

Effect of aqueous extract of *Vernonia amygdalina* on atherosclerosis in rabbits

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Original Article

Abstract

BACKGROUND: Extracts of *Vernonia amygdalina* (*V. amygdalina*) have been shown to affect the serum lipid profile of some laboratory animals in previous studies. Its impact on serum lipid profile and the histological changes in atherosclerosis has not been studied. Our aim was to determine the effects of *V. amygdalina* on atherosclerotic lesions induced in rabbits on high-cholesterol diet.

METHODS: 18 male rabbits were randomly divided into three groups of control, atherogenic diet, and atherogenic diet + 200 mg/kg of *V. amygdalina*. The rabbits were fed a normal diet (control group) or a diet supplemented by 0.5% cholesterol and 1% methionine (second and third groups, respectively) for 12 weeks. The fasting sera of all animals were collected at baseline and at the end of the 12 weeks, to determine the levels of lipid profile and the aortas underwent pathomorphological examination.

RESULTS: The two groups on the atherogenic diet had significantly increased serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) compared to the control group. The serum triglyceride (TG) was not statistically different in all three groups. High-density lipoprotein cholesterol (HDL-C) was significantly increased in the *V. amygdalina* group, compared to the control group but there was no statistically significant difference between the two groups on atherogenic diet. The two groups of rabbits that were on high-cholesterol diet (atherogenic diet group, as well as the atherogenic diet + 200 mg/kg of *V. amygdalina*) developed histological evidence of atherosclerosis. However, there was no histological difference between the lesions observed in these two groups.

CONCLUSION: The use of 200 mg/kg of aqueous extract of *V. amygdalina* in rabbits did not appear to exert a significant effect on the serum lipid profile. It also did not appear to have any beneficial effect on the development of atherosclerotic lesions.

Keywords: *Vernonia*; Rabbits; Atherosclerosis; Cholesterol; Alternative Medicine

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Introduction

Atherosclerosis is a major cause of mortality all over the world. It is characterized by high levels of serum lipids comprising total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) in the serum. Increased serum TC and particularly LDL-C have been implicated in the etiology of atherosclerosis. The atherosclerotic process involves the build-up of a waxy plaque on the inside of blood vessels, and it involves an ongoing inflammatory response. It can involve the entire vascular system and is characterized by plaques in the intima layer of arteries.^{1,2}

High level of serum lipids is related to increased oxidative damage, which affects antioxidant status and lipoprotein levels.^{3,4}

While orthodox medicine is generally accepted and preferred globally, the use of herbs and traditional medicine is often considered an equally acceptable alternative in many regions of the world. The traditional medicine is commonly used in the developing countries where the cost of orthodox medicine and access to medical care is unavailable to part of the populace. According to the World Health Organization (WHO), 80% of people in developing countries use traditional medicine, 85% of which are plant extracts.⁵

Some medicinal herbs have antioxidant effects and can also reduce the blood lipids. Some of these herbs have been shown to prevent atherosclerosis.⁶ *Vernonia amygdalina* (*V. amygdalina*), is a member of the Asteraceae family. It is a shrub that grows in

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tropical Africa.⁷ Leaves from this plant serve as food and culinary herb in soup in many parts of Africa. The aqueous extract of the leaves is used traditionally as treatment for anemia, nausea, diabetes, loss of appetite, dysentery, and other gastrointestinal tract problems.⁸ In Nigeria, the plant is used in the control of tick and treatment of a cough, feverish condition, constipation, and hypertension.⁹⁻¹¹ Extracts of *V. amygdalina* has been shown to reduce serum LDL-C and TC.¹²⁻¹⁴

Some studies have reported that plants which lower serum lipid values are rich in flavonoids and tannins. These compounds play a significant role in the mobilization and metabolism of lipids. Phytochemical analysis of *V. amygdalina* revealed high levels of flavonoids, saponin, tannins, and alkaloids.¹⁵

This study aimed to examine the effect of aqueous extract of *V. amygdalina* on the serum lipid profile and the histological changes in the aorta of rabbits on the atherogenic diet.

Materials and Methods

Preparation of plant extract

V. amygdalina leaves were purchased at a local market, Ibadan, Nigeria and authenticated at Department of Botany, University of Ibadan, Nigeria (Voucher number UIH-22432). The leaves were rinsed with water to remove extraneous materials. The leaves were subsequently spread to dry indoors until a constant dry weight was attained. The size was reduced via grinding it into powder with a mill. At the end of milling, 2.4 kg of ground leaves was obtained.

Aqueous extraction of the leaves was performed at Department of Pharmacy, University of Ibadan. The ground leaves were soaked in 6 liter of distilled water (using 2 glass jars containing 1.2 kg of leaves in 3 liter each) for 24 hours with 2 hourly stirring of the solution. The mixture was subsequently filtered through the first muslin bag and second using a Whatman filter paper. The extract was concentrated using a rotatory evaporator at 45 °C and then dried using a vacuum oven at 45 °C and pressure of 600 mmHg.

The resultant yield of extract was 122 g, giving a percentage yield of 5.1%. The resultant paste was stored in a glass jar in the refrigerator. It was reconstituted with distilled water, on a daily basis as required; to give a solution in which 1 ml contained 100 mg of extract. The extract was administered into the oral cavity of the rabbits with a metal gavage needle.

The dose of extract was set at 200 mg/kg per day based on the doses administered in previous studies conducted on rats which revealed beneficial effects on serum lipid profiles at this dose.^{12,13}

Constitution of the atherogenic diet

Cholesterol powder was procured from AMRESCO (Ohio, USA) and methionine powder from Hard Eight Nutrition LLC (Nevada, USA). The chow was constituted by dissolving 12.5 g of cholesterol in 125 ml of groundnut oil, and mixing this with 25 g of methionine powder and 2350 g of chow. Thus, mixing 2.5 kg of chow was consumed in 2-3 days. The atherogenic diet consisted of chow supplemented with 0.5% cholesterol and 1% methionine and 5% groundnut oil by weight.^{16,17}

A total of 18 male rabbits weighing 750-1200 g and aged 2-3 months were obtained. They were adapted to laboratory handling for a week and then randomly divided into three groups as follows:

- Group 1: Normal chow for 12 weeks
- Group 2: Normal chow and atherogenic diet for 12 weeks
- Group 3: Normal chow, atherogenic diet and 200 mg/kg of extract/day for 12 weeks.

The animals were housed in individual metal cages, in a well-ventilated room with natural 12-hour light/dark cycles. They were fed chow at 5.0% of the body weight (of the largest animal) per day and had free access to water. The weights of the animals at baseline and post-intervention were noted. Animals were handled in compliance with the ethical guidelines of the University of Ibadan.

At the end of 12 weeks, the animals were fasted overnight and a blood sample obtained for fasting lipid profile. Leadman reagents were used to analyze TC, TG and high-density lipoprotein cholesterol (HDL-C) using a Landwind auto-chemistry analyzer. LDL-C was calculated using the Friedewald equation.

Euthanasia was achieved under anesthesia using ketamine and xylazine followed by exsanguination. The aorta was excised from the root of the aorta, distal to the aortic valve to the bifurcation of the aorta. It was split open longitudinally, and the surface of the endothelium was examined. The presence of fatty streaks was seen on gross examination of the vessels of the animals on atherogenic diet.

These fatty streaks were more pronounced at the root of the aorta and around the ostia of the intercostals arteries. Sections of the aorta were obtained from the thoracic aorta and stained with hematoxylin-eosin stain.

Aortic histomorphometric study

The tunica intima of each section was carefully examined with an Olympus light microscope (CX41 model) for the presence of atherosclerotic lesions. The images of each slide were captured. The tunica intima and tunica media thickness were measured using computerized image analyzer (Motic Image plus Version 2.0).

The tunica intima thickness was measured from lumen to the internal elastic lamina while the tunica media thickness was measured from the internal elastic lamina to the external elastic lamina. This was used to calculate the intima-media ratio. Measurements were taken from four sections of the aorta of one rabbit each from the three groups. The average of these measurements was utilized for analysis.¹⁸

Results are expressed as mean \pm standard deviation (SD). A statistical analysis was carried out using SPSS software (version 22, SPSS Inc., Chicago, IL, USA). Paired t-test was used to compare baseline and post-intervention weight and serum lipid profiles. Comparison across the groups of all parameters was done using analysis of variance (ANOVA) test. Bonferroni post-hoc analysis was performed on all parameters which ANOVA showed statistically significant differences (where $P < 0.050$). The level of statistical significance was set at 95% with $P < 0.050$.

Results

The summary of the serum lipid profile at baseline and post-intervention is presented in tables 1 and 2, respectively. The baseline parameters did not show a statistically significant difference across the three groups. The post-intervention parameters showed a significant difference in TC, HDL-C, and LDL-C. Table 3 shows the post-hoc Bonferroni analysis and indicates that TC and LDL-C were significantly different between the groups 1 and 2, also between the groups 1 and 3. However, these parameters were not statistically different between the groups 2 and 3.

Comparison of the weight and serum lipid profile within the experimental groups at baseline and post-intervention is shown in table 4. It revealed a significant change in the weight and TC in the three groups. It also showed that the HDL-C and LDL-C had significant differences between the groups 2 and 3. The mean intima-media ratios in the three groups revealed 0 ± 0 , 0.76 ± 0.13 , and 0.63 ± 0.11 , respectively. The statistical analysis revealed that the difference in the ratios of groups 2 and 3 was not statistically significant.

The hematoxylin-eosin stain of the aorta from the three experimental groups is represented in Figure 1 and reveals the presence of atherosclerotic lesions in groups 2 and 3. The lesions are characterized by several layers of foam cells and pools of extracellular lipid.

Table 1. Summary of mean weights and serum lipid profiles; and comparative ANOVA across the three groups at baseline

Variable	Group 1	Group 2	Group 3	F statistic	P
	Mean \pm SD	Mean \pm SD	Mean \pm SD		
Weight (g)	1000.0 \pm 163.30	966.67 \pm 143.76	808.33 \pm 80.10	3.42	0.640
TC (mmol/l)	1.53 \pm 0.77	2.03 \pm 0.96	2.63 \pm 1.69	0.95	0.410
TG (mmol/l)	2.00 \pm 1.04	1.10 \pm 0.97	1.45 \pm 1.77	0.53	0.600
HDL-C (mmol/l)	0.48 \pm 0.22	0.83 \pm 0.60	0.70 \pm 0.46	0.67	0.530
LDL-C (mmol/l)	1.18 \pm 0.79	1.18 \pm 0.70	1.82 \pm 1.35	0.74	0.500

TC: Total cholesterol; TG: Triglycerides; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; SD: Standard deviation

Table 2. Summary of mean weights and serum lipid profiles; and comparative ANOVA across the three groups post-intervention

Variable	Group 1	Group 2	Group 3	F statistic	P
	Mean \pm SD	Mean \pm SD	Mean \pm SD		
Weight (g)	1525.00 \pm 150.00	1791.00 \pm 253.80	1550.00 \pm 164.32	2.96	0.870
TC (mmol/l)	3.73 \pm 0.75	15.72 \pm 0.27	15.68 \pm 0.42	955.66	< 0.001*
TG (mmol/l)	1.06 \pm 0.09	4.37 \pm 3.53	1.49 \pm 0.36	3.65	0.060
HDL-C (mmol/l)	0.98 \pm 0.39	1.76 \pm 0.44	2.31 \pm 0.70	7.10	0.010*
LDL-C (mmol/l)	2.27 \pm 0.79	11.97 \pm 1.14	12.67 \pm 0.42	213.67	< 0.001*

*Statistically significant

TC: Total cholesterol; TG: Triglycerides; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; SD: Standard deviation

Table 3. Post-hoc multiple comparison analysis of the three groups post-intervention

Variable	Multiple comparison	P
TC	Group 1 vs. Group 3	< 0.001*
	Group 1 vs. Group 2	< 0.001*
	Group 2 vs. Group 3	> 0.999
HDL-C	Group 1 vs. Group 3	0.010*
	Group 1 vs. Group 2	0.140
	Group 2 vs. Group 3	0.310
LDL-C	Group 1 vs. Group 3	< 0.001*
	Group 1 vs. Group 2	< 0.001*
	Group 2 vs. Group 3	0.530

*Statistically significant

TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol;

Discussion

This study evaluated the effects of extracts of *V. amygdalina* on rabbits placed on atherogenic diet with assessments of serum lipid profiles as well as pathomorphological changes. The group exposed to *V. amygdalina* extract did not appear to have had a significantly different serum lipid profile as well as pathomorphological changes. This study is important as previous studies evaluating the effect of *V. amygdalina* had been conducted in rats, rather than rabbits, which are better suited animal models for atherosclerosis research.

At baseline, all the rabbits in the three groups did not have a statistically significant difference in mean weights, which assured that they were comparable

across the groups. This was further buttressed by the mean weight gain across the groups over the course of the 12-weeks intervention, as their feeding regimen was standardized at 5-10% of the body weight of the rabbits. This feeding regimen was in keeping with the recommendations suggested feeding chow for rabbits on atherogenic diet should be restricted to prevent obesity.¹⁹

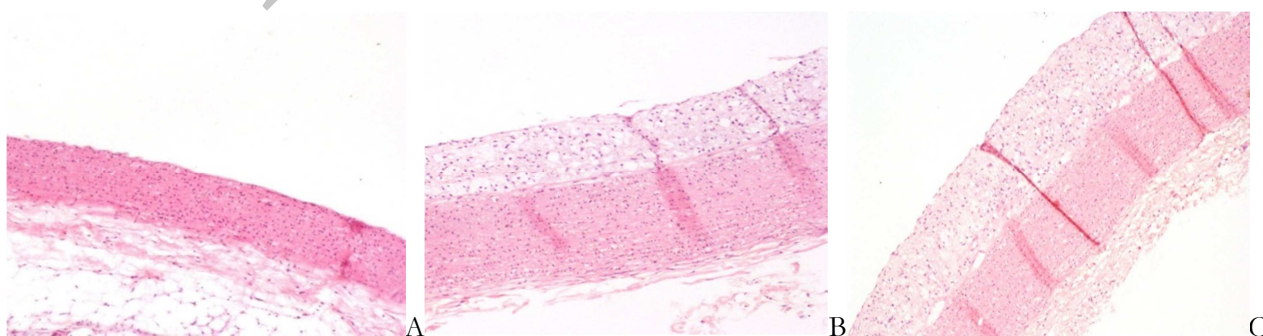
The post-intervention serum lipid profile results revealed a statistically significant increase in TC across all the groups (with baseline values as comparative standard). While this finding was altogether unsurprising for the groups on atherogenic diet, it was unexpected for the group on normal chow. However, a possible explanation is provided by Dontas et al.,²⁰ which reported age-related increase in TC of rabbits confined to cages over time, even when they are on normal chow. Thus, the observed statistically significant increase in TC for the control group of rabbits in this study may simply be an age-related finding. There was also no significant difference between the group on atherogenic diet compared with the group on both atherogenic diet and extracts of *V. amygdalina*. This may suggest that the extracts of the *V. amygdalina* may not be protective against the increase in serum TC. The observed significantly increased mean TC values in groups 2 and 3 after 12 weeks of atherogenic diet is in agreement with previously reported studies such as Zulli and Hare.¹⁷

Table 4. Baseline and post-intervention within group comparison of mean weight and lipid profiles

Variable	Group 1	Group 2	Group 3
	t (P value)	t (P value)	t (P value)
Weight	4.74 (0.020)*	8.20 (< 0.001)*	10.09 (< 0.001)*
TC	6.05 (0.010)*	30.92 (< 0.001)*	16.75 (< 0.001)*
TG	1.87 (0.160)	1.95 (0.110)	0.05 (0.960)
HDL-C	2.27 (0.110)	8.81 (< 0.001)*	4.44 (0.010)*
LDL-C	3.50 (0.040)	22.01 (< 0.001)*	17.53 (< 0.001)*

*Statistically significant

TC: Total cholesterol; TG: Triglycerides; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol

**Figure 1.** Hematoxylin-eosin stain of the aorta $\times 100$ magnificationGroup 1, normal diet (A), Group 2, atherogenic diet (B) and Group 3, atherogenic diet and 200 mg/kg/day *V. amygdalina* extract (C)

The pre- and post-intervention serum TG did not reveal any statistically significant difference across the groups. However, the mean scores of TG were higher in group 2 than both groups 1 and 3. This may suggest that the use of the aqueous extract of *V. amygdalina* may have had some beneficial effect on group 3 in terms of reducing the mean TG scores, even though this was not statistically significant. Previous studies conducted on rats and using 200 mg/kg of *V. amygdalina* reported a reduction in TG levels.^{12,13} To our knowledge, no previous study has evaluated the effects of *V. amygdalina* on rabbits, and it may well be that the usage of an lethal dose 50 (LD50), as well as graded increasing doses of the extract may have shown or definitively confirmed that the extract of *V. amygdalina* has no beneficial effect on serum lipid profiles. This is therefore a limitation of this study.

The HDL-C was the highest in the group on extract as compared to the group on atherogenic diet only as well as the group on normal chow. The HDL-C was the highest in group 3, and there was a statistically significant difference between groups 1 and 3. However, there was no such significance between groups 1 and 2 on the one hand, and between groups 2 and 3 on the other hand. This finding suggests that the HDL-C fraction which is protective against atherosclerosis was highest in the group which received aqueous extract of *V. amygdalina*. This suggestion of some possible benefit from the extract of *V. amygdalina* on HDL-C was not, however, reported by previous studies in rats.^{17,18}

The use of the extract did not have a beneficial effect on the level of LDL-C across the groups. LDL-C was significantly higher in the groups that had atherogenic diet (groups 2 and 3 as compared to group 1). However, there was no significant difference between the values in groups 2 and 3. This implies that the use of the aqueous extract of *V. amygdalina* did not appear to have resulted in a significant reduction of LDL-C, as compared to group 2. This is in contrast with previous studies in rats^{12,13} which reported otherwise. The reason for this observed difference in study findings may be due to the different animals used (rats versus rabbits) and the consequential physiologic differences in the metabolism of the extract.

Post-intervention histology

The post-intervention histological examination of the aorta in the three groups revealed that group 1 animals had a normal aortic wall while there was presence of atherosclerotic lesions in the aortic walls of animals in groups 2 and 3 (comprising

rabbits that had atherogenic diet). A previous study that utilized rabbits as an exploratory model for atherosclerotic studies, and whose atherogenic diet was utilized as a template in this study also found atherosclerotic lesions.¹⁷ Both studies also lasted for an equivalent duration of 12 weeks.

The features of atherosclerosis found in this study were similar in the two groups on atherogenic diet. These lesions consisted of thickened tunica intima with pools of extracellular lipids and several layers of foam cells. These features are consistent with the classification of atherosclerosis type III (intermediate lesions) as categorized by the American Heart Association.²¹ Furthermore, statistical analysis comparing the mean intima-media ratio of these two groups (0.76 ± 0.13 and 0.63 ± 0.11 , respectively) showed there was no statistically significant difference between them. This infers that the use of 200 mg/kg of aqueous extract *V. amygdalina* extract did not appear to have an ameliorating effect on the development of atherosclerosis in these rabbits.

Conclusion

In conclusion, this study showed that the use of atherogenic diet resulted in the induction of atherosclerotic lesions in rabbits. However, the use of 200 mg/kg/day of aqueous extract of *V. amygdalina* did not appear to exert a statistically significant effect on the serum lipid profile. It does not also appear to have exerted any beneficial effect on the lesions of atherosclerosis. Subsequent studies may explore the use of graded and increasing doses of the extract to ascertain if different doses may demonstrate an effect.

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Conflict of Interests

Authors have no conflict of interests.

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