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ORIGINAL RESEARCH

The Effects of Some Medicinal Plants from Southwest Nigeria on Serum Lipids Profile of Adult Male Wistar Rats

Odufuwa Kuburat T¹, Osonuga Ifabunmi O*², Ogunlade Albert ²,
Olukade Baliqis A², Okebule Femi B², Edema Victoria B²,
Taiwo-Ola Dorcas³

¹Department of Biochemistry, ²Department of Physiology, ³Department of Anatomy, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Sagamu Campus, Sagamu, Ogun State, Nigeria

*Correspondence: Dr IO Osonuga, Department of Physiology, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Sagamu Campus, Sagamu, Ogun State, Nigeria.

E-mail: osonuga.bunmi@oouagoiwoye.edu.ng ; ORCID – <https://orcid.org/0000-000159768901>.

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Abstract

Background: The global increase in the prevalence of hyperlipidaemia have become a worldwide health concern. Many herbal remedies have been studied and found to be effective for the treatment of increase in lipids in traditional medicine.

Objectives: To evaluate the efficacy of some medicinal plants on the serum lipid profile of male Wistar rats.

Methods: Sixty-five adult male Wistar rats (7-10 weeks old) were distributed into three groups: control (five rats), test (thirty rats), and recovery (thirty rats). The aqueous leaf extract of *Jatropha curcas*, *Phyllanthus amarus*, *Rauvolfia vomitoria*, *Mormodica charantia*, *Moringa oleifera*, and *Vernonia amygdala* was administered individually at a dose of 150 mg/kg body weight for 21 days. The control group was given normal food and water, the test group (n = 30) received 0.3 mL (150 mg/kg body weight) of the aqueous leaf extract orally for 21 consecutive days and were euthanized immediately after the treatment period. Recovery Group (n = 30) received the same dose and duration of extract administration (21 days) as the test group but were allowed a 21-day recovery period without further treatment before euthanasia.

Results: Oral administration of aqueous leaf extract at the dose of 0.3 ml (150 mg/kg body weight) caused a significant decrease ($p < 0.05$) in high-density lipoprotein levels (HDL) in Wistar rats when compared to control while there was an increase ($p > 0.05$) in low-density lipoprotein levels (LDL) and total cholesterol (TC) in the test and recovery group when compared with the control group.

Conclusions: Aqueous leaves extract from various medicinal plants caused a significant increase in low-density protein levels which can raise the risk for morbidities such as heart disease and stroke in humans.

Keywords: Aqueous Leaves Extract, Lipidaemia, Lipid profile, Medicinal Plants, *Moringa oleifera*, Wistar rats.

Introduction

Herbs from ancient times have been used to prevent and treat different ailments and diseases due to their ready availability and low cost of purchase [1]. A large number of people still depend on plants as a source of medicine [2,3], hence, the World Health Organization, recommended further studies into medicinal herbs, especially in the area of chronic and debilitating illness [1].

Hyperlipidaemia refers to elevated levels of lipids and cholesterol in the blood and it plays an important role in the development of atherosclerosis, the main cause of death in the world [4]. Medicinal plants are known to be a useful in some medical treatment because of their therapeutic effect, safety, and cost of procurement. Many remedies from medicinal plants were used to treat high levels of lipids, they decreased blood lipids through many mechanisms that included inhibition of the expression of fatty acid synthase, decreasing free fatty acid release, inhibition of HMG-CoA reductase, increasing the faecal excretion of fat and cholesterol, inhibition of the activity of pancreatic lipase and inhibition of cholesterol absorption [5-7].

Different approaches are recommended to manage increased levels of lipids, and these include modifications of lifestyle, diet, and pharmacotherapy options [8]. The more effort utilized for the management of high levels of lipids, the less beneficial results the patients receive [9], and with the worldwide use of lipid-lowering agents, the long-term effect is still in dispute [8]. Drugs used for reducing high levels of lipids are known to have various adverse effects such as myopathy, impaired liver function, neuropathy, and declined mental status. [10] Furthermore, the risk of diabetes mellitus has been associated with the use of lipid-lowering medications. [11]

The use of herbal remedies in traditional and folkloric medicine of different regions provides a good way for discovering and introducing

new drugs. [12] Of late, a tremendous effort in the patients' and physicians' desire to reduce high lipid profiles with natural extracts has been detected. [13] Large number of studies performed on the potency and safety of natural products showed auspicious changes in the lipid profile and thus, a reduction of the risk of cardiovascular complications. [13] On the other hand, a smaller number of studies revealed fewer positive effects on this matter or reported adverse effects of herbs, containing active biologic components. [14]

The aim of the present study was to determine the efficacy of some commonly used medicinal plants on hyperlipidaemia.

Methods

Experimental design

The following materials were used for the laboratory experiment and its technique: Weighing Scale, Bench Centrifuge (Model-800RD), Spectrophotometer (BIORAD), Lipid profile reagents (Spectrum Brand), Cuvette, Micro Pipette, and Disposable Pipette tips.

Local names and collection of plants materials

The fresh leaves of the medicinal plants used in the study were purchased from Falawo market, Sagamu, Ogun State. They were subsequently authenticated at the Forestry Research Institute, Ibadan. Voucher specimens have been deposited with the following authentication numbers: *Jatropha curcas* (111883), *Phyllanthus amarus* (112938), *Momordica charantia* (111885), *Moringa oleifera* (111884), and *Rauvolfia vomitoria* (112519).

These species are widely recognized in Nigerian ethnomedicine by the following local names: *Jatropha curcas* (*Lapalapa* in Yoruba, *Binidazugu* in Hausa, *Okwe* in Igbo); *Phyllanthus amarus* (*Ìyèyé/Ehin Olobe* in Yoruba, *Njeru Owa/Ogwu Ofe* in Igbo, *Gègon Hulà* in Hausa); *Momordica charantia* (*Ejinrin/Ejinrin weere* in Yoruba, *Ijiriji/Ndiga* in Igbo, *Garafuni* in Hausa); *Moringa oleifera* (*Zogale/Zogalla* in Hausa, *Ewe*

Igbale/Idagbo monoye in Yoruba, *Okwe Oyibo/Okochi egbu* in Igbo); *Vernonia amygdalina* (*Ewúro* in Yoruba, *Onugbu* in Igbo, *Shiwaka* in Hausa, *Oriwo* in Edo, *Etidot* in Efik/Ibibio); and *Rauvolfia vomitoria* (*Asofeyeje/Ireke abisu* in Yoruba, *Akata/Uta mkpo* in Igbo, *Danya/Dandana* in Hausa, *Ikpogiri* in Edo).

Preparation of aqueous leaf extract

The method of Mukhallad *et al.* [15] was modified for the aqueous extraction. The 60g powdered leaves of each medicinal plant was dissolved in 300 ml of distilled water in a plastic bottle and left for 24 hours. It was then filtered using a white sieve cloth, and the dissolved aqueous leaf extract was collected into an empty plastic. The residue was dried and reweighed to 47g which was finally deducted (60g - 47g=13g); this implied that 13g of the leaf dissolved in 300 ml. The aqueous extract was then refrigerated till it was needed.

Experimental animals

Sixty-five (65) adult male Wistar rats weighing between 180 and 200g were obtained from the Animal House, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ogun State, Nigeria. The rats were kept in thirteen different cages (5 per cage) and allowed to acclimatize to their environment for 14 days before the commencement of extract administration. They were all fed with mouse cubes.

Administration of aqueous leaf extract

The administration was done orally by giving 0.3 ml of each extract to different adult male Wistar rats using an oral cannula for 21 days.

Experimental analysis

Sixty-five (65) adult male Wistar rats were used in the experiment. The rats were divided into three broad groups and kept in different cages:

Group 1: Five rats in the control group received feed and water only.

Group 2: Thirty rats in the test group (5/leaf) received 0.3 ml of aqueous leaf extract of the medicinal plants for 21 days.

Group 3: Thirty rats in the recovery group (5/leaf) received 0.3 ml of aqueous leaf extract of the medicinal for 21 days and were allowed a 21-day recovery period without further treatment.

Collection of blood samples

At the end of 21 days of administration of aqueous leaves extract of the medicinal plants, blood samples were taken from the rats in all the test group into anti-coagulant bottles (Lithium Heparin). After another 21 days of recovery, blood samples were taken from all recovery groups and they were centrifuged and the serum was collected into plain bottles with a micro-pipette.

Procedures for lipid profile determination

Spectrum brand of lipid profile standard methods was strictly followed. Below are the protocols used:

Total Cholesterol

The wavelength was set at 500nm. (i) Blanking was read by mixed of adding 10µl of H₂O (distilled) + 1000µl of R¹ for 10 minutes at 20 - 25°C (ambient temperature). (ii) The standard absorbance was determined with known concentration by mixed of adding 10µl of standard + 1000µl of R¹ for 10 minutes at 20 - 25°C (ambient temperature). (iii) Then sample (serum) absorbance was determined by mixed 10µl of the sample (serum) + 1000µl of R¹ for 10 minutes at 20 - 25°C (ambient temperature). The calculation for Total Cholesterol was determined by:

$$\text{Total cholesterol in serum} = \frac{\Delta A \text{ of sample}}{\Delta A \text{ of standard}} \times \text{conc. of standard (553 mg/dL)}$$

High-Density Lipoprotein (HDL)

(i) Precipitant of HDL was determined by mixing 100µl of standard/sample + 250µl of

diluted precipitate which was allowed to sit for 10 minutes. This mixture was centrifuged for another 10 minutes at 4000rpm, then a clear

supernatant was removed and determination of cholesterol was done. (ii) The cholesterol determined was used to determine HDL cholesterol concentration by a mix of 100µl of H₂O + 1000µl of cholesterol R¹ incubated for 10 minutes at 20 - 25°C, then blank was read at 500nm. (iii) The standard absorbance was determined by a mix of 100µl of precipitated

standard + 1000µl of cholesterol R¹ which was incubated for incubate for 10 minutes at 20 - 25°C. (iv) Then sample (serum) absorbance was determined by a mix of 100µl of precipitated sample + 1000µl of cholesterol R¹ which was incubated for 10 minutes at 20 - 25°C. The calculation for HDL Cholesterol was determined by:

$$\text{HDL in serum} = \frac{\Delta A \text{ of sample}}{\Delta A \text{ of standard}} \times 180 \text{ mg/dL}$$

Triglycerides

A working standard of = R¹a + R¹b was prepared. (i) 5µl of H₂O, 5µl of standard, and 5µl of the sample (serum) were pipetted into separate test tubes. Then the addition of 500µl of working reagent was done which was

incubated at 37°C for 5 minutes. (ii) Absorbance was determined at 500nm. (iii) A plot of the standard curve was used to determine sample concentration at Assay linearity = 1172 mg/dL. The calculation for Triglyceride was determined by:

$$\text{Serum triglycerides conc. (mg/dL)} = \frac{\Delta A \text{ of sample}}{\Delta A \text{ of standard}} \times 200 \text{ mg/dL}$$

Low Density Lipoprotein (LDL)

$$\text{LDL} = \text{Total cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL}$$

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Statistical analysis

Statistical analyses were done using SPSS software package version 25. The data were summarised as Mean ± Standard Error Mean (SEM) and statistical comparisons were carried out using the Student's t-test and ANOVA. Statistical significance was defined by p-values less than 0.05.

Results

Table I presents the total cholesterol concentrations induced by various medicinal plants across control, test, and recovery groups. Notably, *Phyllanthus amarus* showed a significant reduction in cholesterol levels during the test phase (p = 0.020). *Rauvolfia vomitoria* and *Momordica charantia* also exhibited significant decreases in total cholesterol concentrations, (p = 0.001 and 0.002, respectively). In contrast, *Jatropha curcas*, *Moringa oleifera*, and *Vernonia amygdalina* did not demonstrate statistically significant changes in total cholesterol concentrations.

Table II shows the concentrations of high-density lipoprotein (HDL) induced by various medicinal plants. Significantly, *Jatropha curcas* showed a marked decrease in HDL levels during the test phase (p = 0.013). *Phyllanthus amarus* also exhibited a significant reduction (p = 0.009). Moreso, *Rauvolfia vomitoria* and *Momordica charantia* demonstrated extremely low HDL levels (p = 0.001 and 0.000, respectively). Additionally, *Moringa oleifera* and *Vernonia amygdala* also showed significant decreases in HDL (p = 0.001 and 0.000, respectively).

In Table III, the concentrations of low-density lipoprotein (LDL) induced by various medicinal plants are shown. *Phyllanthus amarus* exhibited a significant reduction in LDL levels from the test phase, (p = 0.014), indicating potential adverse effects. *Rauvolfia vomitoria* and *Momordica charantia* showed even more pronounced decreases in LDL levels, (p = 0.000 in each case). Additionally, *Moringa oleifera* and *Vernonia amygdala* demonstrated significant changes (p = 0.043 and 0.014). In contrast, *Jatropha curcas* did not show significant changes in LDL levels (p = 0.136).

Table I: Total Cholesterol (mg/dL) concentrations induced by selected medicinal plants

Groups	Control (Mean $\pm$ SEM)	Test (Mean $\pm$ SEM)	Recovery (Mean $\pm$ SEM)	p-values
<i>Jatropha curcas</i>		201.93 $\pm$ 58.08	142.21 $\pm$ 21.15	0.481
<i>Phyllanthus amarus</i>		268.93 $\pm$ 39.94	72.23 $\pm$ 29.55	0.020
<i>Rauvolfia vomitoria</i>	150.53 $\pm$ 14.67	253.33 $\pm$ 16.97	100.22 $\pm$ 31.94	0.001
<i>Mormodica charantia</i>		234.05 $\pm$ 25.5	112.19 $\pm$ 14.30	0.002
<i>Moringa olifera</i>		273.98 $\pm$ 33.65	191.19 $\pm$ 61.87	0.143
<i>Vernonia amygdala</i>		212.03 $\pm$ 45.59	185.99 $\pm$ 12.01	0.343

SEM - Standard Error Mean

Table II: High-density Lipoprotein (mg/dL) concentrations induced by selected medicinal plants

Groups	Control (Mean $\pm$ SEM)	Test (Mean $\pm$ SEM)	Recovery (Mean $\pm$ SEM)	p-values
<i>Jatropha curcas</i>		106.42 $\pm$ 28.37	42.30 $\pm$ 3.52	0.013
<i>Phyllanthus amarus</i>		94.10 $\pm$ 25.20	33.32 $\pm$ 2.02	0.009
<i>Rauvolfia vomitoria</i>	122.52 $\pm$ 5.73	53.05 $\pm$ 15.95	49.10 $\pm$ 5.24	0.001
<i>Mormodica charantia</i>		61.87 $\pm$ 13.74	44.58 $\pm$ 1.23	0.000
<i>Moringa oleifera</i>		49.13 $\pm$ 18.01	41.20 $\pm$ 6.33	0.001
<i>Vernonia amygdala</i>		64.08 $\pm$ 12.40	36.58 $\pm$ 1.29	0.000

SEM - Standard Error Mean

Table III: Low-density Lipoprotein (mg/dL) concentrations induced by selected medicinal plants

Groups	Control (Mean $\pm$ SEM)	Test (Mean $\pm$ SEM)	Recovery (Mean $\pm$ SEM)	p-values
<i>Jatropha curcas</i>		85.98 $\pm$ 42.66	66.00 $\pm$ 19.02	0.136
<i>Phyllanthus amarus</i>		147.69 $\pm$ 38.92	50.80 $\pm$ 26.86	0.014
<i>Rauvolfia vomitoria</i>	11.06 $\pm$ 11.99	157.80 $\pm$ 27.69	39.98 $\pm$ 13.68	<0.001
<i>Mormodica charantia</i>		140.11 $\pm$ 22.10	39.97 $\pm$ 13.68	<0.001
<i>Moringa olifera</i>		179.76 $\pm$ 65.78	122.10 $\pm$ 65.78	0.043
<i>Vernonia amygdala</i>		103.65 $\pm$ 42.07	113.65 $\pm$ 11.11	0.014

SEM - Standard Error Mean

Table IV shows the relatively lower mean concentrations of triglycerides caused by various medicinal plants, highlighting their potential adverse lipid effects. Significantly, *Jatropha curcas* showed a reduction in triglyceride levels compared to the control to the test phase, with a p-value of 0.046. Similarly, *Phyllanthus amarus* exhibited a significantly lower triglyceride levels (p = 0.006), while *Rauvolfia vomitoria* also demonstrated a significant reduction (p = 0.013). In contrast, *Momordica charantia*, *Moringa oleifera*, and *Vernonia amygdala* did not display significant differences compared with the control.

Table IV: Triglycerides (mg/dL) concentration induced by selected medicinal plants

Groups	Control (Mean $\pm$ SEM)	Test (Mean $\pm$ SEM)	Recovery (Mean $\pm$ SEM)	p-values
<i>Jatropha curcas</i>		167.06 $\pm$ 28.80	174.52 $\pm$ 2.85	0.046
<i>Phyllanthus amarus</i>		135.93 $\pm$ 23.00	174.82 $\pm$ 8.51	0.006
<i>Rauvolfia vomitoria</i>	220.66 $\pm$ 4.95	212.74 $\pm$ 14.25	173.17 $\pm$ 2.63	0.013
<i>Mormodica charantia</i>		159.78 $\pm$ 32.35	168.26 $\pm$ 4.36	0.11
<i>Moringa olifera</i>		187.93 $\pm$ 71.24	139.41 $\pm$ 35.02	0.475
<i>Vernonia amygdala</i>		182.15 $\pm$ 24.71	178.52 $\pm$ 6.33	0.131

Discussion

A large number of the populace rely on plants as a source of medicine, [16] hence, the World Health Organization, recommended further investigation into medicinal plants, especially in the area of chronic and debilitating illness and diseases. [1] It is important to note that a disturbance of the normal concentrations of key lipids: total cholesterol (TC), triacylglycerol (TAG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and total lipid, is indicative of ill health. [17] The present study showed the impacts of some herbal medicines on lipid profiles.

The administration of aqueous leaf extract of 150 mg/kg dose of *Jatropha curcas*, *Phyllanthus amarus*, *Rauvolfia vomitoria*, *Mormodica charantia*, *Moringa olifera*, and *Vernonia amygdala* individually showed a significantly lower serum concentrations of HDL in the test group and recovery group when compared with the control group. The results agree with several documented reports suggesting lower levels of HDL-c concentration in hyperlipidaemia. [18] Current studies have discovered that decreased HDL-c levels may not necessarily be a good sign. There are different ways of explaining the significant decreases in HDL-c as seen in this study. It may suggest being involved in mopping up lipid hydroperoxides induced by oxidative stress. The observed significant decrease in HDL-c concentration in this study may have arisen when there is no lipid hydroperoxides to mop up. HDL-c is generally referred to as “good cholesterol” due to its function in reversing cholesterol transport, removing cholesterol from within the artery, and transporting the same back to the liver for onward excretion and utilization. Oxidative stress has the capacity to modify the protective effects of HDL-c by oxidizing it, thereby making it proatherogenic rather than anti-inflammatory. [19, 20]

However, in contrast to findings in the present study, HDL-c has been observed to have antioxidant and anti-inflammatory properties. [21] Pathological processes such as inflammation can overwhelm the antioxidant and anti-inflammatory functions of HDL-c, changing it to a ‘dysfunctional’ pro-inflammatory, pro-oxidant, prothrombotic, and proapoptotic particle which may affect reverse cholesterol transport and thus lead to cardiovascular diseases. [22]

Also in the present study, the administration of aqueous leaf extract of medicinal plants individually showed significantly higher serum concentrations of total cholesterol and low-density lipoprotein in the test groups treated with *Phyllanthus amarus*, *Rauvolfia vomitoria*, and *Mormodica charantia*, when compared with control group. On the other hand, significantly lower triglycerides concentrations was observed following treatment with aqueous extract of *Jatropha curcas*, *Phyllanthus amarus*, and *Rauvolfia vomitoria* in test groups and the recovery groups when both were compared to the control group. High levels of low-density lipoprotein (LDL) have been linked to an increased risk of atherosclerosis, which may then result in various diseases such as peripheral arterial diseases, coronary artery disease, and stroke. [23] Increased levels of LDL-cholesterol, also known as hypercholesterolaemia, increases the risk of premature atherosclerotic cardiovascular diseases (ASCVD). Documented evidence also has it that decreased HDL-c levels may be due to the inability of efficient clearance of LDL-c from circulation, which might be due to oxidative stress. [19, 20]

Consequently, it is suggested that a significantly higher intake of aqueous extract of *Phyllanthus amarus*, *Rauvolfia vomitoria*, and *Mormodica charantia* may suggest a risk for cardiovascular diseases, which may arise from metabolic disorders such as dyslipidaemia. [9]

Reports have linked the elevation of serum cholesterol, LDL-c, and triacylglycerol concentrations with the aetiology of cardiovascular diseases. [24].

Low-density lipoprotein has been observed to be a major component of total cholesterol and is related to the occurrence of coronary heart disease, as a major atherogenic lipoprotein. Low-density lipoprotein should thus be the main target of therapeutic interventions of any agent that aims to decrease lipid level using medicinal plants. Future studies on significant compound structures of these various plants and their molecular interactions are desired.

Conclusion

The administration of aqueous leaf extract of *Phyllanthus amarus*, *Rauvolfia vomitoria*, and *Mormodica charantia* may cause an elevation in serum low-density lipoprotein levels. The leaf extract, can raise the risk for cardiovascular diseases in humans, though the animals recovered from the effects as observed in the recovery groups in the present study. Therefore, it is advised that this leaf be ingested or consumed with caution.

Authors' Contributions: OKT, OIO, OAA and TDB. OAA and OIO participated in the design of the study and drafted the manuscript. OKT, OIO, OAA, OBA, OBO and EVB analyzed and interpreted the data. OKT, OIO, OAA and TDB revised the draft for sound intellectual content. All the authors approved the final version of the manuscript.

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