The Journal of Phytopharmacology 2023; 12(2):93-99
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2023; 12(2): 93-99
March- April
Received: 23-01-2023
Accepted: 28-02-2023

doi: 10.31254/phyto.2023.12205

EHI-Omosun MB
Department of Anatomy and Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria

EZE GI
Department of Anatomy and Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria

Correspondence:
Dr. Mabel Ehi-Omosun
Department of Anatomy and Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria
Email: mabelehiomosun@gmail.com

Effects of Aqueous Leaf Extract of *Vernonia amygdalina* on High-Fat Diet-Induced Injury in the Heart and Aorta of the Adult Wistar Rat

EHI-Omosun MB, EZE GI

ABSTRACT

The aim of this study was to investigate the effects of aqueous leaf extract of *Vernonia amygdalina* in Wistar rats fed with high-fat diet. 30 adult Wistar rats weighing between 250g and 280g were randomly assigned into 5 groups of 6 rats per group. **Group A** rats were placed on rat food and water only. **Group B** rats received 1000mg/kg body weight / day (BWT/D) of margarine. **Group C** rats received 300mg/kg BWT/D of *V. amygdalina*. **Group D** rats received 1000mg/kg BWT/D of margarine and 150mg/kg BWT/D of *V. amygdalina*. **Group E** rats received 1000mg/kg BWT/D of margarine and 300mg/kg BWT/D of *V. amygdalina.* The dosages were given for 56days via orogastric method. The biochemical outcome showed that **Group B** revealed decrease in high density lipoprotein (HDL) and marked elevation in the total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), and superoxide dismutase (SOD) levels when compared with the other groups. Histologcally, **Group B** showed moderate stenosis, perivascular fibrosis, asymmetrical vascular hypertrophy and mild intimal erosion while **Group A, C, D and E** revealed normal myocardium and vascular architecture. In conclusion, the aqueous leaf extract of *Vernonia amygdalina* have antihyperlipidemic and antioxidant properties against margarine-induced injury in the heart and aorta of Wistar rats.

Keywords: *Vernonia amygdalina*, Margarine, Hypercholesterolemia, Atherosclerosis.

INTRODUCTION

Vernonia amygdalina is a perennial plant that is cultivated in tropical Africa.[1] It ranges in height from very low to the size of a small tree with many broad leaves. It is generally referred to as bitter leaf in English due to its bitter taste. The bitter taste is as a result of its constituent of biologically-active compounds, e.g., alkaloids, glycoside, flavonoids, tannins and saponins [2, 3]

The leaves of *Vernonia amygdalina* were well recognized by herbalists in Esan Central Local Government Area of Edo State, Nigeria for treating their adult obese clients. The leaves are crushed and the resulting liquid can be used to treat obesity, systemic hypertension, diabetes mellitus, dysentery, nausea and emesis. Scientists have opined that the active principles which confer antioxidant and antihypertensive activities on the plant are the flavonoids and its saponins.

Hypercholesterolemia is susceptible to early progression of atherosclerosis.[4,5] Besides hypercholesterolemia, some other conditions can increase susceptibility to the development of atherosclerosis e.g., familial history, hypertension, hyperlipidemia, diabetes mellitus, cigarette smoking, sedentary lifestyle and obesity. [6]

Cholesterol, a waxy, fat-like substance is produced in the body and obtained from food that come from animals particularly egg yolks, meat, fish, poultry and dairy products.[6] Deposition of cholesterol in the arteries causes a hardening and narrowing of the arteries due to the presence of patchy intimal plaques called atheroma [7]

As the plaques get bigger, they clog the arteries especially the coronary arteries thereby restricting the flow of blood to the heart and consequent risk of heart attack and stroke.[8,9] Therefore, a logical long-term strategy to avoid or deal with atherosclerosis and its cardiovascular complications is to aim at the hypercholesterolemia by taking low-fat diet and/or hypolipidemic agents. The present study investigates the effects of aqueous leaf extract of *Vernonia amygdalina* on the heart and aorta of Wistar rats fed with high-fat diet.

MATERIAL AND METHODS

The leaves of *Vernonia amygdalina* that were used in this research work were harvested from a farm in Iguosa Community, Ovia North East Local Government Area Edo State, Nigeria. The harvested plants were identified in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

The fresh matured leaves were oven-dried at 40^{o}Cafter air-drying for about a week. The dried leaves were then grounded using a 2018 model mechanical grinder, manufactured by Dozenmann Group of Company, U.S.A. The powdered material was extracted by cold maceration method.[10] which was done by soaking 500g of powdered *Vernonia amygdalina* leaf in one litre of water for 24 hours at room temperature. Using cotton wool as filter, the soaked *Vernonia amygdalina* was filtered and the filtrate was concentrated over hot water bath using evaporating dishes to obtain a concentrated jellylike extract of *Vernonia amygdalina* leaf which when weighed was found to be 20g and was transferred into a sample bottle for storage inside a refrigerator at 4 ^{o}C.

Phytochemical constituents of *Vernonia amygdalina* include saponins, alkaloids, tannins, proteins, steroids, flavonoids and glycosides [11, 12] Acute oral toxicity of the extract was evaluated. Appropriate doses of the extract were made by diluting with distilled water into 150mg/kg body weight and 300mg/kg body weight which were administered to the rats.

Experimental animals: 30 adult Wistar rats weighing between 250g and 280g were purchased from the Animal House, Department of Anatomy, University of Benin, Benin City Edo State, Nigeria and were utilized for this experimental research. The rats were given a period of 2 weeks to adapt to their new environment before commencement of the experiment. During this period, the animals were allowed access to standard animal feed (Vital grower's feed, manufactured by Bendel Flour Mill, Ewu) and clean water *ad libitum*.

Each animal procedure was carried out in accordance with approved protocols and in compliance with the recommendations for the proper management and utilization of laboratory animals used for **research.**[13] Hyperlipidemic condition was induced by oral administration of 1000mg/kg body weight of margarine once daily for 56 consecutive days.[14] A pilot study was done which confirmed hyperlipidemia in the rats.

Experimental design**:** 30 adult Wistar rats were randomly assigned into 5 groups; (**Group A-E**) comprising of 6 rats per group. **Group A** rats which served as control received 1ml of distilled water daily to compensate for stress of administration procured in the test groups. **Group B**rats were treated daily with oral administration of 1000mg/kg body weight of margarine for 56 consecutive days. **Group C** rats were treated daily with oral administration of 300mg/kg body weight of *Vernonia amygdalina* aqueous leaf extract for 56 consecutive days. **Group D** rats were treated daily with oral administration of 1000mg/kg body weight of margarine and 150mg/kg body weight of *Vernonia amygdalina* aqueous leaf extract for 56 consecutive days while **Group E** rats were treated daily with oral administration of 1000mg/kg body weight of margarine and 300mg/kg body weight of *Vernonia amygdalina* aqueous leaf extract for 56 consecutive days.

Method of sacrifice and sample collection: At the end of the 8th week, the animals were sacrificed under chloroform anaesthesia. Blood samples were collected with plain specimen bottles for biochemical analysis and the heart and the brachiocephalic artery (3rd branch of the aorta) of each rat were harvested and immediately fixed in 10% formal saline for 24 hours before the histological analysis.

The harvested tissues were histologically assessed using the following methods: fixation, embedding and tissue staining for microscopy. The tissue sections were examined under Leica DM750 research microscope with digital camera (Leica ICC50) attached. Digital photomicrograghs of the tissue sections were taken at H & E × 40 and × 100 magnifications.

Total plasma cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL)were assayed during lipid profile analysis using an auto-analizer (2006 model, manufactured by Hoddlier and Stoughton Group of Company, London with a recognized biochemical kit (2010 model, Diadnostical Merek, London. Assay for oxidative stress indicator (superoxide dismutase) also known as SOD was also done according to the method of Marklund and Marklund.[15] The data were subjected to statistical analysis and P values calculated using tne students t-test.

RESULTS

The results are presented as mean and standard error of mean (table 1 and 2). Overall, the weights of the rats in margarine + *Vernonia amygdalina* treated groups (Group D and E) were not significantly different from the control (Group A) (Figure 1). The weight gained by the rats in the *Vernonia amygdalina* treated groups (Group C, D and E) was lower than that of the control group (Group A). There was a significant increase in the weight of the margarine alone treated group (Group B) when compared with the control (Group A). Furthermore, a comparison between *Vernonia amygdalina* alone treated group (Group C) and the control (Group A) reveals a significant decrease in the weight of *Vernonia amygdalina* alone treated group (Group C) (table 1).

There was a significant increase in TC, TG, LDL and SOD and significant decrease in HDL in the margarine alone treated group (Group B) when compared with control (Group A) (table 2). On the other hand, there was a significant decrease in TC, TG, LDL and SOD and a significant increase in HDL in the *Vernonia amygdalina* treated groups (Group C, D, and E) when compared with the control (Group A) (table 2). The lower values of TC, TG, LDL and SOD in comparison with control were more pronounced for the low dose extract (Group D). Furthermore, comparison of HDL between margarine alone treated group (Group B) and *Vernonia amygdalina* treated groups (Group C, D and E) shows that the HDL was significantly higher in the *Vernonia amygdalina* treated groups (Group C, D and E) than those of margarine alone treated group. However, when HDL in *Vernonia amygdalina* treated groups (Group C, D and E) was compared with the control (Group A) there was a significant decrease ($P<0.05$) in the value of HDL in the low dose extract (Group D) but the reduction was not significant (table 2).

Table 1: Changes In The Body Weights of Rats In All The Experimental Groups

Groups	**Duration (weeks)**	**Initial Body Weight (gm)**	**Final Body Weight (gm)**	**% Gain in Body Weight**	**P values**	**Mortality**
roup A (Control)	8	250.05±12.31	273.04±20.13	26.08±5.534	22.22±1.228l	Nil
Group B (Margarine only	8	252.08±4.60	284.06±9.56	32.08±2.292	21.42±0.867	Nil
(Group C (*V. amygdalina* only	8	260.82±4.43	274.07±8.27	14.18±0.866	26.08±5.534	Nil
Margarine+ Low Dose Extract	8	258.02±4.28	280.05±9.05	21.42±0.867	14.18±0.866	Nil
Margarine + High dose Extract	8	270.06±4.44	291.02±8.28	22.22±1.228	32.08±2.292	Nil

n=6, Mean value ± SEM, **P<0.01

Table 2: Comparisons of Biochemical Parameters in all Experimental Groups

Groups -→ Various Biochemical Parameters		**Control (Group A)**	**Margarine Only (Hyperlipidemic) (Group B)**	**Vernonia Amygdalina Only (Group C)**	**Margarine + Low Dose Extract (Group D)**	**Margarine + High Dose Extract (Group E)**	**P-Value**
TC g/gl	Mean±SD	70.67 ±3.28	157.20±15.69*	63.60±3.31*	53.00±3.55*	99.50±11.52*	P<0.001
TG g/dl	Mean±SD	91.33±17.72	324.60±76.70*	80.60±8.87*	61.80±5.33*	105.25±21.03	P<0.001
HDL g/dl	Mean±SD	32.00±4.04	24.80±2.50	34.80±3.84	33.00±2.85	40.75±6.34	P<0.001
LDL g/dl	Mean±SD	17.00±4.04	47.80±9.46*	12.60±2.73	11.00±1.90*	37.75±8.82*	P<0.001
SOD ng/ml	Mean±SD	40.10±6.42	47.53±8.53*	35.96±6.34*	44.32±3.37	38.54±8.67	P<0.00

Figure 1 and 2 are photomicrograghs of a section of rat heart in the control group (Group A) showing bundles of myocardial fibres, coronary artery and interstitial space at H&E × 40 and × 100 respectively.

Figure 3 and 4 are photomicrographs of a section of rat heart in the group given 300mg/kg margarine alone (Group B) showing perivascular fibrosis, asymmetrical vascular hypertrophy, moderate vascular stenosis and mild intimal erosion at H&E × 40 and × 100 respectively.

Figure 5 and 6 are photomicrographs of a section of rat heart in the group given 300mg/kg *Vernonia amygdalina* alone (Group C) showing normal myocardium and vascular architecture at H&E × 40 and × 100 respectively.

Figure 7 and 8 are photomicrograghs of a section of rat heart in the group given margarine and 150mg/kg *Vernonia amygdalina* (Group D) showing normal myocardium, normal vascular architecture and mild congestion and dilatation.at H&E × 40 and × 100 respectively.

Figure 9 and 10 are photomicrographs of a section of rat heart in the group given margarine and 300mg/kg *Vernonia amygdalina* (Group E) showing normal myocardium and vascular architecture and mild congestion and dilatation at H&E × 40 and × 100 respectively.

Figure 11 is a photomicrogragh of a section of rat aorta (brachiocephalic artery) in the control group (Group A) showing the lumen, tunica intima, tunica media and tunica adventitia at H&E × 100.

Figure 12 is a photomicrograph of a section of rat aorta (brachiocephalic artery) in the group given margarine alone (Group B) showing severe intimal ulceration, media hypertrophy and vascular stenosis at H&E × 100.

Figure 13 is a photomicrogragh of a section of rat aorta (brachiocephalic artery) in the group given *Vernonia amygdalina* alone (Group C) showing normal vascular wall and mild vascular congestion at H&E × 100.

Figure 14 is a photomicrograph of a section of rat aorta (brachiocephalic artery) in the group given margarine and 150mg/kg *Vernonia amygdalina* (Group D) showing normal vascular wall and mild vascular dilatation at H&E ×100.

Figure 15 is a photomicrogragh of a section of rat aorta (brachiocephalic artery) in the group given margarine and 300mg/kg *Vernonia amygdalina* (Group E) showing normal vascular wall and mild vascular dilatation at H&E × 100

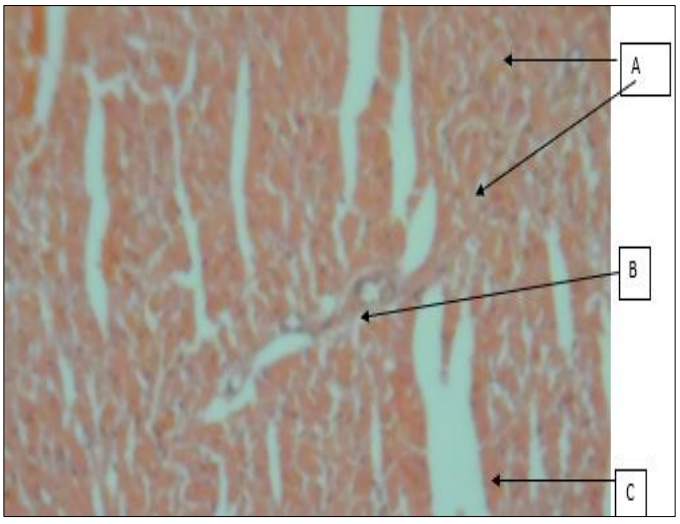

Figure 1: Control (Group A): Rat heart (Group A) composed of: A, bundles of myocardiac fibres, B, coronaryArtery and C, interstitial space (H&E X 40)

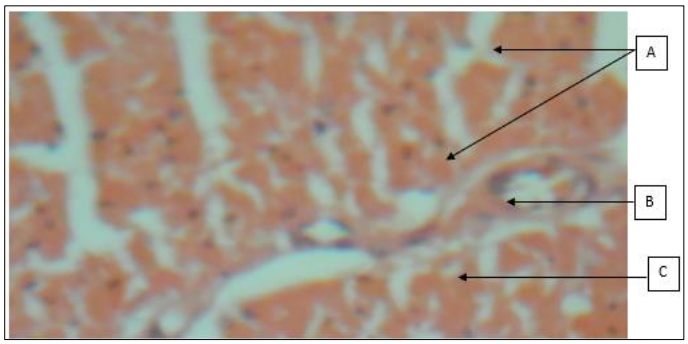

Figure 2: Control (Group A): Rat heart composed of: A, myofibre, B, coronary Artery and C, interstitial space (H&E X 100)

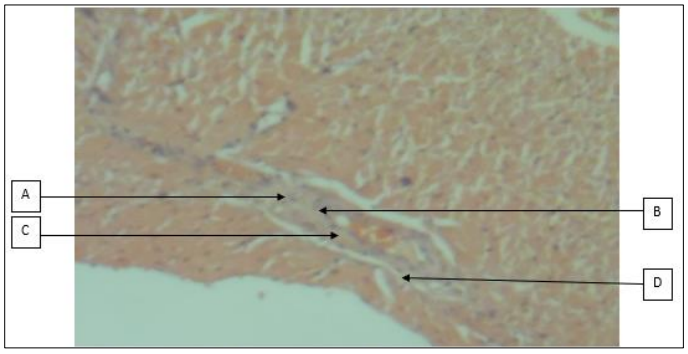

Figure 3: Rat heart given margarine only, (Group B) showing: A, perivascular fibrosis, B, asymmetrical Vascular hypertrophy, C Moderate stenosis and D, mild intimal erosion (H&E X 40)

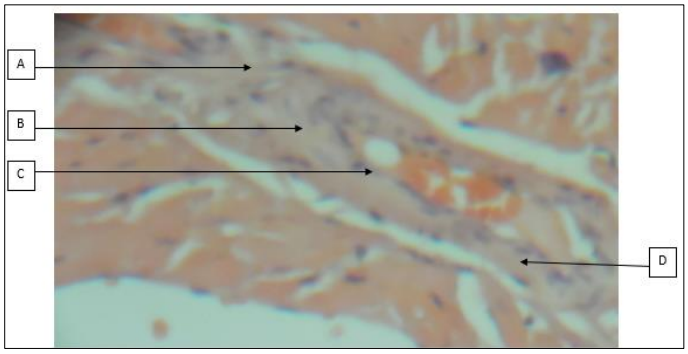

Figure 4: Rat heart given margarine only, (Group B) showing: A, perivascular fibrosis, B, asymmetrical Vascular hypertrophy, C Moderate stenosis and D, mild intimal erosion (H&E X 100)

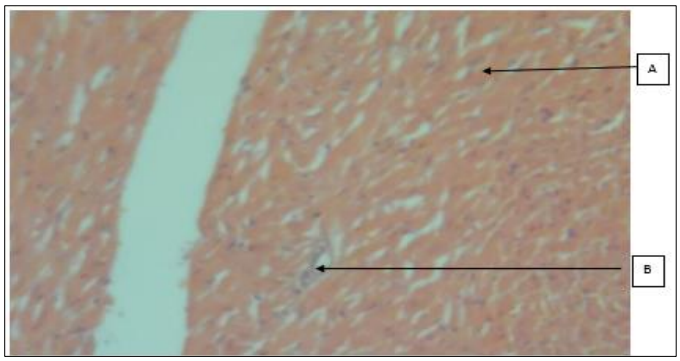

Figure 5: Rat heart given 300mg/kg Vernonia amygdalina only (Group C) showing: A, normal myo- cardium and B, vascular architecture (H&E X 40)

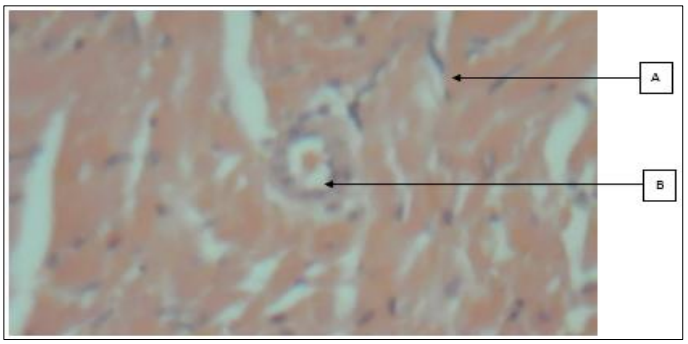

Figure 6: Rat heart given 300mg/kg Vernonia amygdalina only (Group C) showing: A, normal myocardium and B, vascular architecture (H&E X 100)

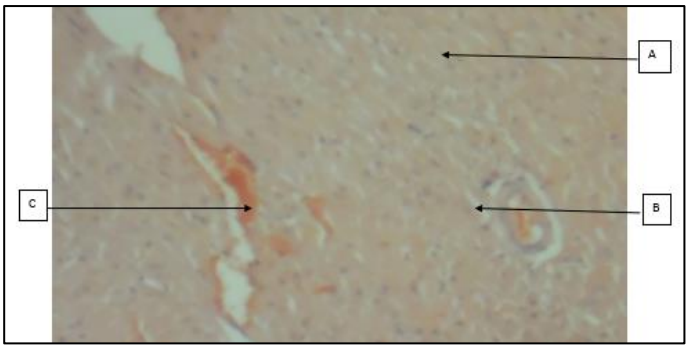

Figure 7: Rat heart given margarine and 150mg/kg V. amygdalina (Group D) showing: A, normal Myocardiam and B, vascular architecture and C, mild congestion and dilatation (H&E X 40)

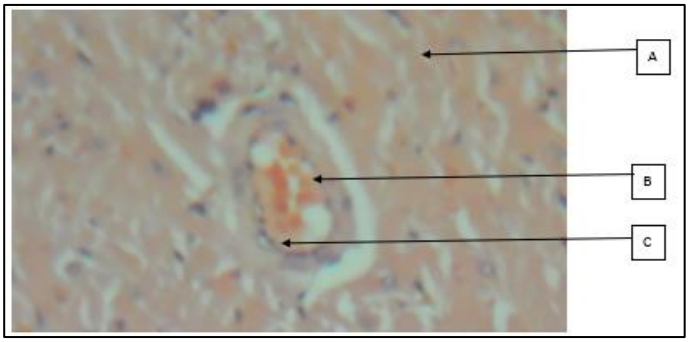

Figure 8: Rat heart given margarine and 150mg/kg V. amygdalina (Group D) showing: A, normal Myocardium and B, vascular architecture and C, mild congestion and dilatation (H&E X 100)

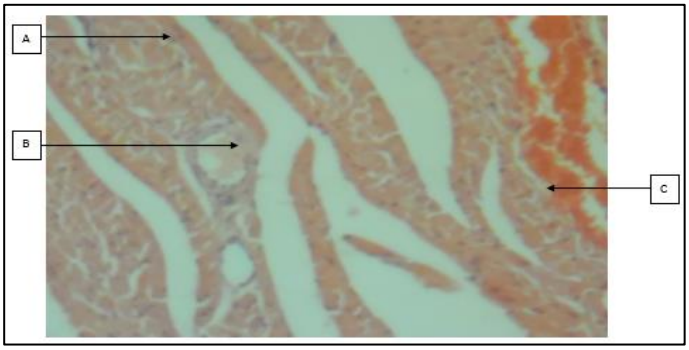

Figure 9: Rat heart given margarine and 300mg/kg V. amygdalina (Group E) showing: A, normal Myocardium and B, vascular architecture and C, mild congestion (H&E X 40)

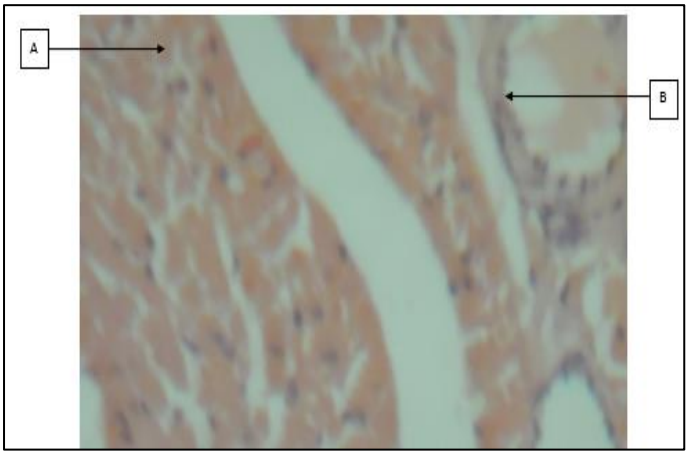

Figure 10: Rat heart given margarine and 300mg/kg V. amygdalina (Group E) showing: A, normal Myocardium and B, vascular architecture (H&E X 100)

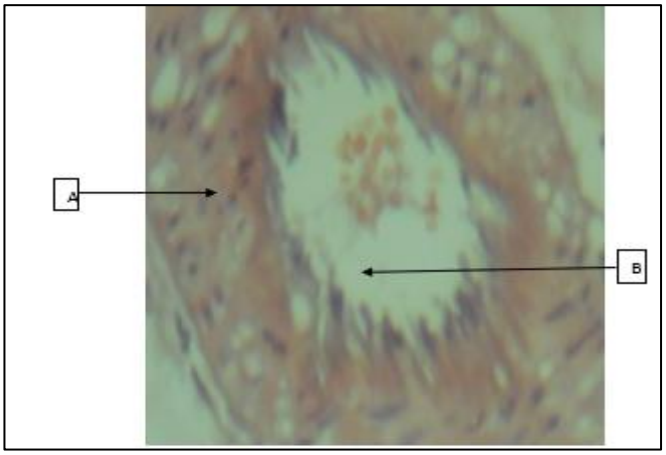

Figure 13: Rat brachiocephalic artery given 300mg/kg V. amygdalina only (Group C), showing: A, normal vascular wall and B, mild vascular congestion (H&E X 100)

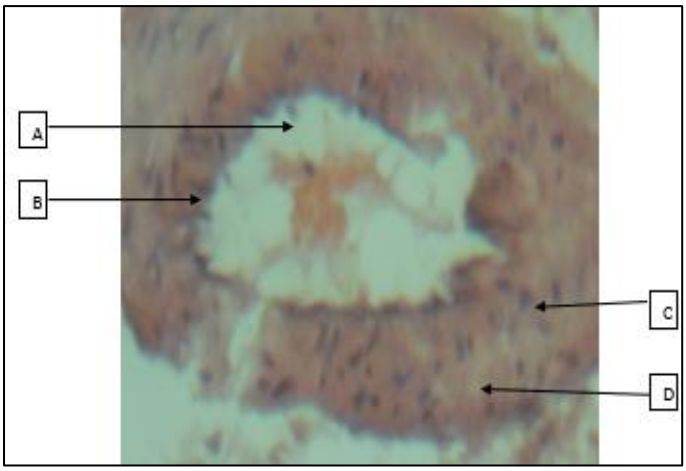

Figure 11: Control (Group A) Rat brachiocephalic artery composed of: A, lumen, B, tunica intima, C, media and D,Adventitia (H&E X 100)

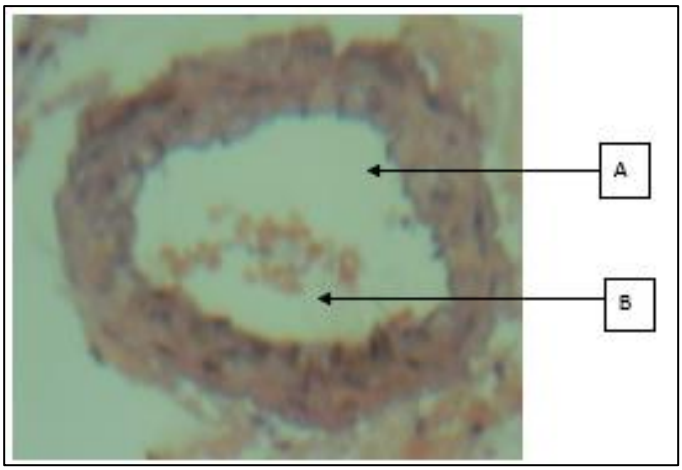

Figure 14: Rat brachiocephalic artery given margarine and 150mg/kg V. amygdalina (Group D) showing: A, normal vascular wall and B, mild vascular dilatation (H&E X 100)

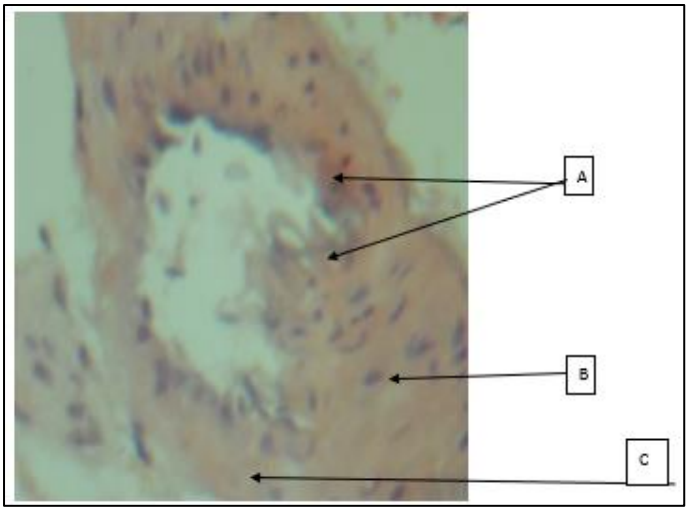

Figure 12: Rat brachiocephalic artery given margarine only (Group B) showing: A, severe intimal ulceration, B, media hypertrophy and C, vascular stenosis (H&E X 100)

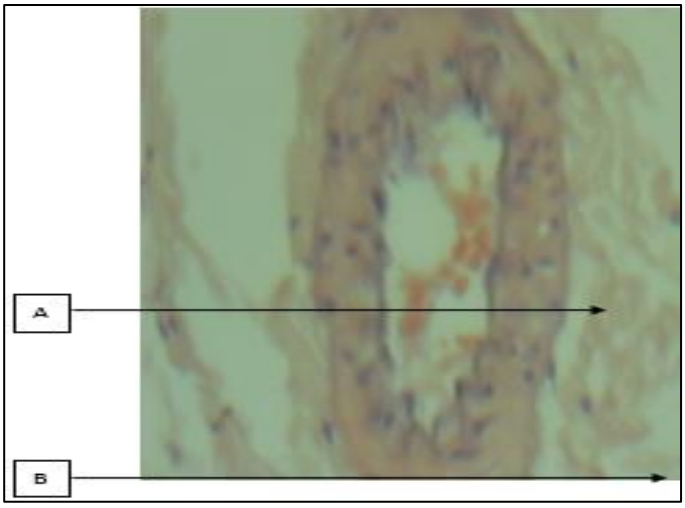

Figure 15: Rat brachiocephalic artery given margarine and 300mg/kg V. amygdalina (Group E) showing: A, normal vascular wall and B, mild vascular dilatation (H&E X 100)

DISCUSSION

Medicinal plants are widely accepted to be a blessing in the developing countries. The present study was designed to examine the effects of aqueous leaf extract of *Vernonia amygdalina* in heart and aorta (brachiocephalic artery) of Wistar rats fed with high-fat diet. Findings for the study of body weight revealed significant increase in body weight of the margarine alone treated rats which concur with previous work (Uchendu, 2018). [7]

Findings of this study for biochemical analysis for serum levels showed significant increase in TC, TG and LDL and SOD in the margarine alone treated rats. However, there was a significant decrease in TC, TG, LDL and SOD in the *Vernonia amygdalina* treated rats which concur with previous work (Zahra and Samaneh, 2012). [6]

Histopathological findings in this study for the margarine alone treated rats showed perivascular fibrosis, asymmetrical vascular hypertrophy, moderate stenosis and mild intimal erosion (Figure 3 and 4) while the *Vernonia amygdalina* treated rats showed normal myocardium, normal vascular architecture and mild vascular congestion (Figure 5, 6, 7, 8, 9 and 10).

The histopathological findings of this study for the histomorphological studies of the 3rd branch of the aorta (brachiocephalic artery) in the margarine alone treated group (Group B) showed severe intimal ulceration, media hypertrophy and vascular stenosis (Figure 12) while the histomorphological studies of the brachiocephalic artery in the *Vernonia amygdalina* treated groups (Group C, D, and E) showed normal vascular wall, mild vascular congestion and dilatation (Figure 13, 14 and 15) which agrees with report by Sumbul and Ahmed,(2012).[14]

In a nutshell, treatment of the margarine-induced injuries in the heart and aorta with graded doses of *Vernonia amygdalina* aqueous leaf extract at a dose of 150mg and 300mg/kg body weight respectively ameliorated these lesions, as revealed by the normal intima of the coronary vessels and myocardial fibres (Figure 7, 8, 9 10, 14 and 15) However, 150mg/kg body weight (low dose) *Vernonia amygdalina* had a slightly more potent effect on the cardiovascular tissues as revealed by normal vasodilatation (Figure 14). Conclusively, the findings of this study for the margarine alone treated group (Group B; Figure 3, 4 and 12) reveal that cholesterol caused injury to the heart and the blood vessels. The injury is represented by myocardial infarction in the heart and by atherosclerosis in the blood vessels. But aqueous leaf extract of *Vernonia amygdalina* protected the heart and the blood vessels from getting these lesions. Therefore, this study revealed a clear protective effect of *Vernonia amygdalina*.

Where else apart from the heart and blood vessels can hypercholesterolemia cause health problems? Hypercholesterolemia can cause health problems in tissues other than the heart and blood vessels. For instance, if cholesterol accumulates in tendons, it causes tendon xanthomas which most often affect the Achilles tendons and tendons in the hands and fingers. Yellow cholesterol deposits can develop under the skin of the eyelids and are known as xanthelasmata. Cholesterol can accumulate at the edges of the cornea leading to a gray-colored ring called an arcus cornealis.[5]

CONCLUSION

Margarine caused increased body weight in the margarine alone treated rats and also caused some derangements in biochemical parameters especially TC, TG, LDL and SOD. Moreover, margarine caused injury to to the heart and blood vessels of the treated rats which were ameliorated with graded doses of *Vernonia amygdalina* aqueous leaf extract.

Recommendation

We therefore recommend that *Vernonia amygdalina* should be developed into a drug that can be used as a hypolipidemic agent.

Conflict of Interest

The authors declare that they have no conflict of interest.

ORCID ID

Mabel Ehi-Omosun: https://orcid.org/0009-0000-5612-8466

REFERENCES

1. Bamidele I, Alabi – Sofunde JA, Efedede BU, Akinwande AI. 'Hepatoprotective and Antioxidant activities of *Vernonia amygdalina* on acetaminophen – induced hepatic change in Mice. *Journal of Medicinal food*. 2006; 9(4):524-530.
2. Farombi EO, Owoeye O. "Antioxidative and chemopreventive properties of *Vernonia amygdalina* and Garcinia biflavonoid" *International Journal of Environ Res public Health*, 2011; 8(6):2533-2537
3. Okoli RI, Aigbe O, Ohafu Obode JO, Mensah JK. "Medicinal herbs used for managing some common ailments among Esan people, Edo State, Nigeria" *Pakistan Journal of Nutrition* 2007; 6(5):470-485.
4. Mark H, Roberts, Thomas V, Justin L, Michael B. *The Merck manualof diagnosis and therapy* Eighteenth edition. Merck Research laboratory publishers, USA. 2006.
5. Ikenna KU, Okechukwu SO, Agu Chidozie, Tochi FN. 'Hypolipidaemic and renoprotective effects of *Glycine max* (Soya bean) against lipid profile and renal biochemical alterations in hypercholesterolemic rat'. University of Nigeria Nsukka, Department of Medical Laboratory Sciences 2016.
6. Zahra S, Samaneh KP. 'Toxicity of margarine on liver enzymes Aspertate, amino transferase and alanine amino transferase) in rats. Iranian Journal of Toxicology 2012; 6(17).
7. Uchendu IK. 'Effects of aqueous extract of bitter leave (*Vernonia amygdalina*) against acetaminophen – induced liver damgae in rats. *Bioequivalenve and Bioavailability Internal Journal* 2018;2(1):000122
8. Harrison RJ. *Textbook of Medicine with relevant physiology and anatomy*. Second edition. Hodder and Stoughton, London. 1980.
9. Guruaja GM, Deepak M, Senthil K, Shekhar MD, Allan JJ, Amit a. 'Evaluation of cholesterol lowering activity of sstandardized extract of *Mangifera indica* in albino Wistar rats' Journal of pharmacognosy Research. 2017;9(1):21-26.
10. Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohammed A, Sahena F, Jahurul MHA, Ghafoor K, Norulaini NAN, Omar AKM. 'Techniques for extraction of Bioactive compounds from plant materials'. A review Journal of food engineering 2013;117(4):426-436.

11. Ugwoke CEC, Nzekwe U, Ameh G. 'Phytochemical constituents and ethnobotany of the leaf extract of bitter leaf (*Vernonia amygdalina*) Del' Journal of Pharmaceutical and Allied Sciences. 2010; 7(3).
12. Offor CE 'comparative chemical analysis of *Vernonia amygdalina* and *Azadirachta indica* leaves'. JOSR Journal of Pharmacy and Biological Sciences (JOSR – JPBS). 2014; 9(5):II, 73-77.
13. Buzek J, Chastel O. 'Directive 2010/63/EU of the European parliament and of the Council. Protection of animals used for scientific purposes (text with EEA relevance)'. *Official Journal of the European Union* L 276/34. 2010.
14. Sumbul S, Ahmed SI Anti-hyperlipidemic activity of *Carissa carandas* (Auct) leaves extract in egg yolk induced hyperlipidemic rats. Journal of Basic and Applied Sciences, 2012;8:124-134.
15. Marklund S, Marklund G. 'Involvement of the superoxide anion radical in the autozidation of pyrogallol and a convenient assay for superoxide dismutase. Euro.J. Biochem. 1974; 4(47):469-74.

HOW TO CITE THIS ARTICLE
EHI-Omosun MB EZE GI. Effects of Aqueous Leaf Extract of *Vernonia amygdalina* on High-Fat Diet-Induced Injury in the Heart and Aorta of the Adult Wistar Rat. J Phytopharmacol 2023; 12(2):93-99. doi: 10.31254/phyto.2023.12205