Nigerian Journal of Biochemistry and Molecular Biology 2018; 33(1&2): 32-43

0189-4757/96 $3.0 + 0.00
Printed in Nigeria

Available online at http://www.nsbmb.com.ng

NJBMB/007/18

Effects of Dietary Substitution with *Vernonia calvoana* Leaf on some Serum Lipid and Haematological Parameters in Male Wistar Rats

Egbung, G. E., Ubaba, M. U., Atangwho, I. J. and Egbung, J. E.

Department of Biochemistry, University of Calabar, P.M.B 1115, Calabar, Nigeria

Abstract: Consumption of green leafy vegetables especially the lesser known types for culinary and medicinal values has been on the increase in the tropics. The present study was designed to evaluate the haemotopoetic and hypolipidaemic effects of dietary substitution with *Vernonia calvoana* (VC) leaf in male Wistar rats. Twenty-four male Wistar rats weighing 80-140 g were randomly assigned into 4 groups of 6 rats each. Group 1(normal control) received normal rat chow, groups 2, 3 and 4 were maintained on standard rat chow substituted with 5%, 15% and 30% of *V. calvoana* leaf respectively for 30 days. At the end of the experimental period, the animals were sacrificed and blood samples collected for some biochemical and haematological studies. There was non-significant ($p > 0.05$) change in serum triacylglycerol, total cholesterol, very low density lipoprotein-cholesterol (VLDLC) and low density lipoprotein-cholesterol (LDLC) concentrations in groups 2, 3 and 4 relative to the control group while serum high density lipoprotein cholesterol increased significantly($p < 0.05$) in the group fed 30% *V.calvoana* supplemented diet relative to the control. The result showed no significant effect ($p > 0.05$) on the levels of packed cell volume, haemoglobin concentration, lymphocytes and total white blood cell. This finding suggests that the leaf of *V. calvoana* are non-haematotoxic and may possess positive modulatory effect on serum lipid profile by increasing HDL-C especially at 15% and 30% level of supplementation, hence might be beneficial to the diabetics and obese.

KEYWORDS: Cardioprotective, Haematopoetic, Hypolipidemic, *Vernonia calvoana*

1.0 Introduction

Most tropical countries have a wide array of foodstuff and vegetables that are important in nutrition and healthy development (Ejor *et al*., 2007). One of such plants with a highly nutritive and ethno-botanical/pharmacological value is a member of astereaceae family called *Vernonia calvoana*. It has a close taxonomic relationship with *Vernonia amygdalina* (Igile *et al.*, 2013). It is widely consumed among the indigenes of southwestern Cameroun, southeastern and northern Cross River in Nigeria as a local delicacy and used as ingredients in soup and stew, complementing most cereals and tuber staples like potatoes, yam and plantain (Ejor *et al*., 2007; Igile *et al.*, 2013; Egbung *et al*., 2016). In Yakurr local government area of Cross River State, it is consumed raw with or without oil palm and there is unsubstantiated folkloric claim that this method of consumption is responsible for the long life span of the indigenes of Yakurr.

Anti-nutrient contents of *V. calvoana* such as oxalates, phytates and cyanates are relatively non-significant content (Igile *et al*., 2013). The side effects often associated with synthetic drugs in the management of lipid disorders has warranted the search for neutraceuticals that could elicit an explicit lipid modulating effect as observed in *V. amygdalina* leave which share similar ethno -medicinal and nutritive value with *V. calvoana* (Egedigwe, 2010; Ugwu *et al*., 2010, Atangwho *et al*., 2012). These researchers have reported the serum lowering effect on triacylglycerol and LDL-cholesterol with increased concentration of HDL-cholesterol following dietary incorporation of *V. amygdalina* in non-diabetic and diabetic albino rats. Despite the ethnomedicinal and highly nutritive value of the plant, there is little or no

***Corresponding Author**
Tel.: +234 8038375557
E-mail: eneji.egbung@unical.edu.ng

documented research on the hypolipidaemic, haematopoeitic and cardioprotective effects of dietary supplementation with *V. calvoana* leaf in Wistar rat. The present study was designed to investigate the effects of supplementation of *V. calvoana* leaf in the diet on some serum lipid indices and haematological parameters of Wistar rats.

2.0 Materials and Methods

2.1 Plant material

Fresh leaf of *V. calvoana* was purchased from a local market in Asiga town in Yakurr Local Government Area of Cross-River State, Nigeria. The leaf was authenticated by Dr Mike Eko of the Department of Botany, University of Calabar, Calabar, Cross-River State, Nigeria and voucher specimen (BOT/VC/1/2015) was deposited in the herbarium of the same Department.

2.2 Chemicals

All the chemicals used for this experiment were of analytical grade. Diagnostic kits for the determination of lipid profile (total cholesterol, triacylglycerol, VLDL-C, LDL-C and HDL-C) were obtained from Agappe Diagnostics, Switzerland.

2.3 Animals

Twenty four male Wistar rats weighing 80 – 140 g were obtained as a disease-free stock from the Animal House, Department of Zoology, University of Calabar. The animals were acclimatized for two weeks on pelletized rat chow and water provided *ad libitum*.

2.4 Processing of plant materials

The leaf was washed to remove dust and other forms of dirt and afterwards air-dried at $27^0C \pm 2^0C$ for 14 days. The dried leaf was blended to a fine powder using a dry Moulinex Super Blender (LM2070 - 4A, Dubai, United Arab Emirates) and stored in air-tight containers. The coarse powder was weighed and used for feed supplementation.

2.5 Animal grouping

The animals were randomly assigned into four groups of six animals each as shown in Table 1. The feed were substituted with pulverised leaf of *V. calvoana* at 5%, 15% and 30% inclusion level. The experimental feeding lasted for 30 days. The experiment was conducted in accordance with the internationally accepted principles for laboratory animal use and care (NIH, 1996). Permission and approval for the use of the animals to carry out the study were obtained from the College of Medical Sciences Ethical Committee, University of Calabar, Nigeria.

Table 1: Animal Grouping

Groups	Description
1 (Control)	Animal Feed (100%)
2 (95% Feed)	5% inclusion of *V. calvoana*
3 (85% Feed)	15% inclusion of *V. calvoana*
4 (70% Feed)	30% inclusion of *V. calvoana*

Number of rats in each group = 6

2.6 Sample collection and pre-treatment

The animals were fasted 12 hours overnight prior to the time of sacrifice. They were sacrificed under anaesthesia and blood samples collected via cardiac puncture. The blood samples were collected into a set of tubes (ethylenediamine tetra acetic acid [EDTA] and plain) for the determination of full blood count and serum lipid indices respectively.

2.7 Determination of haematological parameters

Whole blood was collected from each experimental animal through cardiac puncture and put into to sterile EDTA sample tubes and used for the determination of haematological indices (haemoglobin, red blood cell, white blood cell, platelet, packed cell volume and

white blood cell differentials). The Sysmsex® Automated Haematology Analyzer KX-21N, Sysmex Corporation, Kobe-Japan was used for the haematological assay. The pre-diluted (PD) sample method was used where blood was diluted manually and then fed into the transducers. The transducer chamber has a minute hole called the aperture. On both sides of the aperture, there were electrodes through which direct current flows. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes. As direct current changes, the blood cell size is detected as electric pulses. Blood cell count was then calculated by counting the pulses, and a histogram of blood cell sizes was plotted by determining the pulse sizes.

2.8 Determination of serum lipid indices

The blood was collected into samples tubes without anticoagulant and allowed to clot for 2-3 hours after which it was centrifuged (3000 rpm for 15 minutes). The obtained serum was stored at 4^0C for the determination of serum total cholesterol, triacylglycerol, low density lipoprotein-cholesterol and high density lipoprotein-cholesterol. These determinations were carried out according to the manufacturer's protocol. In this method, the activity of cholesterol esterase results in the enzymatic hydrolysis of cholesterol ester to yield cholesterol and fatty acid. The resulting cholesterol was then oxidized following the activity of cholesterol oxidase to yield hydrogen peroxide as a by-product together with cholestene -3-one. The concentration of the resulting hydrogen peroxide was proportionally related to the initial concentration of cholesterol in the sample and it was determined via a quinoneimine indicator that was formed from the reaction between H_2O_2 and 4-aminoantipyrene in the presence of phenol and peroxide. Determination of triacylglycerol involved lipase-induced enzymatic hydrolysis. Quninoneimine formed from the peroxidation reaction between hydrogen peroxide, phenol and 4-aminoantipyrine served as the indictor in this method.

2.9 Statistical Analysis

Data obtained were analyzed using one-way analysis of variance and differences between pairs of groups were determined using *Post-hoc* multiple comparisons. Results were expressed as mean ± standard error of mean (SEM). Data obtained were considered significant at $p < 0.05$. Computer software SPSS (version 17.0) by Norman Nie Dale Bent and Hadlai Tex Hull and Microsoft Excel (version 2007) were used for the analyses.

3.0 Results

The results of the study on the effect of dietary substitution of *V. calvoana* leaf on serum lipid profile, haematological indices in Wistar rats are presented in Figures 1 – 12.

Results of the haematological indices of Wistar rats fed on 5%, 15% and 30% of *V. calvoana* substituted diets are presented in Figures 1-4. Figures 1 and 2 revealed non-significant change in the packed cell volume and haemoglobin concentration of rats fed on 5%, 15%, and 30% of *V. calvoana* leaf substituted diet when compared with the control animals. The 15% and 30% of *V. calvoana* leaf substituted diet did not affect ($P>0.05$) the white blood cell count (Figure 3). However, only the decrease caused by 15% *V. calvoana* leaf substituted diet was significant ($p<0.05$) when compared with the control animals. The result of the neutrophil count as shown in Figure 4, revealed a significant increase ($p < 0.05$) at 5% while the 15% and 30% of *V. calvoana* leaf substituted diet had non-significant increases relative to the controls.

The lymphocytes revealed non-significant decreases in the treatment groups relative to the controls (Figure 5). A reverse pattern was obtained in Figure 6 for the mixed lymphocytes. Result of the red blood cell count as presented in Figure 7, revealed a significant ($p < 0.05$) increase in RBC count for the experimental animals fed on 15% and 30% *V. calvoana* leaf substituted diet when compared with the control animal. However, experimental animals fed on 5% of *V. calvoana* leaf diet showed a significant decrease amongst the treatment

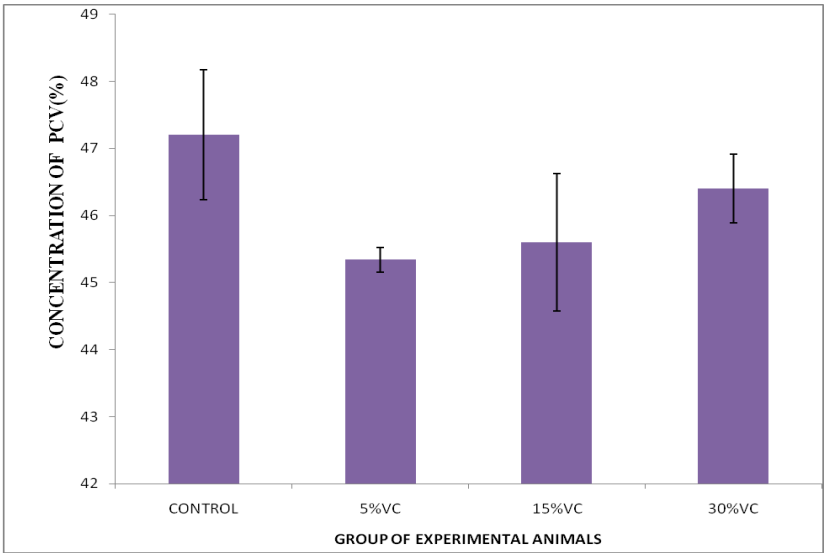

Figure 1: Packed cell volume (PCV) of rats maintained on 5%, 15% and 30% of *V. calvoana* leaf substituted diet. Values are expressed as mean ± SEM (n=5).

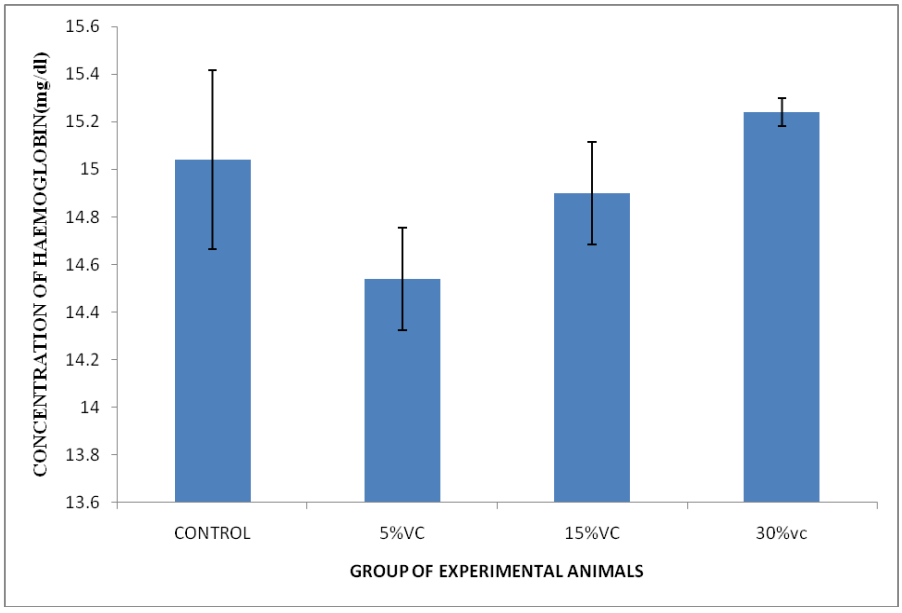

Figure 2: Haemoglobin concentration of rats maintained on 5%, 15% and 30% of *V. calvoana* leaf substituted diet. Values are expressed as mean ± SEM (n=5).

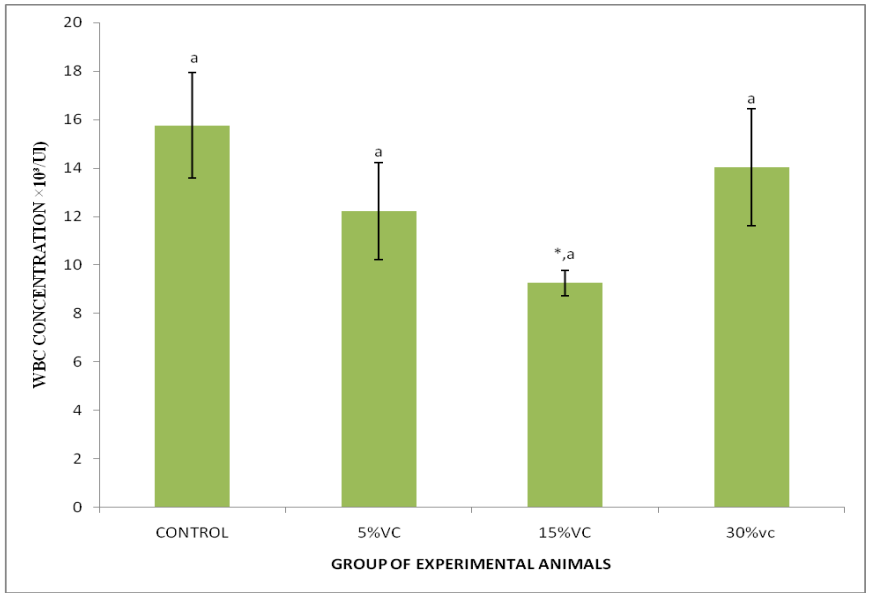

Figure 3: White blood cell count of rats maintained on 5%, 15% and 30% of *V. calvoana* leaf substituted diet. Values are expressed as mean ± SEM (n=5); *p<0.05 vs control; [a]p<0.05 among groups

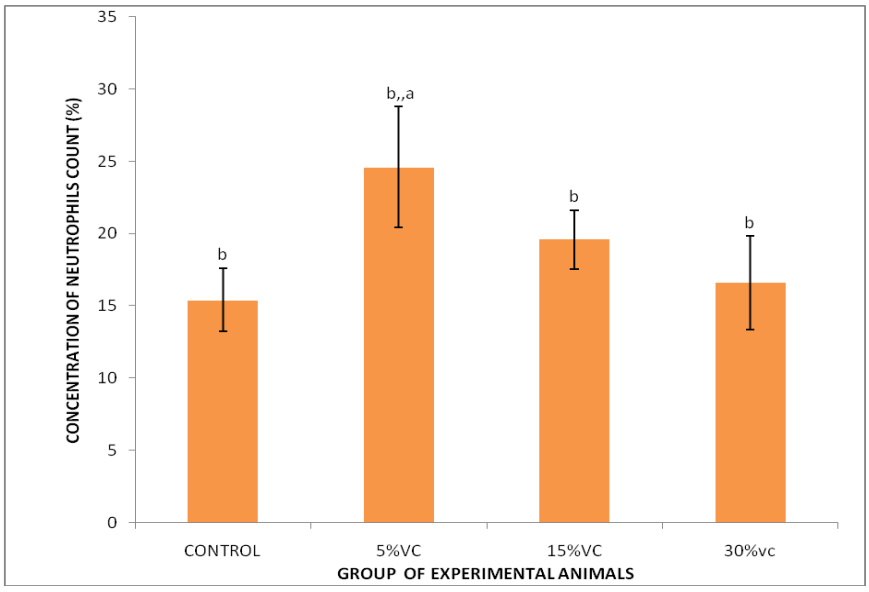

Figure 4: Neutrophil count of rats maintained on 5%, 15% and 30% of *V. calvoana* leaf substituted diet. Values are expressed as mean ± SEM (n=5); [a]p<0.05 vs control; [b]p>0.05 among groups

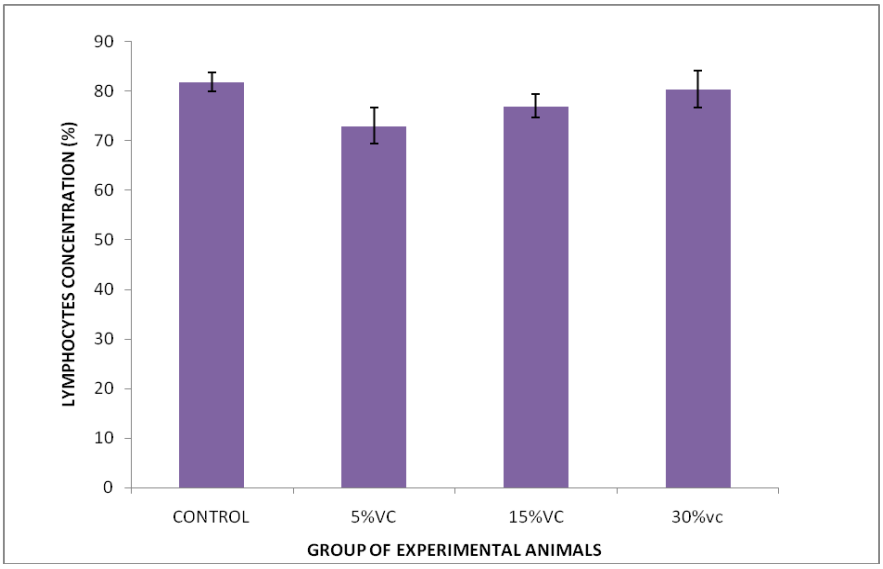

Figure 5: Lymphocyte count of rats maintained on 5%, 15% and 30% of *V. calvoana* leaf substituted diet. Values are expressed as mean ± SEM (n=5).

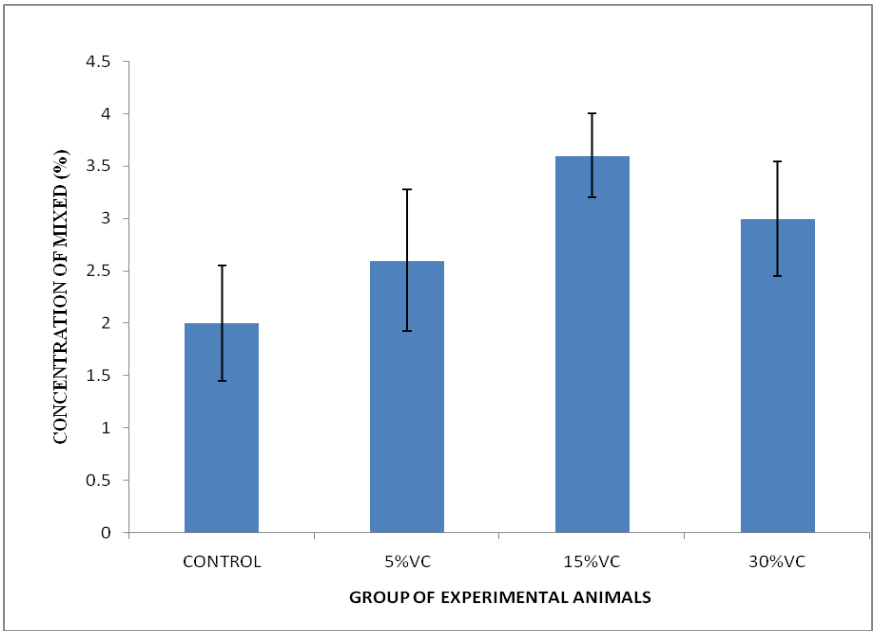

Figure 6: Mixed lymphocyte count of rats maintained on 5%, 15% and 30% of *V. calvoana* leaf substituted diet. Values are expressed as mean ± SEM (n=5).

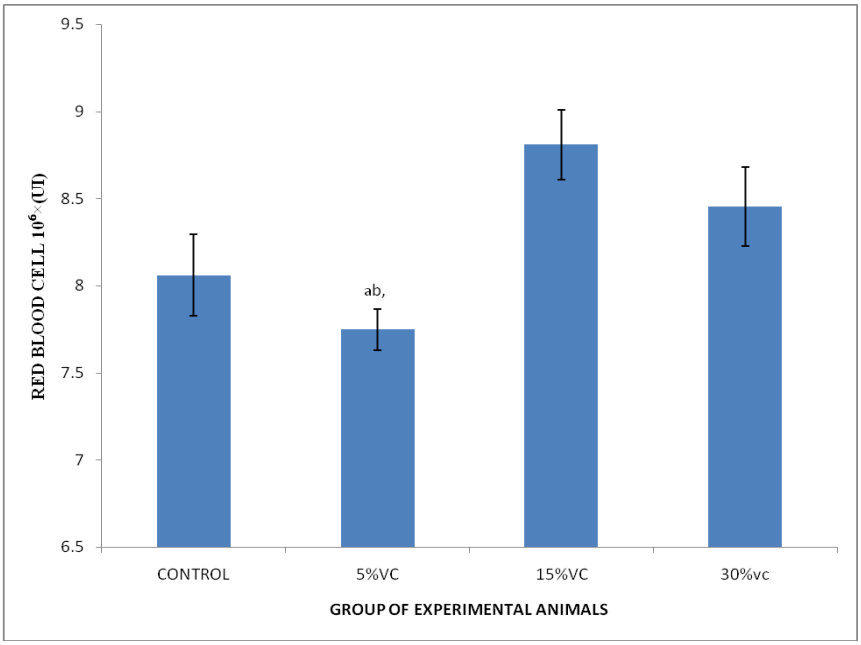

Figure 7: Red blood cell count of rats maintained on 5%, 15% and 30% of *V. calvoana* leaf substituted diet. Values are expressed as mean ± SEM (n=5).

group and when compared to control animals.

There were insignificant changes amongst experimental animals maintained on 5%, 15% and 30% *V. calvoana* leaf substituted diets relative to the control animals (Figure 8). Figure 9 revealed a non-significant increase in high density lipoprotein cholesterol of experimental animals maintained on 5% and 15% of *V. calvoana* leaf substituted diet when compared with the control animals. However, experimental animal fed with 30% of *V. calvoana* leaf substituted diet showed a significantly ($p < 0.05$) higher levels of high density lipoprotein-cholesterol when compared to the control animals. Result of the serum low density lipoprotein cholesterol and total cholesterol shown in Figures 9 and 10 respectively revealed non-significant decrease at 15% and 30% of *V. calvoana* leaf substituted diets and a non-significant increase at 5% of *V. calvoana* leaf substituted diet when compared with the control animals. Figure 12 showed a non-significant change in the serum very low density lipoprotein-cholesterol of the rats in the experimental groups when compared with the control animals.

4.0 Discussion

Vernonia calvoana leaf substituted diets at the various percentage of incorporation did not impact any change in the serum triacylglycerol. Unlike dietary supplementation with *V. amygdalina*, a related specie of similar ethnobotanical and nutritive potentials as reported by Atangwho *et al*. (2012) who indicated reduction in serum triacylglycerol level of obese and diabetic rats respectively. Dietary supplementation with *V. colorata* leaf, a wild type also demonstrated decrease in triacylglycerol level of Wistar rats (Ijeh and Ededigwe, 2010). The study by Iwara *et al*. (2015) showed lowered triacylglycerol following administration of methanolic extracts of *V. calvoana* in diabetic rats. Fatty acids from adipose tissues are mobilised for energy purpose and excess fatty acids are accumulated in the liver, which are converted to triacylglycerol (Carmena *et al*., 2004). This indicated that incorporation of *V. calvoana* leaf in diets may have a regulatory effect on serum triacylglycerol level in the blood and may therefore be useful in controlling body weight. The experimental animals did not show any change in serum total

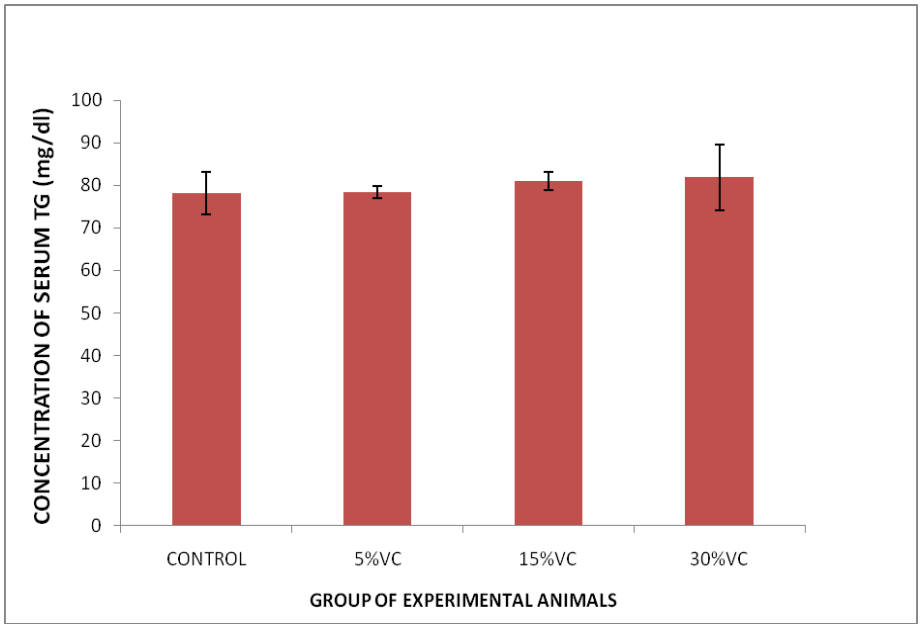

Figure 8: Triacylglycerol levels of rats maintained on 5%, 15% and 30% of *V. calvoana* leaf substituted diet. Values are expressed as mean ± SEM (n=5).

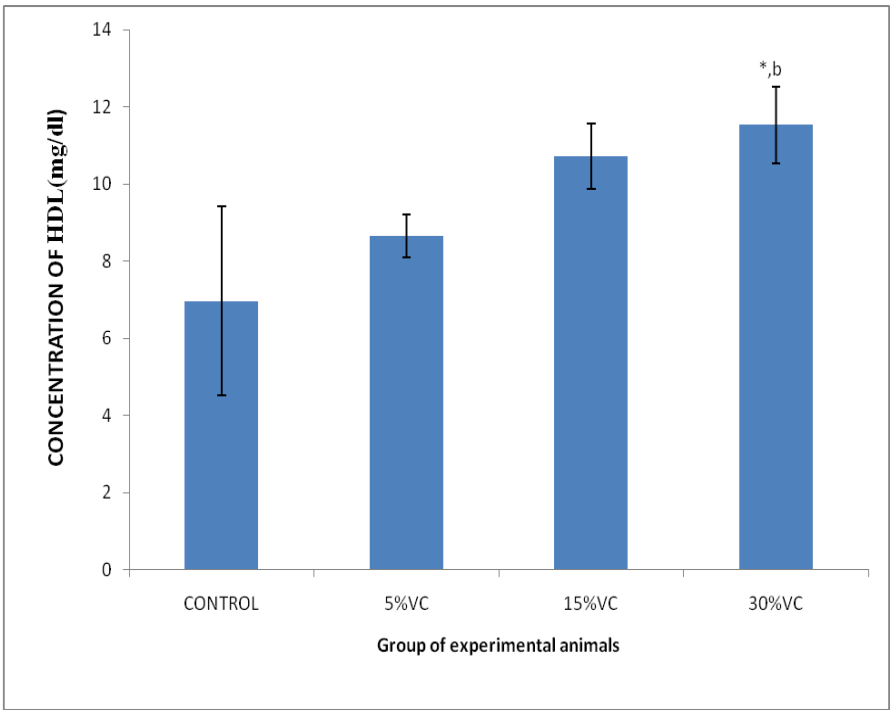

Figure 9: High density lipoprotein-cholesterol levels of rats maintained on 5%, 15% and 30% of *V. calvoana* leaf substituted diet. Values are expressed as mean ± SEM (n=5).

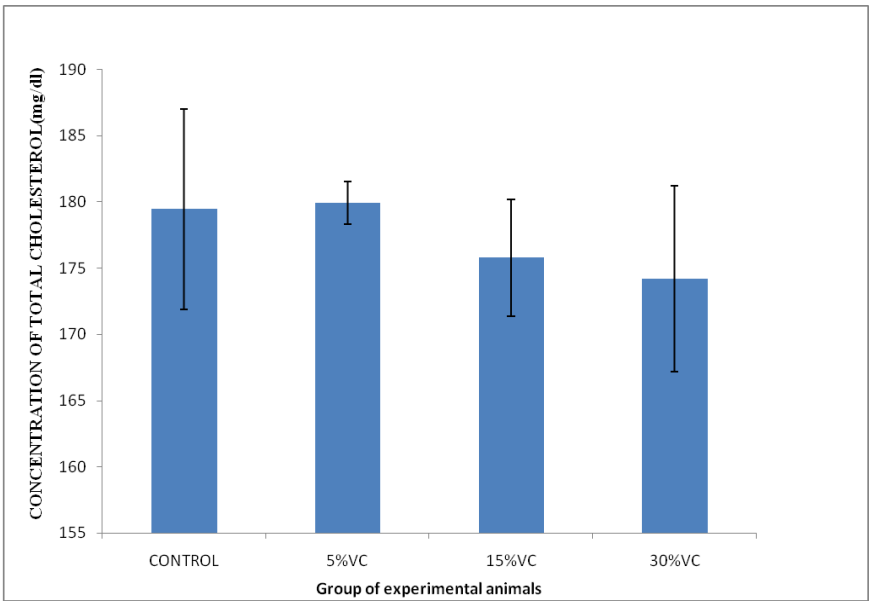

Figure 10: Serum total cholesterol levels of rats maintained on 5%, 15% and 30% of *V. calvoana* leaf substituted diet. Values are expressed as mean ± SEM (n=5).

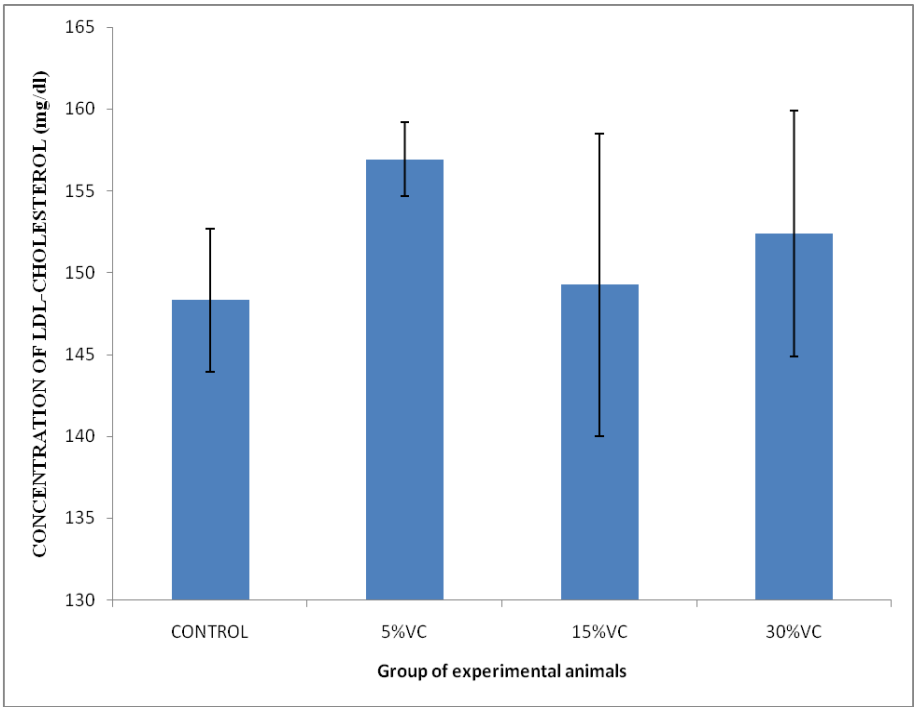

Figure 11: Low density lipoprotein-cholesterol levels of rats maintained on 5%, 15% and 30% of *V. calvoana* leaf substituted diet. Values are expressed as mean ± SEM (n=5).

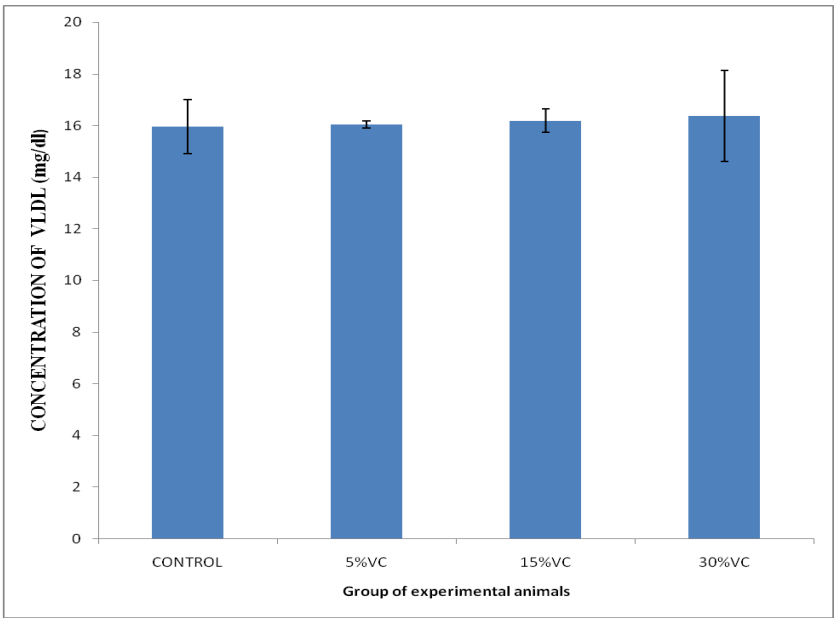

Figure 12: Very low density lipoprotein-cholesterol levels of rats maintained on 5%, 15% and 30% of *V. calvoana* leaf substituted diet. Values are expressed as mean ± SEM (n=5).

cholesterol, LDL-C, and VLDL-C, supporting that the rapid lipid lowering effect of *V. calvoana* may be confined to its crude extract when compared to the substituted diets. Saponins present in these leaves have been reported to have hypocholesterolemic effects (Price *et al*., 1987). The serum total cholesterol, triacylglycerol, LDL-C and VLDL-C were not affected by *V. calvoana* leaf substitution, hence, requiring possibly a long term feeding in order to effect its hypolipidaemic action (Atangwho *et al*., 2012).

High density lipoprotein-cholesterol has been associated with reduced risk of cardiovascular events (Khera *et al*., 2011; Kosmas *et al*., 2018). It plays a key role in reverse transport of cholesterol by inducing the efflux of excess accumulated cellular cholesterol and prevents the generation of an oxidized modified LDL-C (Yokozawa *et al*., 2006). The dietary substitution with *V. calvoana* leaf at 30% level of incorporation resulted in an increased serum HDL-C, however, substitution at 5% and 15% levels did not show any effect on serum HDL-C. This suggests that feeding experimental animals with *V. calvoana* leaf may probably play an anti-atherogenic role that may be dependent on its level of dietary incorporation as well as the duration of the study.

Haematological parameters are good indicators of physiological status of humans and animals. Alterations in these indices can be used to assess *in vivo* responses to different physiological situations (Esonu *et al*., 2006). There were increases in the red blood cell counts of experimental rats fed 15% and 30% diet substitution of *V. calvoana* leaf compared to the control animals. On the other hand, there were no changes in the levels of packed cell volume and haemoglobin count at all levels of *V. calvoana* leaf dietary incorporation in the experimental rats compared to the control animals. This suggests a positive effect of *V. calvoana* leaf substituted diets on the haemtopoietic system of Wistar rats. Increases in the red blood cell counts of the rats fed with substituted diets could have been due to high content of zinc and iron (minerals) in the plant (Ejor *et al*., 2007; Igile *et al*., 2013). The white blood cell counts in the experimental rats fed with 5% and 30% *V. calvoana* leaf substituted diets showed no change while those fed 15% *V. calvoana* leaf were decreased compared to the control animals. This decrease in white blood

cell counts suggests that the immune system was not compromised. The suppressive action of dietary *V. calvoana* leaf at 15% level of substitution on WBC suggests that the plant possess anti-inflammatory properties. This finding agrees with the report of Atangwho *et al*. (2012). The study concluded that *V. calvoana* leaf dietary substitution modulated blood lipid profile positively with probable cardio-protective effects at 15% and 30% level of incorporation. Also the leaf was not haematotoxic. The findings from our study though obtained from experimental animals could be extrapolated to humans especially in diabetes, obesity and other associated cardiovascular conditions. It is therefore, recommended that long term supplementation with *V. calvoana* leaves in rat models.

Acknowledgements

The authors are grateful to Professor P. E. Ebong of the Department of Biochemistry, University of Calabar, Nigeria, for allowing us access to the facilities of the Endocrine Research Laboratory.

References

Atangwho, I. J., Edet, E. E., Uti, D. E., Obi, A. U., Asmawi, M. Z. and Ahmad, M. (2012). Biochemical and histological impact of *Vernonia amygdalina* supplemented diet in obese rats. Saudi Journal of Biological Sciences 19:385-392.

Carmena, R., Duriez, P. and Fruchart, J. C. (2004). Atherogenic lipoprotein particles in atherosclerosis. Circulation 109:27-?.

Duffy, D. and Rader, D. J. (2009). Update on strategies to increase HDL quantity and function. Nature Reviews Cardiology 6:455-463.

Egedigwe, C. A. (2010). Effect of dietary incorporation of *Vernonia amygdalina* and *Vernonia colorata* on blood lipid profile and relative organ weights in albino rats. MSc. Dissertation in the Department of Biochemistry, Michael Opara University of Agriculture, Umudike, Nigeria.

Egbung, G. E., Atangwho, I. J., Kiasira, Z. B., Iwara, A. I. and Igile, G. O. (2016). Antioxidant activity of the inflorescents of *Vernonia calvoana* growing in Yakurr Local Government Area of Cross River State, Nigeria. Global Journal of Pure and Applied Sciences 22(*2*):141-146.

Ejor, A. R., Nkonga, D. V., Innocent, G. and Moses, M. C. (2007). Nutritional components of some non-conventional leafy vegetables consumed in Cameroun. Pakistan Journal of Nutrition 6:712-717.

Esonu, B. O., Onuoha, M. N., Okoli, I. C., Obiakaonu, H. O., Udedibie, C. and Iheshiulor, O. O. (2006). Physiological responses of laying birds to Neem (*Azadirachta indica*) leaf meal-based diets, body weight, organ characteristics and haematology. Online Journal of Health and Allied Sciences 5(*2*):2- 4.

Igile, G. O., Iwara I. A., Mgbeje B. I. A., Uboh F. E. and Ebong P. E. (2013). Phytochemical, proximate and nutrient composition of *Vernonia calvaona* Hook (Asteraceae): A Green-leafy vegetable in Nigeria. Journal of Food Research 2:6-10.

Ijeh, I. I. and Ededigwe, A. C. (2010). Effect of dietary incorporation of *Vernonia colorta* (wild) leaves on blood profile of albino wistar rats. International Journal of Biological and Chemical Sciences 4(1):100-106.

Iwara, I. A., Igile, G. O., Uboh, F. E., Eyong, E. U. and Ebong, P. E. (2015). Hypoglycemic and Hypolipidemic potentials of extract of *Vernonioa calvoana* on alloxan-induced diabetic albino Wistar rats. European Journal of Medicinal Plants 8(*2*): 78-86.

Khera, A. V., Cuchel, M. M., Rodrigues, A. and Burke, M. F. (2011). Cholesterol efflux capacity, High-density lipoprotein function, and atherosclerosis. New England Journal of Medicine 364:127-135.

Kosmas, C. E., Martinez, I., Sourlas, A., Bouza, K. V., Campos, F. N., Torres, V., Montan, P. D. and Guzman, E. M. (2018). High-density lipoprotein (HDL) functionality and its relevance to atherosclerotic cardiovascular disease. Drugs in Context 7: 212525.DOI: 10.7573/dic.21252

NIH Guide (1996). Revised Guide for the Care and use of Laboratory Animals, 25:28-31.

Price, K. R., Johnson, I. T. and Feriwick, G. R.. (1987). The chemical and biological significance of saponins in foods and feeding stuffs. Critical Review. Food Science and Nutrition 26(1):27-135.

Ugwu, C. E., Olajide, J. E., Alumana E. O. and Ezeanyika, L. U. S. (2010). Comparative study on the effects of the leaves of *Gongronema latifolium*, *Vernonia amygdalina* and *Telfairia occidentalis* incorporated diets on the lipid profiles of rats. Global Journal of Pure and Applied Sciences 16(3):319-324.

Yokozawa, T., Cho, E. J., Sasaki, S., Satoh, A., Okamoto, T. and Sei, Y. (2006). The protective role of Chinese prescription *Kangenkaryu* extract on diet-induced hypercholesterolemia in rats. Biological and Pharmaceutical Bulletin 29:760-765.