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Hypolipidaemic Effects and Body Weight Changes of Male Wistar Rats Administered Crude Saponin Extracts from *Vernonia amygdalina* and *Vernonia colorata* Leaves

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Abstract: The effect of oral administration of crude saponins extracted from leaves of *Vernonia amygdalin (V.a) and Vernonia colorata (V.c)* on serum lipid profile and body weight changes in male Wistar rats was studied. Twenty five male albino rats aged 8-10 weeks old were used in the study. The animals were placed in five groups of five animals each. After four weeks of feeding, serum lipid profile was analyzed and daily body weight changes was recorded. *Vernonia amygdalina* and *Vernonia colorata* extracts significantly ($p< 0.05$) decreased serum cholesterol levels. Triacylglycerol concentration decreased significantly extract treated animals in a concentration dependent manner LDL-cholesterol reduced significantly relative to control whereas the HDL-cholesterol concentration decreased significantly in the animals that was administered 100mg/kg body weight (high dose) of the extract. Body weight was significantly reduced in all extract treated animals. These results suggest that the crude saponin extracts may contain bioactive agents that can reduce blood lipid fractions with high atherogenic indices.

KEYWORDS: *Vernonia amygdalina*, *Vernonia colorata*, serum lipids, saponins, hypolipidaemia, body weight

1.0 Introduction

A decline in the incidence of infectious diseases in developing countries has been witnessed and a rise in the toll taken by cardiovascular diseases, including stroke and myocardial infarction (Forrester *et al*., 1998). Cardiovascular disease (CVD) is the leading worldwide cause of death in all developing regions with the exception of sub-Saharan Africa (Opie and Mayosi, 2005). Hypertension remains the most important cardiovascular (CV) risk factor in Africa (Mahmoud, 2007). Another major cardiovascular disease susceptible to the changing environment in Africa is diabetes mellitus (Kengne *et al*., 2005). Opie and Seedat (2005) reported that urbanization and adopting Western lifestyles contribute greatly in the rising incidence of hypertension in sub-Saharan Africa. Thus CVDs and its risk factors could be checked and prevented in Africa by adopting or consuming indigenous leafy vegetables which are cheap sources of readily available nutrients. Awareness and an increased consumption of African leafy vegetables could have a positive effect on nutrition, health and economic wellbeing of both rural and urban populations (Darkwa and Darkwa, 2013). Indigenous vegetables are cultivated and consumed by various localities in Nigeria either as sources of food or used as drugs to treat various ailments.

Vernonia amygdalina and *Vernonia colorata* are among such vegetables grown widely in Nigeria, West Africa. It is called "Onugbu" or "Olugbu" (Ibo),"Ituyna" (Tiv), "Oriwo" (Edo) and "Chusa-doki" (Hausa) (Egedigwe, 2010). *Vernonia amygdalina* Del, commonly called bitter leaf, is a perennial shrub of 2-5m in height that grows throughout tropical Africa, it is drought resistant and thrives in humid environments (Ijeh and Ejike, 2011). *Vernonia colorata* Wild belongs to the family Asterecea.

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It is relatively non bitter and can be chewed raw as vegetable or sliced for direct use in soups without prior washing (Ijeh *et al*., 1996). *Vernonia colorata* has been reported to be one of the local species of *Vernonia* used to treat infectious diseases in Ghana though regarded as a wild species (Lengani *et al*., 2010, Nadembega *et al*., 2011). They are inexpensive sources of cheap and abundant source of protein, carbohydrate, minerals, vitamins and fibre to most vulnerable groups (Mensah *et al*., 2008). *Vernonia amygdalina* and *Vernonia colorata* are leafy vegetables that have been used to alleviate the problem of micronutrients, malnutrition and it is very prominent in tropical Africa (Ejoh *et al*., 2007).

Our earlier studies showed that incorporation of the leaves in the diet of experimental rats resulted in significantly lower cholesterol levels in normolipidemic rats (Egedigwe and Ijeh, 2010a; Ijeh and Egedigwe, 2010). Researchers have reported on whole leaf extracts of both leafy vegetables. Therefore, there is need to investigate the hypolipidaemic effect of saponins from both leafy vegetables in normoglycemic albino rats.

The present study seeks to investigate the effect of oral administration of crude saponins extracted from the leaves on blood lipid profile in experimental rats with a view to establishing how the saponin fraction is responsible for the hypolipidemic effect as a preliminary step towards characterization of active principles responsible for hypolipidemic activity.

2.0 Materials and Methods

2.1 Plant materials

Fresh matured leaves of *Vernonia amygdalina* and *Vernonia colorata* were harvested from farms around the Forestry Research Institute, Ahiaeke, Abia State. Both leaves were identified by Mr. Ibe .K. Ndukwe a taxonomist at the Herbarium unit of Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State. Voucher specimens were deposited at the herbarium (FHI 28786- *Vernonia amygdalina* and FHI 4873- *Vernonia colorata*).

2.2 Animals

Twenty five male albino rats of the Wistar strain, aged 8 to 10 weeks were purchased from the Animal Breeding unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. The animals were carefully transported to the Animal House Breeding Unit of the Biochemistry Department in Michael Okpara University of Agriculture, Umudike. The animals were exposed to 12 h light/dark cycles and supplied feed and water *ad libitum*.

2.3Extraction of saponins

The leaves of both plants were separately air dried at room temperature for 3 to 4 days to attain a constant weight after rinsing with distilled water. Both leaves were also separately ground into fine powder using an electric blender and stored in an air tight plastic container. The dried leaves of *Vernonia amygdalina* and *Vernonia colorata* were used for saponin extraction as described by Obadoni and Ochuko (2001).

A portion of the leaves (100 g) of each plant was put into a conical flask and 100 cm^3 of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 hours with continuous stirring at 55^0C. The mixture was filtered and the residue re-extracted with 200ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at 90^0C. The concentrate was transferred into a 250ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight, weighed and the saponin content was calculated as percentage. For *Vernonia colorata*, the crude saponin extract yield was 2 g (2%) while for *Vernonia amygdalina*, the crude saponin extract yield was 3 g (3%).

2.4 Animal grouping

The animals were separated into five groups of five animals each and housed in stainless steel cages with plastic base under humid tropical conditions. The animals were allowed to acclimatize on the basal diet (Vital Growers Feed) and clean tap water for one week. The body weights of the experimental animals were recorded. After one week of acclimatization, the experimental albino rats were assigned into five groups and administered the saponin extract and normal saline three times weekly as follows:
Group 1: Control group received orally 2 mls of normal saline /kg body weight of the animal.
Group : received saponin extract from *Vernonia colorata* (50 mg/kg body weight)
Group 3: received saponin extract from *Vernonia colorata* (100 mg/kg body weight)
Group 4: received saponin extract from *Vernonia amygdalina* (50 mg/kg body weight)
Group 5: received saponin extract from *Vernonia amygdalina* (100 mg/kg body weight).

The protocol conformed to the guidelines of the National Institute of Health (NIH) (NIH Publication 85-23, 1985) for Laboratory animal care and use.

2.5 Preparation of serum

After 28 days of extract administration, the animals were sacrificed and bled by cardiac puncture using a disposable sterile syringe. The blood was transferred into non-EDTA tubes. It was centrifuged at 4000 rpm for 30 minutes. The serum was collected and used for the assay of biochemical parameters.

2.6 Determination of lipid profile

Serum lipids were assayed using commercial kits purchased from RANDOX Laboratories Limited, Crumlin, County Antrim, UK. Total cholesterol (TC) was determined by the enzymatic end-point method using Cholesterol Oxidase-Peroxidase (CHOD-PAP) reagent for hydrolysis and oxidation (Richmond, 1973). Triacylglycerols were determined after enzymatic hydrolysis with lipases by the Glycerol-3-phosphate Oxidase - Peroxidase (GPO-PAP) method (Jacobs and Vandemark, 1960). HDL-Cholesterol concentrations were determined also as described by Richmond (1973). Low density Lipoprotein concentration in the blood was calculated as follows: LDL Cholesterol (mg/dl) = Total Cholesterol – (Triacylglycerol ÷ 5 + HDL Cholesterol) (Friedewald *et al.*, 1972).

2.7 Statistical analysis

All values were expressed as Mean ± S.D. Data were analyzed by One-way ANOVA, and then differences among means were analyzed using Duncan's Multiplicity Range Test (DMRT). Statistical analysis of data was performed using SPSS version 17.0 (SPSS Inc Chicago IL). Differences were considered significant at $p < 0.05$.

3.0 Results and Discussion

The results show that saponin extracts of both *Vernonia amygdalina* and *Vernonia colorata* administered to albino rats at 50 and 100 mg/kg body weight resulted in a significant ($p<0.05$) decrease in serum total cholesterol and triacylglycerol concentrations relative to the control (Figures 1 and 2). This decrease was concentration dependent as both extracts administered at high doses (100 mg/kg body weight) resulted in a greater decrease in total cholesterol concentration than the 50 mg/kg body (low dose) weight treated animals. Saponins present in leafy vegetables have been reported to have hypocholesterolemic effects (Price *et al.*, 1987; Offor *et al*., 2009). Saponins bind to cholesterol, and make it unable to be reabsorbed into the system and therefore are excreted from the body (Mayes, 1996). This findings also agrees with other workers (Adaramoye *et al*., 2008) who reported the lipid lowering of methanolic extract of *Vernonia.amygdalina.*

Saponins may have potential in human health issues because they reduce serum cholesterol by preventing its reabsorption after it has been excreted in the bile. It is hypothesized that the saponins either bind with bile salts or cause the bile salts to bind to the polysaccharides in dietary fiber. Either way, the bile salts are

unavailable to bind with cholesterol. Saponin has been known to elicit serum cholesterol lowering activity by causing resin-like action, thereby reducing the enterohepatic circulation of bile acids (Topping *et al.,* 1980). In the process, the conversion of cholesterol to bile acid is enhanced in the liver resulting in concomitant hypocholesterolemia (Kritchevsky, 1977). The suggested mechanism by which saponins exert their hypolipidemic effect is commonly attributed to disruption in cholesterol metabolism (Sidhu and Oakenfull, 1986).

Several workers such Kayode *et al* (2013) and Zhao *et al* (2008) have also reported on the hypolipidaemic effects of saponins from *Parkia Biglobosa* leaves and saponins from *Platycodon grandiflorum* respectively. Saponins are steroids or triterpenoid glycosides, common in a large number of plants and plant products. Several biological effects have been ascribed to saponins. Research evidence suggest that some saponins possess membrane–permeabilising, immunostimulant, hypocholesterolemic and anticarcinogenic properties (William and Gong, 2007).

Figure 3 shows the HDL Cholesterol concentrations in albino rats administered saponin extracts at 50 and 100 mg/kg body weight from *Vernonia amygdalina* and *Vernonia colorata.* There was a non significant ($p<0.05$) decrease in HDL Cholesterol concentrations observed when saponins from both leafy vegetables were administered at low dose relative to the control. At high doses of saponin administration, there was a significant ($p<0.05$) decrease observed relative to the control. HDL cholesterol promotes the reverse cholesterol transport pathway by removing cholesterol from the atheroma within the arteries and transports it back to the liver for excretion or re-utilization.

Serum LDL cholesterol (Figure 4) concentrations decreased significantly ($p<0.05$) at low and high doses of saponin extract administration given to rats relative to the control group. LDL-cholesterol poses a risk for CVDs as it transports cholesterol to the arteries, invades the endothelium and becomes oxidized. Cromwell and Otvos (2004) reported that increased levels of LDL cholesterol are associated with atherosclerosis, heart attack, stroke and peripheral vascular diseases.

Figure 5 depict the daily body weight changes in albino rats administered saponin extracts from *Vernonia amygdalina.* Days1-7 showed a significant ($p<0.05$) decrease in weight observed in albino rats administered extracts from *Vernonia amygdalina* when compared to the Days 8-14. Days 1-7 of saponin extract from *Vernonia colorata* administered to albino rats at both low and high doses resulted in a significant ($p<0.05$) decrease in weight when compared to the Days 7-14 of saponin administration (Figure 6). This is in line with the findings reported by several workers such as Igile *et al* (1995), Egedigwe and Ijeh (2010a); Egedigwe and Ijeh (2010b) and Offor (2013) who reported significant decrease in weight of animals either administered extracts from these leafy vegetables or incorporation of *Vernonia amygdalina* and *Vernonia colorata* in their diets. The weight loss observed in albino rats could be attributed to inhibition of pancreatic lipase and delay the intestinal absorption of fat.

Administration of crude saponin extracts from *Vernonia amygdalina* and *Vernonia colorata* resulted in significant decreases in body weight, serum cholesterol, LDL-Cholesterol and total Triacyglycerol.

These findings suggest that crude saponin extracts may contain bioactive fractions that could be useful in the management of disease conditions associated with hyperlipidaemia.

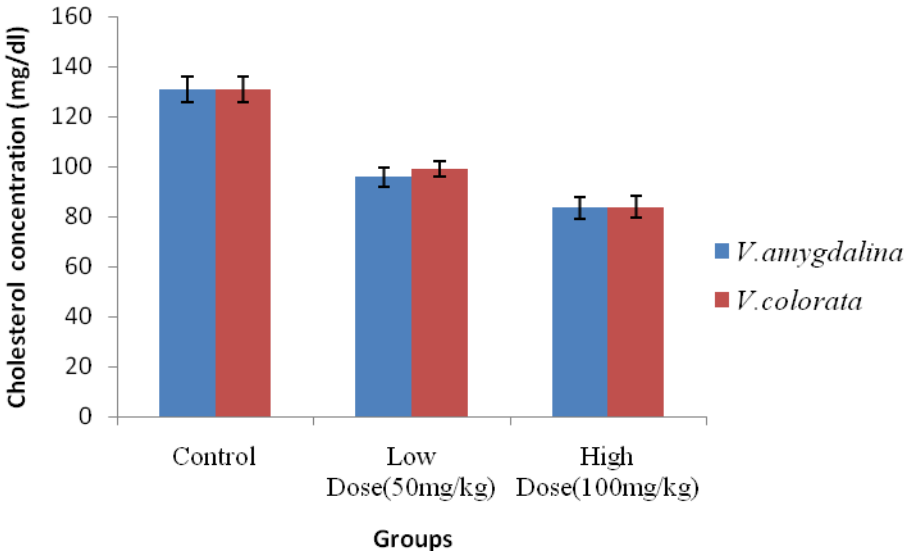

Fig. 1: Effect of saponin extract of *Vernonia amygdalina* and *Vernonia colorata* on serum total cholesterol concentration in albino rats

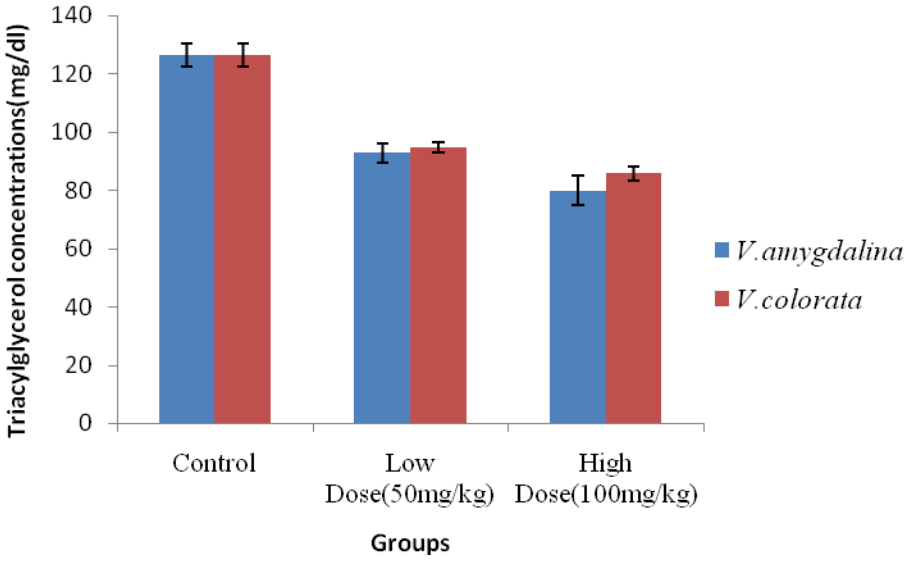

Fig. 2: Triacylglycerol concentrations in rats administered saponins from *Vernonia amygdalina* and *Vernonia colorata*

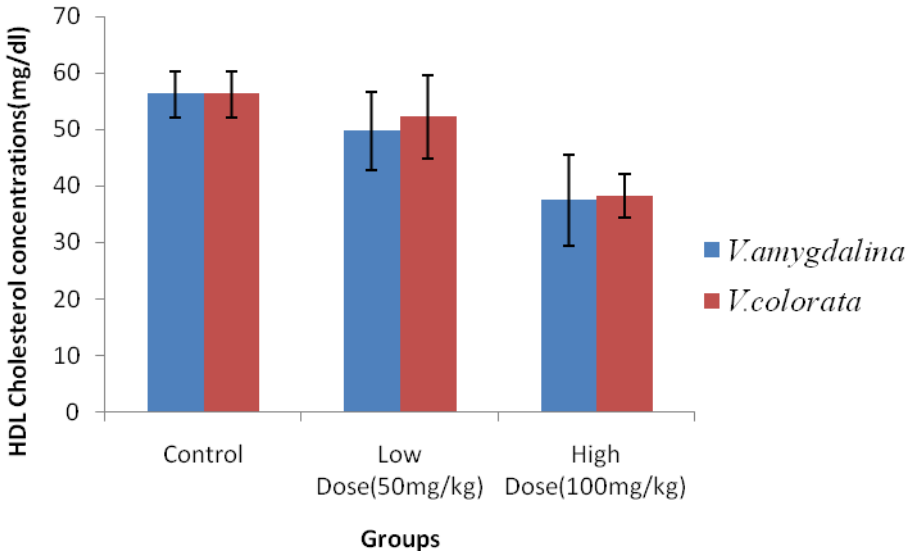

Fig. 3: HDL-Cholesterol concentrations in rats adminstered saponin extracts from *Vernonia amygdalina* and *Vernonia colorata*

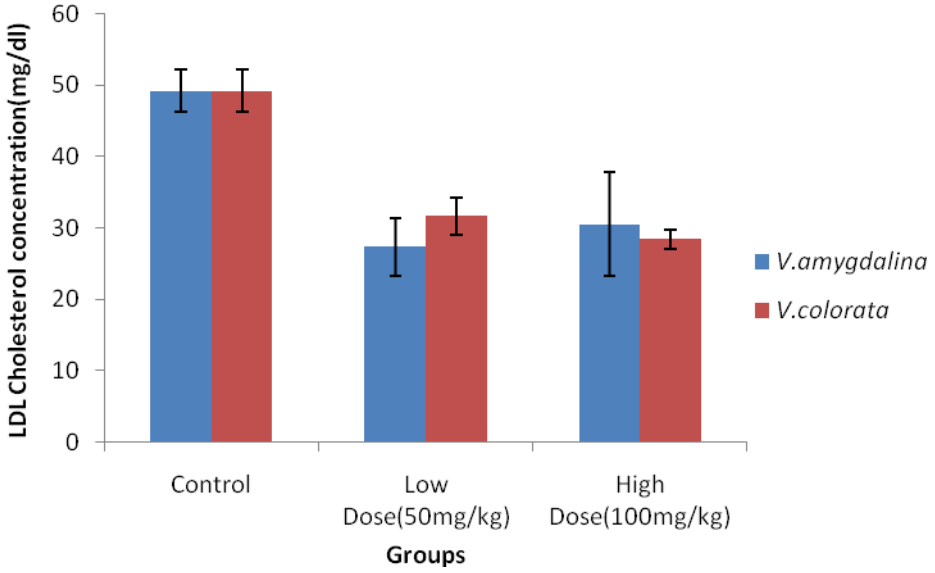

Fig. 4: LDL-Cholesterol concentrations in rats administered saponin extract of *Vernonia amygdalina* and *Vernonia colorata*

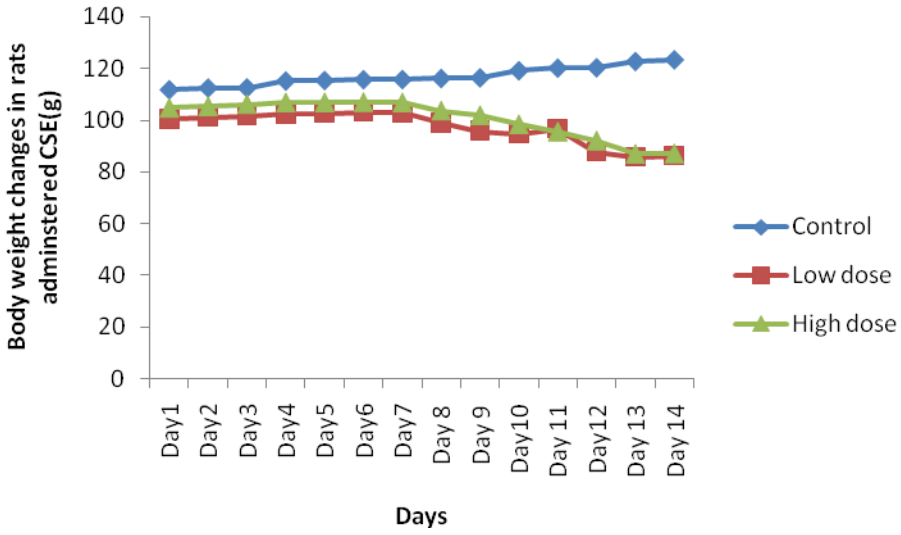

Fig. 5: Body weight changes in rats adminstered crude saponin extracts (CSE) of *Vernonia amygdalina*

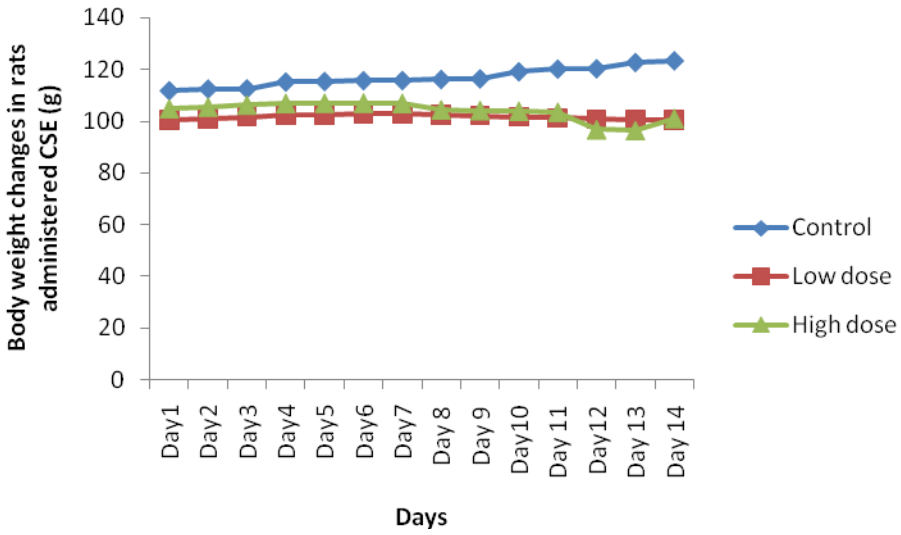

Fig. 6: Body weight changes in rats adminstered crude saponin extracts of *Vernonia colorata*

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