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Hypolipidaemic Potentials of *Vernonia amygdalina* (Bitter Leaf) in male albino rats fed high-sucrose diet

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Abstract

Background: Increasing prevalence of obesity, overweight and metabolic syndrome secondary to excessive consumption of a high-sucrose and high-fat diet is a major global health problem.
Objective: This study was meant to determine the hypolipidaemic potentials of phytochemicals present in*Vernonia amygdalina*(Bitter leaf) in obese rats**.**
Method: Thirty-two (6 weeks old, weighing 150-160g) male Wistar rats, were randomly selected and grouped into four: with eight rats in each group. The rats in the control group were fed normal rat chow and water while the three experimental groups were fed the various compounded diets and water *ad libitum*. At the end of 12th week, the animals fasted overnight, and Oral Glucose Tolerance Test (OGTT) was performed in some while others were anaesthetized using diethyl ether. Fasting plasma, liver tissue lipid and lipoprotein levels were determined, and LDL-C was calculated.
Result: Rapid weight gain (89.9%) was recorded in rats fed High Sucrose Diet [HSD] when compared with the control group (55.3%) without significant differences in the liver weight. The mean plasma glucose, triglyceride, total cholesterol and LDL-C levels were significantly higher ($p = 0.000$, $p = 0.010$, $p = 0.000$ and $p = 0.002$ respectively) while mean plasma HDL-C was significantly lower ($p = 0.043$) in rats fed HSD when compared with rats fed normal rat chow. A significantly lower plasma lipid and higher liver tissue HDL-C ($p = 0.03$) were observed in the *Vernonia amygdalina*-treated rats.
Conclusion: HSD induces hepatic steatosis and the related biochemical changes while dietary *Vernonia amygdalina* ameliorates the biochemical changes in hepatic steatosis in rats.

Keywords: Hepatic steatosis; Hypolipidaemia, Obesity; Sucrose; Vernonia amygdalina.

Introduction

Globally, the prevalence of obesity, overweight and metabolic syndrome is increasing, mainly from the excessive consumption of high-fat diet (HFD) and high sucrose diet (HSD), both of which ultimately leads to elevated plasma triglyceride and cholesterol levels. [1 - 3] The increasing incidence of

overweight and obesity in Africa, in particular among the middle-class workers, is mainly due to the adoption of Western diet, sedentary lifestyle and reduced dietary fibre intake. The westernised diet entails the consumption of high-calorie diet which has been linked to the development of many metabolic disorders such as hyperglycaemia, hyperinsulinaemia, obesity, hypertension and dyslipidaemia. [4]These diet-induced metabolic disorders occur consequent upon the generated excess Reactive Oxygen Species (ROS), which increases protein carbonylation levels. [5]

The global consumption rate of fresh vegetables is low, especially in the developed countries, due to preferences for the consumption of conveniently processed vegetables rather than the fresh ones. [6] Most dietary plant materials are also medicinal, but a lot of these medicinal values are yet to be scientifically determined. Most of the known medicinal values are presently exploited in the traditional treatment of some ailments.

Vernonia amygdalina (Bitter leaf) is a plant that is widely grown in Nigeria, especially in the south-eastern and south-western parts of the country. It is a shrub 2 to 5 meters tall with petiolate leaves about 6.0 mm wide. [7] The leaves of the plant are consumed as vegetables while the leaf extract is used to treat certain common ailments. Some of the several medicinal values of *Vernonia amygdalina* had been reported; these include antiparasitic, antiviral, antibacterial, antimalarial, antifungal actions as well as antihyperglycaemic effect in diabetes mellitus. [8 - 14] The leaf extract of the plant is also locally used to treat abdominal cramps and other gastrointestinal tract disorders such as constipation and as anxiolytic for insomnia. Therefore, it appears *Vernonia amygdalina* has a lot of potential therapeutic values yet to be harnessed. [15]

The plasma lipid profile is an important diagnostic tool, particularly in conditions such as obesity, diabetes mellitus, coronary heart disease, atherosclerosis, hypertension, infertility, obstructive jaundice, hepatitis and other diseases characterised by abnormalities of lipid metabolism. Atherogenicity of Low-Density Lipoprotein-Cholesterol (LDL-C) is rightly implicated in the pathogenesis of cardiovascular diseases (myocardial infarction, acute coronary syndrome, cerebrovascular accident) through the formation of intra-arterial plaques and atherosclerotic lesions. [16] The risk for the progression of cardiovascular diseases has been reported high in obesity, hypertension and hypercholesterolaemia. [17]
The drugs (Statins) that are used to reduce plasma lipid in clinical settings act by reducing the LDL-C level through competitive inhibition of HMG-CoA reductase enzyme, an enzyme that catalyses the rate-limiting step in the biosynthesis of cholesterol. [18] These drugs have several side effects such as hepatotoxicity, muscle toxicity such as myopathy, rhabdomyolysis and acute renal failure. [19, 20] Therefore, attention is shifting in scientific circles towards phytochemicals with relatively little or no unwanted effects in the management of metabolic disorders. *Vernonia amygdalina* is one source of such phytochemicals. This study aimed to determine the effect of *Vernonia amygdalina* on the plasma and hepatic tissue lipids in overweight and obese male albino rats.

Methods

Plant material
Fresh leaves of *Vernonia amygdalina* were harvested from a local farm in Ago-Iwoye, Ogun State, Nigeria. The leaves were washed and blended to obtain the extract.

Feed composition
The rat chow pellets obtained from FA Feeds, Ijebu-Ode, were ground to a powder using pestle and mortar. The obtained powder was mixed with sucrose and extract of *Vernonia amygdalina* leaves respectively in different proportions. The obtained mixture was moistened with distilled water, pelletized, oven-dried at 60°C for two days to

maintain the initial water content of pellets. This mixture was kept in air-tight polythene bags for feeding.

Preparation of animals

Thirty-two (six weeks old, weighing150 to160g) male Wistar rats were randomly selected from a population of forty rats. These selected rats were grouped into four; each group comprised eight rats. The rats were acclimatised for one week, during which period they were fed standard rat chow and water *ad libitum*. Afterwards, the rats were grouped as follows:

a) Group 1 (G1): 100%Rat chow (Normal group)
b) Group 2 (G2): 100% Rat chow + 100mg/kg Bitter leaf feed
c) Group 3 (G3): 60% Rat chow + 40% Sucrose
d) Group 4 (G4): 60% Rat chow + 40% Sucrose + 100mg/kg Bitter leaf feed

The rats were allowed access to the feed *ad libitum* for twelve weeks. The rats were also weighed weekly. At the end of 12th week, the animals were fasted overnight and were anaesthetized using diethyl ether. Blood was collected by cardiac puncture, and the liver was also harvested. The obtained blood was dispensed into a heparinized bottle, centrifuged at 3000rpm for 5 minutes to get the plasma which was further stored for analysis. The harvested liver tissue was put into a mortal, 2ml phosphate buffer added and macerated till homogenate was obtained. The homogenate was then centrifuged at 10,000rpm for 10 minutes, and the supernatant was collected into a clean labelled test tube for analysis. [21]

Oral glucose tolerance test

Oral Glucose Tolerance Test (OGTT) was performed at Week 12 as follows: 50% Glucose was administered orally at the dose of 1g/kg body weight to overnight fasted rats. [22] Thereafter, blood was collected from the tail vein at 0, 30, 60, 90, and 120 minutes after glucose administration to determine the fasting blood glucose level.

Biochemical parameters

(a) Fasting plasma glucose determination

The fasting plasma glucose was determined using Accu-chek® glucometer. A drop of fasting blood sample obtained from rat tail vein and also by cardiac puncture was put on the test strip for the glucometer, and the value read off.

(b) Plasma and Tissue lipid profile estimation

Fasting plasma and liver tissue triglyceride (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) were determined enzymatically using test kits obtained from Randox Laboratories®, Crumlin, England, based on the standard methods. [23 - 25] The levels of low-density lipoprotein cholesterol (LDL-C) and the atherosclerotic index (LDL-C/HDL-C) were consequently calculated. [24, 26]

Statistical Analysis

The data were analysed using SPSS version 21. The variables were all expressed as the mean and standard error of the mean (Mean ± SEM) and represented as tables and graphs. One-way ANOVA was used to compare mean differences between the groups, and the level of significance was assessed using Duncan Multiple Range Test at $P < 0.05$.

Results

Body and liver weights

Rats fed high-sucrose diet (HSD) only (G3) gained more weight (89.9%) compared with (55.3%) among the control group fed standard rat chow diet (G1) although the food intake remained unchanged in the two groups over the period of the experiment (Table I). The weight gain among the rats fed HSD, and bitter leaf was observed to be slower. There was no significant difference in the mean liver weight between the groups.

Table I: Mean values of total body and liver weight of *Vernonia amygdalina*- treated rats and the control groups

Groups	Initial Body Weight (g)	Final Body Weight (g)	%Weight change	Liver weight (g)	Hepatosomatic Index (%)
G1	158.1 ± 5.6	245.5 ± 11.5[a]	55.3	8.1[c]	3.29
G2	155.3 ± 5.4	230.5 ± 10.5[b]	48.4	7.5	3.25
G3	162.1 ± 5.8	308.5 ± 15.2[a]	89.8	10.1[c]	3.27
G4	157.9 ± 5.6	265.8 ± 13.2[b]	68.3	8.7	3.27

Superscripts "a" "b" and "c" represent significant increase, decrease and no significance between comparison groups respectively.

Plasma glucose

The patterns of OGTT are shown in Figure 1. A significantly higher (p = 0.000) mean plasma glucose level was observed among HSD-fed rats (G3) compared with the group fed on rat chow only (G1). A slight reduction in mean plasma glucose was noted in the *Vernonia amygdalina*-treated groups (G2, G4) (Table II).

Plasma and Liver lipid profile

The mean plasma TG, mean total cholesterol, mean LDL-C were significantly higher (p = 0.01, p = 0.000, p =0.002) while the mean plasma HDL-C was significantly lower (p = 0.012) among the rats fed HSD (Group 3) compared with those fed normal rat chow (Group 1). The mean plasma TG, total cholesterol and LDL-C were significantly higher (p = 0.01, p = 0.000, p = 0.002) while mean plasma HDL-C was significantly lower (p = 0.012) among the rats fed HSD plus bitter leaf (Group 4).

The mean liver TG, total cholesterol, LDL-C were significantly lower (p = 0.027, p = 0.000, p = 0.002) while the mean liver HDL-C was significantly lower (p = 0.001) among the rats fed HSD plus bitter leaf (G4) compared with those fed HSD alone (G3) as shown in (Table III). In addition, the mean liver LDL-C was significantly higher (p = 0.000) among rats fed normal rat chow diet plus bitter leaf (G2) compared with rats fed normal rat chow diet alone (G1) whereas the mean liver HDL-C were insignificantly lower (p = 0.082) in the former group of rats compared with the latter. Lower mean liver cholesterol, mean LDL-C and higher mean liver TG and mean liver HDL-C were observed among rats fed normal rat chow diet plus bitter leaf (G2) (Table III).

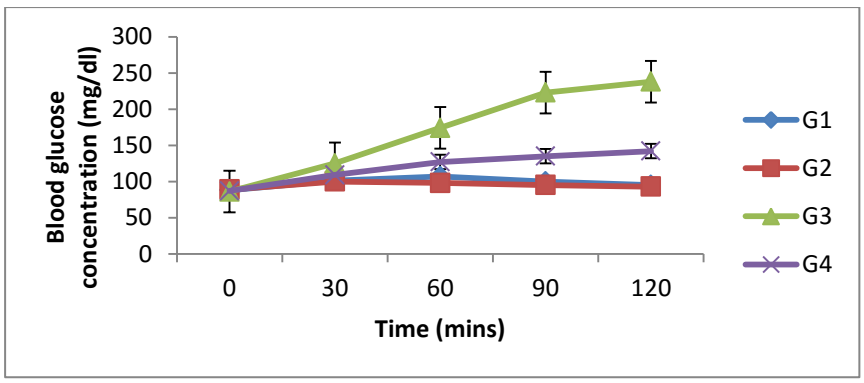

Figure 1: Oral Glucose Tolerance Test patterns in Bitter leaf-treated groups and controls

Discussion

Diet-induced animal models are usually used to study the pathogenesis and metabolic turnover of substances in the body using methods not applicable to human. This method is used to investigate some metabolic disorders such as obesity, diabetes mellitus and metabolic syndrome. The effect of diet on the body mass depends on the total amount and the type of food ingested. This effect of diet is also influenced by the amount of calories obtainable from such diet. Food consumption in the right proportions maintains body carbohydrate, protein, lipid, and lipoproteins stores resulting in normal blood levels of body lipid and lipoproteins. This study was designed to involve a higher energy intake among rats fed with sucrose than controls.

Table II: Mean values of plasma glucose and lipid profile levels of study groups

Groups	Glucose (mg/dL)	Triglyceride (mg/dL)	Total Cholesterol (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	AI
G1	92.60 ± 8.67	120.60 ± 5.69	158.76 ± 12.38	24.56 ± 2.79	110.08 ± 14.16	6.46
G2	85.80 ± 7.29	141.80 ± 6.91[a]	154.33 ± 6.49	27.85 ± 1.06	98.12 ± 6.52[b]	5.54
G3	168.30 ± 8.13[a]	159.96 ± 7.03[a]	173.20 ± 8.36[a]	8.23 ± 0.94[b]	132.97 ± 6.97[a]	21.04
G4	96.30 ± 6.81	128.99 ± 5.98[b]	160.41 ± 9.25[b]	22.4 ± 1.16[a]	112.20 ± 9.36[b]	7.15

HDL-C – High-Density Lipoprotein-Cholesterol; LDL-C – Low-Density Lipoprotein-Cholesterol; AI – Atherosclerotic Index
Superscripts "a" and "b" represent a significant increase and decrease between comparison groups respectively.

Table III: Mean values of liver tissue lipid profile in the study groups

Group	Triglyceride (mg/dL)	Total Cholesterol (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
G1	79.01 ± 6.39[b]	128.50 ± 1.75	30.99 ± 2.21	81.71 ± 2.65
G2	111.74 ± 3.82[a]	127.24 ± 1.27	51.05 ± 1.11	53.84 ± 2.35[b]
G3	127.05 ± 1.77[a]	117.11 ± 3.37[a]	26.66 ± 3.10	65.04 ± 4.15
G4	97.73 ± 2.27[b]	98.12 ± 2.83[b]	36.84 ± 2.10	41.73 ± 3.43[b]

HDL-C – High-Density Lipoprotein-Cholesterol; LDL-C – Low-Density Lipoprotein-Cholesterol
Superscripts "a" and "b" represent a significant increase and decrease among comparison groups respectively.

HSD plays a critical role in the onset of oxidative stress-induced insulin resistance. [27] The source of oxidative stress, in this instance, is post-prandial hyperglycaemia resulting in overproduction of superoxide by the mitochondrial electron-transport chain. [28] There is a resultant cellular redox impairment which leads to inactivation of the insulin signalling pathway. [29]

The reports from previous researches on HSD intake had revealed an increase in body biophysical and some biochemical features. While Hallfrisch *et al*. [30] reported increase in

body weight in Wistar rats fed on 30% (w/w) sucrose over a period of eight to nine weeks, Huang *et al*. [31] reported an increase in body weight, fasting blood glucose and plasma cholesterol. [32, 33] Increased body weight, plasma insulin, plasma triglyceride, plasma cholesterol, mild fasting hyperglycaemia and impaired oral glucose tolerance test were noted in HSD-fed rats over a period of four weeks. [34] From the present study, the HSD-fed group (G3) had a significantly increased body and liver weights when compared with the control group. Contrary to this finding, a decrease in body weight of HSD-fed rats had earlier been reported. [35]

At the end of experimental period, HSD-fed rats weighed about 26% higher than the control group. The observed increase in liver weight of HSD-fed rats is probably due to the accumulation of lipids in the liver compared to rats fed on standard rat chow. On the other hand, a significant reduction was observed in the body weight and liver weight of the groups fed on the *Vernonia amygdalina.* An indication of reserve energy status in the rats was expressed as the hepatosomatic index (ratio of liver weight to body weight). [36] The hepatosomatic index (HSI) at the end of 12 weeks showed no significant difference among all the groups, though the liver of the HSD-fed rats was the largest.

The result of mean plasma glucose was higher among the HSD-fed group compared with the group fed on rat chow but slight lower among *Vernonia amygdalina*-treated groups. A previous study had reported increased fasting plasma insulin, insulin resistance, glucose intolerance and increased fasting plasma triglyceride levels in HSD-fed rats over a six-week period. [37]

The pattern of OGTT in the present study showed that HSD-fed rats had higher blood glucose than the rats fed rat chow alone. This pattern reflected impairment of glucose handling mechanisms and suggested the development of impaired response to insulin action in the HSD-fed group of rats. The deterioration in OGTT results among HSD-fed rats indicated that the ingestion of HSD might have contributed to the worsened glucose tolerance observed in that group. However, the rats in the *Vernonia amygdalina*-treated groups showed a normal response to OGTT suggesting that *Vernonia amygdalina* assisted with the maintenance of plasma glucose homoeostasis in the event of high oxidative stress. The improvements in OGTT response observed in the *Vernonia amygdalina*-treated groups may be associated with decreased fasting plasma insulin. This observation implied that *Vernonia amygdalina* might have the potential to improve insulin sensitivity and reduce insulin resistance following a high carbohydrate diet. This potential is probably a function of the flavonoids present in *Vernonia amygdalina*. [38]

Hypertriglyceridaemia is an important risk factor for coronary heart disease while cholesterol is the major lipid constituent of atherosclerotic plaques. [39] Hypertriglyceridaemia was observed in the HSD-fed groups in the present study while reductions in plasma lipid and lipoproteins were observed in the group fed rat chow and *Vernonia amygdalina*. The administered bitter leaf appeared to have reduced plasma triglyceride, cholesterol, LDL-C and caused an increase in plasma HDL-C. Therefore, it is attractive to infer that dietary bitter leaf improved associated dyslipidaemia in obese rats. The observed hypertriglyceridaemia among rats fed HSD in the present study may be related to the high sucrose concentration in the diet. [40]

High intake of refined sugar (dietary content up to 60% of calories) had been reported to induce elevated plasma triglyceride levels. [41] Hypertriglyceridaemia is a resultant effect of the

breaking down of sucrose into glucose and fructose by sucrase enzyme. Thereafter, fructose is absorbed and converted to lipogenic precursors resulting in hypertriglyceridaemia. [42] The observed reduction in plasma triglyceride and LDL-C and increase in HDL-C level in rats fed HSD and bitter leaf in the present study may be a measure of the effectiveness of *Vernonia amygdalina* in the prevention or modification of dyslipidaemic events in high energy state. This probably reduces plasma macrovascular disease and thus, the mortality in subjects with metabolic syndrome. The observed decrease in the lipid profile corroborated earlier reported hypolipidaemic effects of *Vernonia amygdalina*. [15, 41]

Higher levels of liver triglyceride, total cholesterol and LDL-C and reduction in HDL-C were observed among HSD-fed rats compared with the *Vernonia amygdalina*-treated rats. Also, the *Vernonia amygdalina*-treated rats demonstrated significantly higher levels of the liver triglyceride and HDL-C but significantly lower levels of compared with rats fed on rat chow alone. These observations may imply high metabolic dynamics (lipolysis, glycogenolysis and gluconeogenesis) in the liver due to its central role in metabolism.

The Atherosclerotic Index (AI) may indicate the deposition of foam cells or plaque or fatty infiltration of the liver, heart, coronary vessels, the aorta and the kidney. The higher the atherosclerotic index, the greater the risk of oxidative damage to the organs. From the present study, AI was observed to be significantly lower in *Vernonia amygdalina*-treated rats. This effect can be directly related to the reduction of plasma lipid and lipoproteins, thus, suggesting the possible benefit of *Vernonia amygdalina* in the prevention of atherosclerotic lesions and vascular dysfunctions.

Therefore, the current observations indicate that *Vernonia amygdalina* has a strong anti-obesity property in obese rats. The probable mechanism for this anti-obesity property of bitter leaf is probably a modulating effect on the expression of obesity-related genes and possibly, through increased phosphorylation of adenosine monophosphate-dependent kinase (AMPK) in the visceral adipose tissue. [38]

Conclusion

The present study provided evidence that HSD-induced biochemical changes which suggested that sucrose-induced hypertriglyceridaemia was associated with abnormal OGTT in rats. Dietary consumption of *Vernonia amygdalina* ameliorated the biochemical changes in the liver and improved OGTT. Therefore, *Vernonia amygdalina* could be considered as a promising complementary treatment against the development of glucose impairment and dyslipidaemia associated with high sucrose diet.

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Authors' Contribution: OWE conceived and designed the study and OOE participated in designing the study. OOO procured the rats and conducted most of the laboratory aspects of the research. OWE drafted the manuscript. OOE and AAO participated in drafting the manuscript. All the authors approved the final version of the manuscript.
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