Effects of Methanol Leaf Extract of *Clerodendrum violaceum* on Serum Lipid Profile of Mice after 14 days of Administration

Treatment	Total cholesterol (mmol/L)	Triglycerides (mmol/L)	HDLc (mmol/L)	LDLc (mmol/L)	Atherogenic index
Control	4.40 ± 0.69^{a}	2.53 ± 4.03^{a}	1.88 ± 0.09^{a}	1.37 ± 0.59^{a}	2.34 ± 1.54^{a}
31.25 mg/kg b.wt	4.37 ± 1.17^{a}	2.50 ± 1.84^{a}	1.86 ± 0.05^{a}	1.36 ± 0.08^{a}	2.35 ± 0.23^{a}
62.5 mg/kg b.wt	4.35 ± 1.26^{a}	2.51 ± 0.54^{a}	1.87 ± 0.07^{a}	1.34 ± 0.61^{a}	2.33 ± 0.14^{a}
125 mg/kg b.wt	4.36 ± 1.15^{a}	2.49 ± 1.49^{a}	1.85 ± 0.08^{a}	1.35 ± 0.65^{a}	2.35 ± 0.16^{a}
250 mg/kg b.wt	3.70 ± 0.76^{b}	0.98 ± 3.66^{b}	2.73 ± 0.04^{b}	0.53 ± 0.03^{b}	1.36 ± 0.08^{b}
500 mg/kg b.wt	3.55 ± 2.41^{c}	0.74 ± 0.49^{b}	2.81 ± 0.08^{b}	0.41 ± 0.09^{b}	1.27 ± 0.04^{b}

Values are means of 5 replicates ±SD. Means in the same column with different superscripts are significantly different (p<0.05). High density lipoprotein cholesterol: HDLc, Low density lipoprotein cholesterol: LDLc

Table 3. Effects of Methanol Leaf Extract of *Clerodendrum violaceum* on Serum Lipid Profile of Mice after 28 Days of Administration

Treatment	Total cholesterol (mmol/L)	Triglycerides (mmol/L)	HDLc (mmol/L)	LDLc (mmol/L)	Atherogenic index
Control	4.99 ± 0.66^{a}	2.95 ± 1.16^{a}	2.04 ± 0.11^{a}	1.61 ± 0.57^{a}	2.45 ± 1.23^{a}
31.25 mg/kg b.wt	3.73 ± 2.02^{b}	1.48 ± 0.12^{b}	2.25 ± 0.06^{b}	0.81 ± 0.03^{b}	1.66 ± 0.97^{b}
62.5 mg/kg b.wt	3.64 ± 1.39^{b}	1.29 ± 0.05^{b}	2.35 ± 0.16^{b}	0.70 ± 0.04^{b}	1.55 ± 1.09^{b}
125 mg/kg b.wt	3.61 ± 2.69^{bc}	1.03 ± 0.07^{b}	2.58 ± 0.06^{b}	0.56 ± 0.04^{b}	1.40 ± 0.67^{c}
250 mg/kg b.wt	3.59 ± 1.76^{c}	0.97 ± 0.02^{b}	2.62 ± 0.07^{b}	0.53 ± 0.05^{b}	1.37 ± 0.98^{c}
500 mg/kg b.wt	3.49 ± 1.27^{c}	0.85 ± 0.06^{b}	2.64 ± 0.09^{b}	0.46 ± 0.06^{b}	1.32 ± 0.07^{c}

Values are means of 5 replicates ±SD. Means in the same column with different superscripts are significantly different (p<0.05). High density lipoprotein cholesterol: HDLc, Low density lipoprotein cholesterol: LDLc

Table 4. Effects of Methanol Leaf Extract of *Clerodendrum violaceum* on Serum Lipoprotein (a) Concentration in Mice after 14 and 28 days of Administration

Treatment	Day 14 (mg/dL)	Day 28 (mg/dL)
Control	1.02 ± 0.09^{a}	1.38 ± 0.22^{a}
31.25 mg/kg b.wt	1.03 ± 0.31^{a}	1.40 ± 0.20^{a}
62.5 mg/kg b.wt	1.03 ± 0.46^{a}	1.42 ± 0.28^{a}
125 mg/kg b.wt	1.05 ± 0.19^{a}	1.41 ± 0.49^{a}
250 mg/kg b.wt	1.06 ± 0.64^{a}	1.42 ± 0.32^{a}
500 mg/kg b.wt	1.07 ± 0.41^{a}	1.44 ± 0.25^{a}

Values are means of 5 replicates ±SD. Means in the same column with the same superscripts are not significantly different (p>0.05).

Cellular enzymes

Alkaline phosphatase

There was a dose-dependent significant increase (p<0.05) in ALP activity in the serum with significant increase (p<0.05) in heart ALP activity only at the doses of 250 and 500 mg/kg body weight compared to controls after 14 days of extract administration (Figure 1).

After 28 days of extract administration, there was a dose-dependent significant increase (p<0.05) in ALP activity in serum compared to control (Figure 2). However, there was no significant alteration (p>0.05) in ALP activity in the heart of mice compared to control (Figure 2).

Alanine aminotransferase

There was significant increase (p<0.05) in ALT activity in the heart and serum at the doses of 62.5, 125 and 250 mg/kg body weight while the least dose significantly increased (p<0.05) only heart ALT activity compared to controls after 14 days of extract administration (Figure 3). There was however, significant decrease (p<0.05) in ALT activity in the heart and serum at the dose of 500 mg/kg body weight compared to controls (Figure 3).

After 28 days of extract administration, there was significant increase (p<0.05) in ALT activities in the serum at all doses compared to control (Figure 4). There was a significant decrease (p<0.05) in the activity of the enzyme in the heart at 500 mg/kg body weight with no significant change (p>0.05) at other doses compared to control.

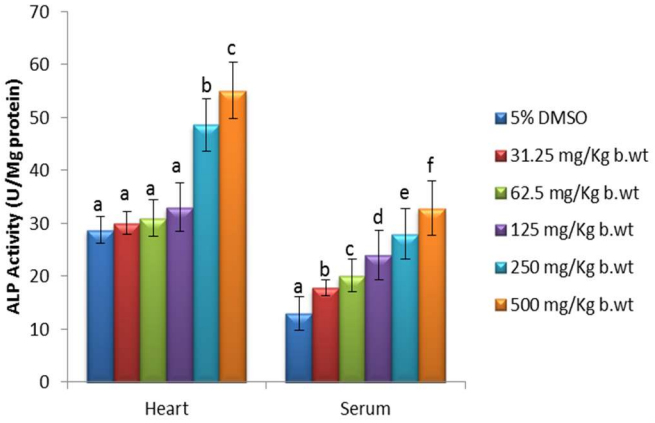

Figure 1. Effects of Methanolic Extract of *Clerodendrum violaceum* Leaf on Alkaline Phosphatase Activities in Serum and Heart of Mice after 14 days of Administration

Values are means of 5 replicates ± SD. Bars for heart/serum with different letters are significantly different (p<0.05).

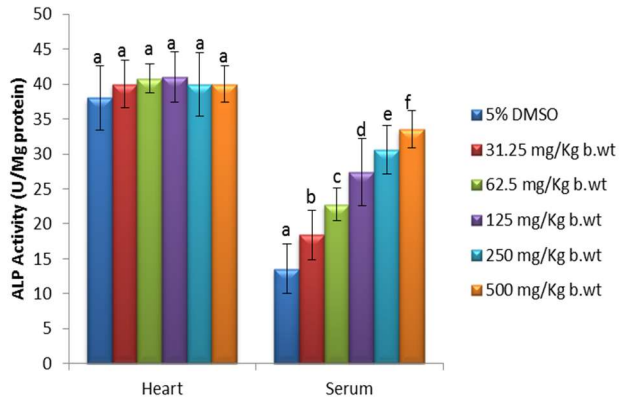

Figure 2. Effects of Methanolic Extract of *Clerodendrum violaceum* Leaf on Alkaline Phosphatase Activities in Serum and Heart of Mice after 28 days of Administration

Values are means of 5 replicates ± SD. Bars for heart/serum with different letters are significantly different ($p<0.05$).

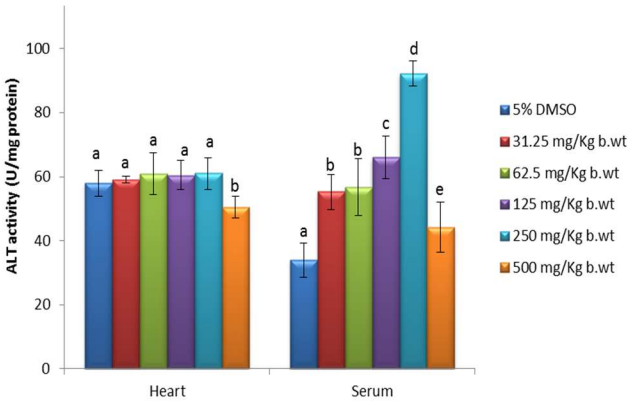

Figure 4: Effects of Methanolic Extract of *Clerodendrum violaceum* Leaf on Alanine Aminotransferase Activities in Serum and Heart of Mice after 28 days of Administration

Values are means of 5 replicates ± SD. Bars for heart/serum with different letters are significantly different ($p<0.05$).

Aspartate aminotransferase

There was significant decrease ($p<0.05$) in AST activity at 500 mg/kg body weight in the heart and serum compared to controls after 14 days of administration of extract (Figure 5). There was however, a significant increase ($p<0.05$) in AST activity in the serum, with no significant change ($p>0.05$) in heart AST activity at the other doses compared to controls after 14 days of extract administration (Figure 5).

After 28 days of administration, there was a significant decrease ($p<0.05$) in AST activity at the dose of 500 mg/kg in the serum and heart compared to controls. Its activity in the serum was significantly increased ($p<0.05$) with no significant change ($p>0.05$) in heart AST activity at the other doses compared to control (Figure 6).

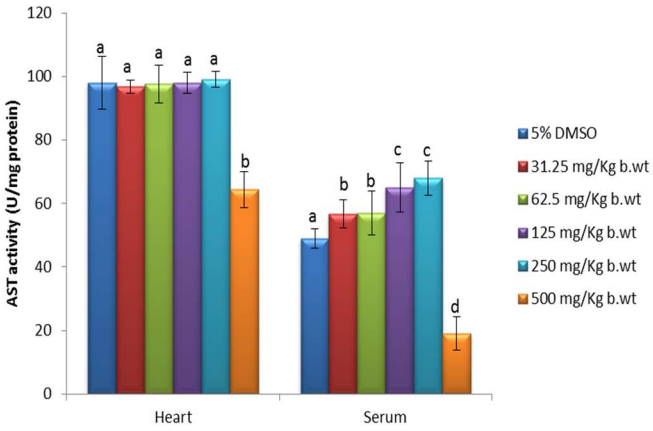

Figure 5. Effects of Methanolic Extract of *Clerodendrum violaceum* Leaf on Serum and Heart Aspartate Aminotransferase Activities in Mice after 14 days of Administration

Values are means of 5 replicates ± SD. Bars for heart/serum with different letters are significantly different ($p<0.05$).

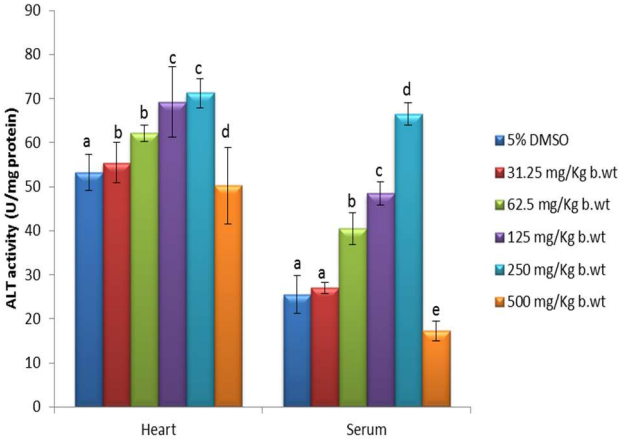

Figure 3. Effects of Methanolic Extract of *Clerodendrum violaceum* Leaf on Alanine Aminotransferase Activities in Serum and Heart of Mice after 14 days of Administration

Values are means of 5 replicates ± SD. Bars for heart/serum with different letters are significantly different ($p<0.05$).

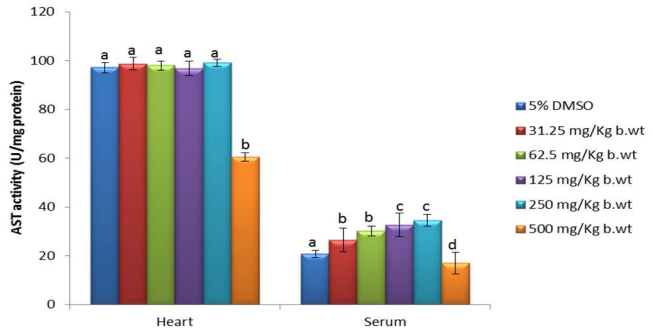

Figure 6. Effects of Methanolic Extract of *Clerodendrum violaceum* Leaf on Serum and Heart Aspartate Aminotransferase Activities in Mice after 28 days of Administration

Values are means of 5 replicates ± SD. Bars for heart/serum with different letters are significantly different ($p<0.05$).

Creatine kinase

Extract administration for 14 days caused a significant increase ($p<0.05$) in creatine kinase activity at all doses in the serum and heart compared to control (Figure 7).

After 28 days of extract administration, the activity of the enzyme was not significantly altered ($p>0.05$) at all doses in the serum and heart compared to controls (Figure 8).

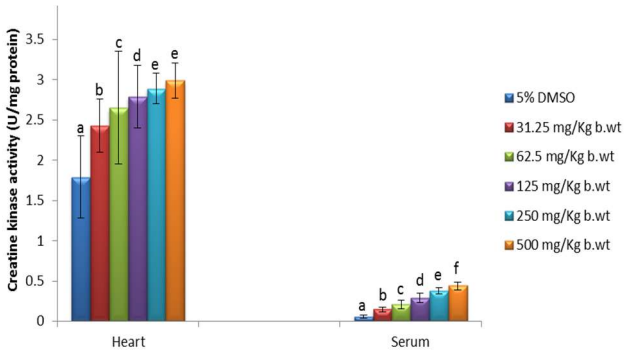

Figure 7. Effects of Methanolic Extract of *Clerodendrum violaceum* Leaf on Serum and Heart Creatine Kinase Activities in Mice after 14 days of Administration

Values are means of 5 replicates ± SD. Bars for serum/heart with different letters are significantly different ($p<0.05$).

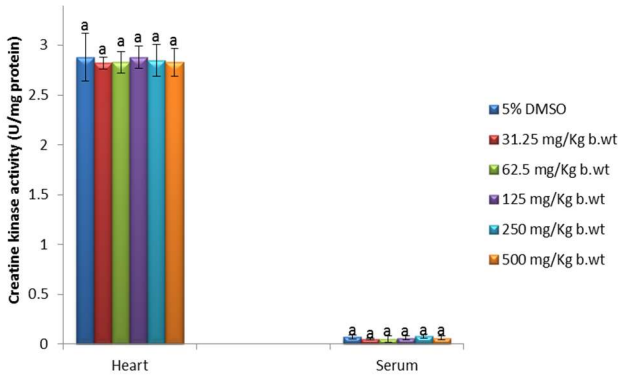

Figure 8. Effects of Methanolic Extract of *Clerodendrum violaceum* Leaf on Serum and Heart Creatine Kinase Activities in Mice after 28 days of Administration

Values are means of 5 replicates ± SD. Bars for heart/serum with the same letters are not significantly different ($p>0.05$).

Histopathological studies

Histopathological investigation of the heart was done for all experimental groups on days 14 and 28 after extract administration. There was no difference in the normal architecture of the heart throughout the experimental period (Plates 1 and 2).

Discussion

The ratio of the organ weight to body weight is an important endpoint for identification of potentially harmful effects of drugs/chemicals (Bailey *et al.*, 2004); organ weight changes are accepted as a sensitive indicator of chemically induced effects on organs and are commonly assessed (Lazic *et al.*, 2020). The absence of any significant alteration in the heart-body weight ratios of the mice suggests that the extract at the doses used in this study did not adversely affect the size of the heart in relation to the weight of the animals (Table 1).

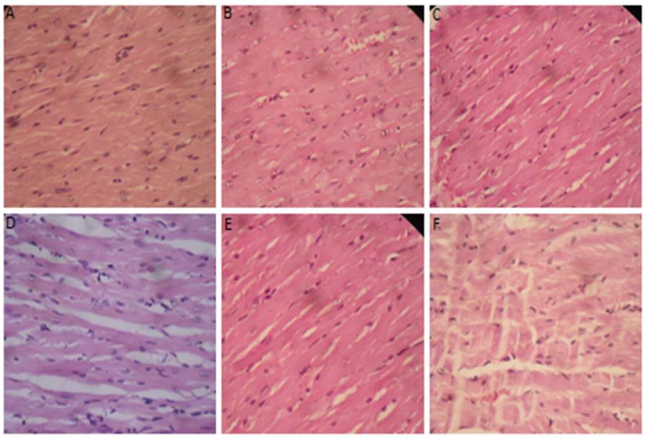

Plate 1. Photomicrographs of the Hearts of Mice Administered Various Doses of Methanol Leaf Extract of *Clerodendrum violaceum* for 14 days. A, B, C, D, E and F: Control, 31.25, 62.5, 125, 250 and 500 mg/kg b.wt respectively (H and E x 400).

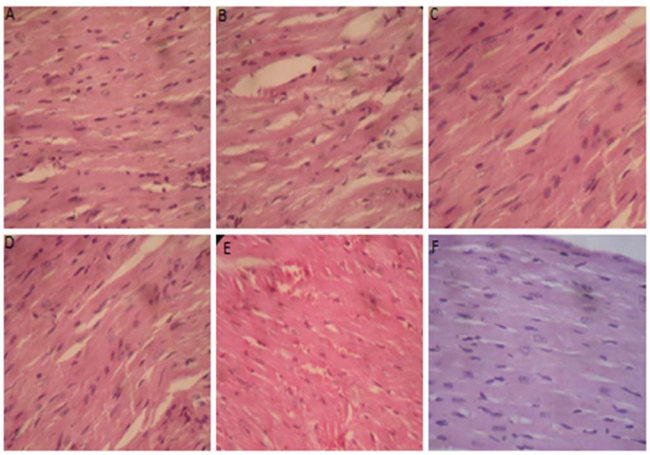

Plate 2. Photomicrographs of the Hearts of Mice Administered Various Doses of Methanol Leaf Extract of *Clerodendrum violaceum* for 28 days. A, B, C, D, E and F: Control, 31.25, 62.5, 125, 250 and 500 mg/kg b.wt respectively (H and E x 400).

The major serum lipids include cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) and triglycerides. Any alteration in the concentration of these lipids can give useful information to the predisposition of subjects to atherosclerosis and coronary heart disease (Abolaji *et al.*, 2007; Rafiee *et al.*, 2017). High blood cholesterol concentration is an important risk factor for cardiovascular disease (Ashafa *et al.*, 2009; Al-Snafi, 2017). Therefore, the reduced concentration of serum total cholesterol at 250 and 500 mg/kg body weight on day 14 (Table 2) and at all doses on day 28 (Table 3) may be clinically beneficial as the extract may reduce risk of

cardiovascular disease by decreasing vascular resistance through improvement of endothelial function (Adebayo *et al*., 2005). Triglycerides are the main storage form of fatty acids. The significant decrease in the concentration of serum triglycerides at 250 and 500 mg/kg body weight on day 14 (Table 2) and at all doses compared to control on day 28 (Table 3) suggests that the extract increased the breakdown of triglycerides or impaired incorporation of triglycerides into lipoproteins in the liver (Adebayo *et al*., 2007). However, this effect was only at higher doses (Table 2) or after prolonged administration (Table 3).

LDL-c is often termed the "bad" cholesterol because it is the primary carrier of plasma cholesterol and builds up slowly in the walls of arteries feeding the heart; this results in formation of plaques that clog the arteries leading to atherosclerosis and increased risk of high blood pressure which may eventually lead to stroke (Chia, 1991, Yakubu *et al*., 2008, Saikia and Lama, 2011). Extract administration led to a significant decrease in the concentration of LDL-c at the doses of 250 and 500 mg/kg body weight on day 14 (Table 2) and at all doses on day 28 compared to controls (Table 3). The significant decrease in plasma LDL-c may be due to increased hepatic LDL receptors and decreased conversion of VLDL to LDL (Olatunji *et al.,* 2005). This reduction could be beneficial since high LDL concentrations may predispose to coronary heart disease. The reduction was also seen at higher doses (Table 2) or after prolonged administration (Table 3).

HDL transports excess or unused cholesterol from the tissues back to the liver, where it is broken down to bile acids and is then excreted making it beneficial (Adebayo *et al*., 2011). The significant increase in HDL-c concentration at 250 and 500 mg/kg body on day 14 (Table 2) and at all doses on day 28 (Table 3) suggests that the extract at higher doses or when administered for a prolonged period may be beneficial since it has been demonstrated that an increase in the concentration of HDL-c correlates inversely with coronary heart disease (Nicholls and Nelson, 2019). This potential benefit is further shown by a decrease in the computed atherogenic index which followed a similar pattern of LDL-c concentration (Tables 2 and 3).

Lp(a) particles are like LDL consisting of a cholesterol-rich core, with an apoB-100 protein attached. However, Lp (a) uniquely differs from LDL in that it also has an apo(a) protein attached via a disulfide bond. Elevated Lp(a) levels associate robustly and specifically with increased cerebrovascular disease (CVD) risk. This association is continuous and does not depend on high levels of LDL or non-HDL cholesterol, or the presence of other CVD risk factors. Lp(a) levels, like elevated LDL, is causally related to premature development of atherosclerosis and CVD (Nordestgaard *et al*., 2010). The non-significant alteration in the concentration of Lp(a) (Table 4) throughout the study period suggests that the extract may not predispose subjects to atherosclerosis and CVD.

The observed effect of the extract on the cardiovascular disease indices may be attributed to its reported phytochemical contents which include, phenolic compounds, flavonoids, tannins, and phlobatannins (Adebayo *et al*., 2022). Secondary metabolites present in medicinal plants have been shown to possess potent cardio-protective activity due to their antioxidant activities (Rafiee *et al*., 2017; Afsheen *et al*., 2018). We have previously shown that the methanol leaf extract of *Clerodendrum violaceum* possessed significant antioxidant activity (Balogun *et al*., 2014). Therefore, the presence of these antioxidant phytochemicals may play an important role in the prevention and treatment of chronic diseases caused by oxidative stress including cardiovascular diseases.

The significant increase in the activity of ALP in the heart on day 14 (Figure 1) could be attributed to induction of enzyme synthesis or activation of the enzyme *in situ* in the heart at those doses by extract components. However, this effect was short lived as the activity of the enzyme was reverted to the range of control on day 28 (Figure 2). The significant increase in serum ALP activity on days 14 and 28 suggests activation of the enzyme *in situ* rather than leakage from the heart. However, leakage from other tissues may not be ruled out. The significant increase in the activity of ALT in the heart at the doses of 62.5, 125 and 250 mg/kg body weight on day 14 (Figure 3) indicates that the extract might have caused an increase in *de novo* synthesis of the enzyme molecules or activated the enzyme *in situ* (Yakubu *et al*., 2005). However, the sustained increase in the serum activity of ALT and the reverting of heart ALT to the range of control after 28 days suggests that there was no leakage from the heart but possibly other tissues, like the liver. This still supports the fact that the increase in ALT activity is due to the activation of the enzyme *in situ*. The reduction in ALT activities at 500 mg/kg body weight in the heart and serum on both days suggests inactivation of the enzyme *in situ* at the highest dose (Figures 3 and 4). Heart AST activity was not significantly changed while serum AST activity was significantly increased at doses less than 500 mg/kg body weight on both days suggesting that the increase in serum AST activity was not due to leakage from the heart but activation of the enzyme *in situ* in the serum. However, reduction in the activity of heart and serum AST activities on both days at 500 mg/kg body weight also suggests inactivation of the enzyme *in situ* and possibly reduced synthesis of the enzyme in the heart at the highest dose.

Creatine kinase is an enzyme present in skeletal muscle, brain and smooth muscle. There was a significant increase in the activity of this enzyme in the serum and heart of experimental animals at all doses on day 14 (Figure 7). This increase could have resulted from activation of the enzyme *in situ* in the serum and heart. The increased activity of this enzyme could lead to increased creatine phosphate production in the heart which could be of benefit to heart function because it will enhance availability of ATP molecules for muscle contraction. Serum creatine kinase activity has been shown to be decreased in malaria patients compared to normal patients (Baloch *et al*., 2010). This early observed increase in creatine kinase activity could be beneficial in counteracting the effects of malaria (which the plant extract is used to treat) in patients. The activities of this enzyme in the serum and heart on day 28 (Figure 8) were not significantly different from controls suggesting that the earlier increase on day 14 was a mechanism of adaptation to cope with the assault from extract components.

The absence of histopathological lesions in the heart throughout the experimental period (Plates 1 and 2) suggests that the extract at the doses investigated did not adversely affect the normal architecture of the heart.

CONCLUSION

The results of the study showed that the methanol leaf extract of *Clerodendrum violaceum* was able to significantly reduce some selected cardiovascular disease indices in mice at higher doses of the extract and after prolonged administration; thus, it may not predispose subjects to atherosclerosis and cardiovascular diseases.

AUTHORS' CONTRIBUTIONS

EAB and JOA designed the study and helped with data interpretation; AHZ carried out the laboratory investigations and drafted the manuscript. All authors proofread and approved the manuscript.

FUNDING STATEMENT

The study was partially funded by the University of Ilorin Central-Based Senate Research Grant (2010).

CONFLICT OF INTEREST

The authors declare no conflict of interest

ACKNOWLEDGEMENT

The authors thank Mal. Lawan Raji and Ibrahim Hayatu, Laboratory Technologists of the Department of Biochemistry and the Central Laboratory of Modibbo Adama University, Yola, Nigeria respectively for their assistance in the course of this work.

REFERENCES

Abolaji, A. O., Adebayo, A. H., & Odesanmi, O. S. (2007). Effect of ethanolic extract of Parinari polyandra (Rosaceae) on serum lipid profile and some electrolytes in pregnant rabbits. *Research Journal of Medicinal Plants,* 1, 121-127.

Adebayo, J. O., Yakubu, M. T., Egwim, C.E., & Owoyele, B. O. (2003). Effect of ethanolic extract of *Khaya senegalensis* stem bark on some biochemical parameters of rat kidney. *Journal of Ethnopharmacology*, 88, 69-72.

Adebayo, J. O., Adesokan, A. A., Olatunji, L. A., Buoro, D. O., & Soladoye, A. O. (2005). Effect of ethanolic extract of Bougainvillea spectabilis leaves on haematological and serum lipid variables in rats. *Biokemistri,* 17, 45-50.

Adebayo, J. O., Akinyinka, A. O., Odewole, G. A., & Okwusidi, J. I. (2007). Effect of caffeine on the risk of coronary heart disease- A re-evaluation. *Indian Journal of Clinical Biochemistry*, 22(1), 29-32.

Adebayo, J. O., Igunnu, A., Arise, R. O., & Malomo, S. O. (2011). Effects of co-administration of artesunate and amodiaquine on some cardiovascular disease indices of rats. *Food and Chemical Toxicology*, 49, 45-48.

Adebayo, J. O., Balogun, E. A., & Zailani, A. H. (2022). *In vitro* and *in vivo* antiplasmodial activities and cytotoxicities of *Clerodendrum violaceum* leaf extracts. *African Journal of Biomedical Research,* 25(2), 249-255.

Allain, C.C., Poon, L., Chan, S.G., Richmond, W., & Fu, P. (1974). Enzymatic determination of total serum cholesterol. *Clinical Chemistry*, 20, 470.

Al-Snafi, A. E. (2017). Medicinal plants for prevention and treatment of cardiovascular diseases - A review. *IOSR Journal of Pharmacy*, 7(4), 103-163.

Ashafa, A. O. T., Yakubu, M. T., Grierson, D. S., & Afolayan, A. J. (2009). Effects of aqueous extract from the leaves of *Chrysocomaciliata* L on some biochemical parameters of Wistar rats. *African Journal of Biotechnology*, 8(8), 1425-1430.

Afsheen, N., Rehman, K., Jahan, N., Ijaz,M., Manzoor, A., Khan,K. M., & Hina, S. (2018). Cardioprotective and metabolomic profiling of selected medicinal plants against oxidative stress. *Oxidative Medicine and Cellular Longevity*. https://doi.org/10.1155/2018/9819360.

Bachorik, P.S., Walker, R., Brownell, K.D., Stunkard, A.J., & Kwiterovich, P.O. (1980). Determination of high density lipoproteincholesterol in stored human plasma. *Journal of Lipid Research,* 21, 608- 616.

Bailey, S. A., Zidell, R. H., & Perry, R. W. (2004). Relationships between organ weight and body/brain weight in the rat: What is the best analytical endpoint. *Toxicologic Pathology*, 32, 448–466.

Baloch, S., Gachal, G. S., & Memon, S. A. (2010). Investigation of creatine phosphokinase (CPK)

concentration in serum of malaria patients. *Sindh University Research Journal (Science Series)*, 42(2), 71-72.

Balogun, E.A., Adebayo, J.O., Zailani, A.H., Kolawole, O.M., & Ademowo, O.G. (2010). Activity of ethanolic extract of *Clerodendrum violaceum* leaves against *Plasmodium berghei* in mice. *Agriculture and Biology Journal of North America*, 1, 307-312.

Balogun, E.A., Zailani A. H., & Adebayo, J. O. (2014). Augmentation of antioxidant system: Contribution to antimalarial activity of *Clerodendrum violaceum* leaf extract. *CELLMED*, 4(4), 26-31.

Chia, B. C. (1991). Cholesterol and coronary artery disease-issue in the 1990's. *Singapore Medical Journal,* 32, 29-294.

Di Witt, C., & Trendelenburg, J. (1982). Quantitives determination of creatine kinase in serum. *Clinical Chemistry and Clinical Biochemistry*, 20, 235.

Eran, B.A., Noah, S., Lee, H. G., Kamer, M., Suha, O., & Elad, S. (2016). Potential risks associated with traditional herbal medicine use in cancer care: A study of Middle Eastern oncology health care professionals. *Cancer*, 122, 598–610.

Erukainure, O. L., Narainsprasad, N., Singh, M., Olakunle, S., & Islam, M. S. (2018). *Clerodendrum volubile* inhibits key enzymes linked to type 2 diabetes but induces cytotoxicity in human embryonic kidney (HEK 293) cells via exacerbated oxidative stress and pro inflammation. *Biomedicine and Pharmacotherapy,* 106, 114-1152.

Friedwald, W. T., Levey, R. I., & Friedrickson, D. S. (1972). Estimation of the concentration of LDL in plasma without the use of preparative concentrating. *Clinical Chemistry,* 18, 499-502.

Gornall, A. G, Bardawill, C. T., & David, M. M. (1949). Determination of serum protein by means of Biuret reaction. *Journal of Biological Chemistry,* 177, 751.

Jacobs, N. J., & VanDemark, P. J. (1960). Enzymatic determination of serum triglyceride. *Archives of Biochemistry and Biophysics*, 88, 250-255.

Krause, W. J. (2001). The art of examining and interpreting histologic preparations. A student handbook. Partheton Publishing group, U.K. Pp 9-10.

Lazic, S. E., Semenova, E., & Williams, D. P. (2020). Determining organ weight toxicity with Bayesian causal models: Improving on the analysis of relative organ weights. *Scientific Reports*, 10, 6625. doi: 10.1038/s41598-020-63465-y.

Marcovina, S. M., Albers, J. J., Wijsman, E., Zhang, Z., Chapman, N. H., & Kennedy, H. (1996). Differences in lipoprotein (a) and apo(a) polymorphs between black and white Americans. *Journal of Lipid Research,* 37, 2569-2585.

Nath, P., & Yadav, K.A. (2015). Acute and sub-acute oral toxicity assessment of the methanolic extract from leaves of Hibiscus rosa-sinensis L. in mice. *Journal of Intercultural Ethnopharmacology*, 4, 70–73.

Nicholls, S. J., & Nelson, A. J. (2019). HDL and Cardiovascular disease. *Pathology*, 51(2), 142-147.

Nordestgaard, B. G., Chapman, M. J., Ray, K., Borén, J., Andreotti, G. F., Watts, H., Ginsberg, P., Amarenco, A. L., Catapano, O. S., Descamps, E., Fisher, P. T., Kovanen, J. A., Kuivenhoven, P., Lesnik, L., Masana, Z., Reiner, M. R., Taskinen, L., Tokgözoglu, A., & Tybjærg-Hansen, A. (2010). Lipoprotein (a) as a cardiovascular risk factor: current status. *European Heart Journal*, 31(23), 2844-2853.

Olatunji., L. A., Adebayo, J. O., Oguntoye, O. B., Olatunde, N. O., Olatunji, V. A., & Soladoye, A.O. (2005). Effects of aqueous extracts of petals of red and green *Hibiscus sabdariffa* on plasma lipid and haematological variables in Rats. *Pharmaceutical Biology,* 43(5), 471-474.

Porwal, M., Ali Khan, N., & Maheshwari, K. K. (2017). Evaluation of acute and subacute oral toxicity induced by ethanolic extract of Marsdenia tenacissima leaves in experimental rats. *Scientia Pharmaceutica*, 85, 29. doi:10.3390/scipharm85030029.

Rafiee, L., Keshvari, M., Atar, A. M., Hamidzadeh, Z., Dashti, G. R., Kopaei, M. R., & Asgary, S. (2017). Effect of Gundelia tournefortii L. on some cardiovascular risk factors in an animal model. *Journal of Herbmed Pharmacology*, 6(4), 191-195.

Razmpoosh, E., Abdollahi, S., & Mousavirad, M. (2022). The effects of olive leaf extract on cardiovascular risk factors in the general adult population: a systematic review and meta-analysis of randomized controlled trials. *Diabetology and Metabolic Syndrome*, 14, 151. https://doi.org/10.1186/s13098-022-00920-y

Reitman, S., & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *The American Journal of Clinical Pathology*, 28(1), 56–63.

Remya, K., Kaushik, C., & Musuvathi, M. B. (2021). Hepatoprotective and antioxidant capacity of *Clerodendrum paniculatum* flower extract against carbon tetrachloride-induced hepatotoxicity in rats. *ACS Omega*, 6(40), 26489-26498.

Roth, G. A., Mensah G. A., & Fuster, V. (2020). The global burden of cardiovascular diseases and risks: a compass for global action. *Journal of the American College of Cardiology*, 76, 2980-2981.

Saikia, H., & Lama, A. (2011). Effect of Bourgainvillea spectabilis leaves on serum lipids in albino rats fed with high fat diet. *International Journal of Pharmaceutical Sciences and Drug Research*, 3(2), 141-145.

Shendge, A. K., Basu, T., & Mandal, N. (2021). Evaluation of anticancer activity of *Clerodendrum viscosum* leaves against breast carcinoma. *Indian Journal of Pharmacology*, 53(5), 377-383.

Shrivastatva, N., & Patel, T. (2007). *Clerodendrum* and Healthcare: An overview Part II Phytochemistry and Biotechnology. *Medicinal and Aromatic Plant Science and Biotechnology*, 42, 209-217.

Tiwari, R. K., Chanda, S. M. U., Singh, M., & Agarwal, S. (2021). Anti-inflammatory and anti-arthritic potentials of standardized extract of *Clerodendrum serratum* (L). Moon. *Frontiers in Pharmacology*, 12, 629607.

Tsao, C., Aday, A. W., Beaton, A. Z., Bittencourt, M., Boehme, A. K., Carson, A. et al., (2022). Heart Diseases

and Stroke Statisitics-2022 update: A report from the American Heart Association. *Circulation,* 45, e153-e639

Vaduganathan, M., Mensah, G., Turco, J. V., Fuster, V., & Roth, A. G. (2022). The global burden of cardiovascular diseases and risk. *Journal of the American College of Cardiology*, 80(25), 2361–2371. https://doi.org/10.1016/j.jacc.2022.11.005

Wright, P. J. Walker, S., & Plummer, D.T. (1972). Enzymes in rat urine alkaline phosphatase. *Enzymologia,* 42, 317-327.

Yakubu, M. T., Adebayo, J. O., Egwim, E. C., & Owoyele, V. B. (2005). Increased liver alkaline phosphatase and amino transferase activities following administration of ethanolic extract of *Khaya senegalensis* stem bark torats. *Biokemistri,* 17(1), 27-32.

Yakubu, M. T., Akanji, M. A., & Oladiji, A. T. (2008). Alterations in serum lipid profile of male rats by oral administration of aqueous extract of *Fadogia agrestis* stem. *Research Journal of Medicinal Plants*, 2(2), 66-73.

Zhang, Y., Pletcher, M., J., & Vittinghoff, E. (2021). Association between cumulative low-density lipoprotein cholesterol exposure during young adulthood and middle age and risk of cardiovascular events. *JAMA Cardiology*, 6, 1406-1413.