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Comparative Effects of the Leaves of *Gongronema latifolium* and *Vernonia amygdalina* Incorporated Diets on the Lipid Profiles of Rats

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Abstract

The hypolipidaemic effects of the leaves of *Gongronema latifolium* (GL) *and Vernonia amygdalina* (VA) diet preparations on the lipid profile of rats were compared. The rats were fed for 28 days on diet specially formulated to contain 5%, 15% and 30% by weight of the leaves of each plant respectively while the control group was fed standard rat diet. The serum total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein cholesterol (HDL-C) and low- density lipoprotein cholesterol (LDL-C) were determined on blood samples collected on the 28th day. The results show that the VA diet induced a significantly lower serum total cholesterol level at the 15% and 30% concentrations relative to the GL diet preparation. The results also show that there was no significant difference between the effects of GL and VA diet preparations at the various levels of treatments. The comparison of the effects of the two diet preparations show that VA produced higher levels of HDL-C compared to GL which was significant at 5% and 15% concentrations respectively. It appears from our results that VA diet preparation may have better hyperlipidaemic effect than GL diet preparation. Therefore, VA may have better therapeutic promise in preventing lipid related pathologies compared to GL

Keywords: *Gongrnema latifolium, Vernonia amygdalina,* lipid profile.

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INTRODUCTION

Lipids play a critical role in almost all aspects of biological life. They are the structural components in cells and are involved in metabolic and hormonal pathways[1]. Lipid and lipoprotein abnormalities are well known risk factors for heart disease. Elevated levels of triacylglycerols (TG), cholesterol, and low density lipoprotein-cholesterol (LDL-C) are documented as risk factors for atherogenesis[2-7]. The blood level of high density lipoprotein-cholesterol (HDL-C) in contrast bears an inverse relationship to the risk of atherosclerosis and coronary heart disease. The higher the level, the smaller the risk[8,9]. Genetic factors and diet both play a major role in regulating cholesterol and triacylglycerols levels in the plasma[5,10]. High levels of cholesterol, particularly LDL-cholesterol, are mainly responsible for hypercholesterolaemia[5]. Hypercholesterolemia is known to be associated with enhanced oxidative stress related to increased lipid peroxidation[11]. Increased generation of oxidized LDL-C is a major factor in the vascular damage associated with high cholesterol levels[12]. There is a need for a drug that may control both hypercholestrolaemia and hypertriglyceridaemia. Hence the nutritional inhibition or reduction of hypercholestrolaemic conditions is considered to be an important therapeutic approach and efforts have been made to identify medicinal plants with these functions[7,13-15].

Vernonia amygdalina (Compositae) is a shrub that grows predominantly in the tropical Africa. In Nigeria, the plant is locally called bitter leaf because of its bitter taste. The leaves have found relevance in traditional folk medicine as antihelmint, a laxative herb and an antimalarial as they are known as quinine substitute[16]. It is also used in the treatment of cough and hypertension[17-19]. *Gongronema latifolium* (Asclepiadaceae) is also a tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine[20, 21]. Reports by various authors showed that it contains essential oils, saponins and pregnans among others[22-24]. The leaves of *Gongronema latifolium* have protective role against diabetes, hypertension, stomach upsets and pains, and typhoid fever[25]. Following the recorded pharmacological functions of these plants, the present study was designed to compare the effect of the two diet preparations on the lipid profile of rats.

MATERIALS AND METHODS

Animals

Twenty five male albino rats (Wistar strain) weighing between 93-120g were used as experimental animals. The rats were kept in cages for two weeks to acclimatize, and were allowed free access to food and water *ad libitum*. The protocol is in line with the guidelines of the National Institute of Health (NIH) (NIH Publication 85-23, 1985) for laboratory animal care and use.

The experimental animals were randomly distributed into four groups of five animals each. Group 1 rats were fed standard rat diet (Vital feeds, Nigeria), and served as the control, while groups 2,3,4 were fed on diets containing 5%, 15% and 30% by weight of *Gongronema latifolium* leaves for 28 days. The protocol was repeated with *Vernonia amygdalina* leaves for another set of rats.

Feed formulation

The leaves of *Gongronema latifolium* and *Vernonia amygdalina* were purchased from a local market in Anyigba, Kogi state, Nigeria. The botanical identification and authentication was confirmed at the Department of Biological Sciences, Kogi State University, Anyigba. The leaves were dried at room temperature for 2 weeks to a constant weight and then powdered. The standard rat diet was similarly milled. The feed for each leaf type was mixed to contain 5%, 15% and 30% by weight of the leaves for groups 2, 3, and 4 respectively.

Sample collections

Overnight prior to treatment, the animals were starved of food. Blood was collected from the ocular median-cantus vein of the rats with the aid of capillary tubes and transferred to test tubes and allowed to clot and subsequently centrifuged to obtain the serum component used for lipid analysis.

Lipid analysis

The lipid profiles were determined using kits manufactured by TECO Diagnostics Lakeview Ave, Anaheim, CA, USA. Serum total cholesterol (TC) was determined by the

method of Aliain *et al.*[26], while triacylglycerols was determined by the method of Burstein *et al.*, [27]. The lipoproteins, VLDL and HDL were precipitated using phosphotungstic acid and magnesium chloride. After centrifugation, the supernatant contained the high-density lipoprotein cholesterol (HDL-C) fraction which was assayed for cholesterol by the method of Grove[28]. The low-density lipoprotein cholesterol (LDL-C) was calculated using the method of Friedewald *et al.* [29].

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) using the paired T- test. The mean ± SD of each parameter was taken for each group. Test probability value of $p< 0.05$ was considered significant. The analysis was carried out on SPSS for windows version 10.

RESULTS

The effects of *Gongronema latifolium* and *Vernonia amygdalina* diet preparations on the serum total cholesterol and triacylglycerols concentrations are shown in Table 1. From the results the two diet preparations decreased the serum total cholesterol levels as the concentrations of the vegetables were increased. The observed decrease was significantly lower than the control ($p<0.05$) in both the *Gongronema latifolium* and *Vernonia amygdalina* diet preparations. There was no difference between the effects of the two diet preparations at the 5% level of treatment though at the 15% and 30% levels of treatment there was a significant difference between the effect of the vegetable diets preparations. The *Vernonia amygdalina* diet induced a significantly lower serum total cholesterol level at the 15% and 30% concentrations relative to the *Gongronema latifolium* diet preparation.

The results of the effect of *Gongronema latifolium* and *Vernonia amygdalina* diet preparations on the serum triacylglycerols levels show that both preparations lowered the triacylglycerols levels as the concentrations of the vegetables increased (Table 1). The reduction in the triacylglycerols levels was dose dependent and was significantly lower compared to the control ($p<0.05$). The results show that there was no significant difference between the effect of *Gongronema latifolium* and *Vernonia amygdalina* diet preparations relative to each other at the 5%, 15%, and 30% treatments respectively($p>0.05$).

Table 1: Effects of *Gongronema latifolium* and *Vernonia amygdalina* diet preparations on the serum total cholesterol and triacylglycerols (mg/dl)

	Total cholesterol(mg/dl)		**Triacylglycerols(mg/dl)**	
	Gongronema latifolium	***Vernonia amygdalina***	***Gongronema latifolium***	***Vernonia amygdalina***
Control	133.80±5.95	133.80±5.95	150.73±6.8	150.73±6.8
5%	120.30±7.04^{*b}	118.35±5.95^{*b}	141.00±4.53^{*a}	144.00±2.39^{*a}
15%	116.06±2.07^{*b}	112.55±4.42^{*a}	120.00±4.44^{*a}	121.17±2.40^{*a}
30%	110.94±5.09^{*b}	101.92±8.24^{*a}	114.53±3.46^{*a}	111.62±4.89^{*a}

*Results are mean ±SD. Values with different alphabetical superscript for the same parameter in a row are significant with respect to each other ($p<0.05$). Values with the superscript * in a column are significant with respect to the control ($p<0.05$).*

Table2: Effects of *Gongronema latifolium* and *Vernonia amygdalina* diet preparations on the serum LDL-C and HDL-C (mg/dl)

	LDL-C(mg/dl)		**HDL-C(mg/dl)**	
	Gongronema latifolium	***Vernonia amygdalina***	***Gongronema latifolium***	***Vernonia amygdalina***
Control	127.33±5.64	127.33±5.64	22.13±2.49	22.13±2.49
5%	86.35±8.59^{*b}	85.99±8.86^{*b}	127.85±4.46^{*a}	214.65±9.98^{*b}
15%	41.20±5.03*^{a}	25.51±7.96^{*b}	191.78±2.76^{*a}	236.35±4.14^{*b}
30%	8.36±1.76*^{a}	34.82±9.89^{*b}	256.85±2.77^{*a}	275.75±3.68^{*a}

*Results are mean ±SD. Values with different alphabetical superscripts for the same parameter in a row are significant with respect to each other ($p<0.05$). Values with the superscript * in a column are significant with respect to the control ($p<0.05$)*

Table 2 depicts the effects of *Gongronema latifolium* and *Vernonia amygdalina* diet preparations on the serum LDL-C and HDL-C concentrations. The results show that there was a decrease in the serum LDL-C values as the concentration of each vegetable diet was increased except with 30% incorporation of *Vernonia amygdalina*. The decrease in the LDL-C levels was significantly lower ($p<0.05$) than the control at the 5%, 15% and 30% treatments respectively. At the 5% treatment, *Vernonia amygdalina* induced a lower decrease in the serum LDL-C level compared to *Gongronema latifolium* but the decrease was not significant ($p>0.05$). The results also show that at 15% treatment *Vernonia amygdalina* produced a significantly lower ($p<0.05$) effect in the serum LDL-C compared to *Gongronema latifolium* while at the 30% treatment, *Gongronema latifolium* produced a significantly lower effect compared to *Vernonia amygdalina*. The results show that the two diet preparations induced an increase in the serum HDL-C level which was significantly higher than the control ($p<0.05$) (Table2). The comparison of the effects of the two diet preparations show that *Vernonia amygdalina* produced higher serum levels of HDL-C compared to *Gongronema latifolium* which was significant at the 5% and 15% concentrations respectively.

DISCUSSION

The presence of a high amount of cholesterol in the diet has been shown to increase plasma cholesterol and may elevate aortic atherosclerosis[2]. Many investigations have shown that diet treatment or drug therapy to regulate cholesterol can decrease subsequent cardiovascular disease (CVD) -associated mortality and morbidity[30]. On the basis of this, great efforts have been made to reduce the risk of CVD through the regulation of cholesterol, thus the therapeutic benefits of plant foods have been the focus of many extensive dietary studies[6,31]. Traditional plant remedies have been used for centuries in the treatment of diseases[32], but only a few have been scientifically evaluated. Therefore, the effects of the diet preparations of *Gongronema latifolium* and *Vernonia amygdalina* leaves on the lipid profile of rats were studied and compared. The two diet preparations reduced the serum total cholesterol levels but the *Vernonia amygdalina* diet induced a significantly lower ($p<0.05$) serum total cholesterol when compared to the *Gongronema latifolium* diet preparation.

Nwanjo[33] has shown that the administration of aqueous leaf extract of *Vernonia amygdalina* produced hypoglycaemic, hypolipidaemic and antioxidant effects in rats. The result is also in line with the findings of Ugochukwu *et al.* [21], and Adaramoye *et al.*[34] The cholesterol lowering effects of these diets preparations could be beneficial in preventing lipid abnormalities which may arise in certain metabolic disorders[35]. Ezekwe and Obidoa[37] have reported that flavoniods, tannins and saponins may play some roles in the hypolipidaemic effect of some plants. The mechanism of the hypocholesterolaemic action of these plant leaves may be due to inhibition of the absorption of dietary cholesterol in the intestine or its production by the liver[38] or stimulation of the biliary secretion of cholesterol and cholesterol excretion in faeces[39].

Triacylglycerols are partly taken up with the diet and partly synthesized in the liver[40]. Triacylglycerols as major components of various very low density lipoproteins (VLDL) and chylomycrons, play a significant role in metabolism as energy sources and transport of dietary fats. High blood triacylglycerols have been linked to atherosclerosis, and by extension, the risk of heart disease and stroke[41]. In this study, the diet preparations showed a triacylglycerol lowering effects in rats. The study also compared the effect of equal concentrations of the plant leaves diet preparations in rats. The lowering of the serum triacylglycerols by the two diet preparations was not significant to each other at equal concentration when compared. The result is in line with the results obtained by Nwanjo[33] and Ugochukwu *et al.*[21]. The results suggest that the plants could reduce hepatic triacylglycerols biosynthesis and favor the redistribution of cholesterol among the lipoprotein molecules. Adaramoye *et al.*, [34] observed no significant difference in plasma triacylglycerol levels of rats fed on *Telfairia occidentalis* supplemented diets when compared to cholesterol-fed rats.

Low density lipoprotein cholesterol (LDL-C) is another primary target of CVD risk

reduction therapy[30]. In this study, the effects of *Gongronema latifolium* and *Vernonia amygdalina* diets on the serum LDL-C were compared. The results show that both preparations significantly lowered the serum LDL-C values though the *Vernonia amygdalina* diet preparation produced a significantly lower serum LDL-C concentration relative to the *Gongronema latifolium* diet up to 15% dietary incorporation. LDL-C is associated with CVD because they transport cholesterol to the arteries which could lead to the formation of plaque. Therefore, plasma LDL-C level may be used for monitoring the treatment of patients with elevated cholesterol levels. From the results obtained, the plants elicited beneficial effects by lowering the serum LDL in rats.

Beyond the role of LDL-C in the development of atherosclerosis, growing evidence suggests that high density lipoprotein cholesterol (HDL-C) is a powerful predictor of CVD. Indeed, epidemiological, mechanistic and intervention studies suggest that low HDL-C is a major CVD risk factor and that increasing HDL-C plasma levels may be beneficial, particularly in patients with low HDL-C levels[42].

The results from this study show that the treatment with *Gongronema latifolium* and *Vernonia amygdalina* diets led to a significant increase in serum HDL-C, showing their promising protective role against CVD. The comparison of the effects of the vegetable leaves diet preparations on serum HDL-C showed that *Vernonia amygdalina* induced a significantly higher HDL-C concentration compared to *Gongronema latifolium*. This implies that *Vernonia amygdalina* could have a better therapeutic application as it may reduce atherogenic process better than *Gongronema latifolium.* The protective role of HDL-C against CVD has been suggested to occur in various ways[42]. HDL exerts part of its anti-atherogenic effect by counteracting LDL oxidation and, recent studies show that HDL promotes the reverse cholesterol transport pathway, by inducing the efflux of accumulated cellular cholesterol and prevents the generation of an oxidatively modified LDL[6]. Furthermore, HDL inhibits the oxidation of LDL by transition metal ions, but also prevents12-lipoxygenase mediated formation of lipid hydroperoxides[42]. On the basis of the results from this study, the plant leaves could play anti-atherogenic role through the increase of HDL-C.

Evidence from the present study confirms the effects of *Gongronema latifolium* and *Vernonia amygdalina* diets preparations on lipid levels in experimental animals. *Gongronema latifolium* and *Vernonia amygdalina* were found to be highly effective in reducing the levels of serum cholesterol, triacylglycerols and LDL-C thereby exhibiting hypocholestrolaemic effects. They also increased the levels of serum HDL-C in the experimental animals.

It appears from our results that the *Vernonia amygdalina* diet preparation may have better hypolipidaemic effect than the *Gongronema latifolium* diet preparation. Therefore, *Vernonia amygdalina* may have better therapeutic promise in preventing lipid related pathologies compared to *Gongronema latifolium*. However, because the search for natural anti-lipidaemic compounds to replace synthetic ones is gaining ground, further research is required to elucidate the exact mechanism responsible for the hypo-lipidaemic effects shown by the two plants.

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