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Abstract

Background: Obesity is currently a global epidemic. Conventional treatments have not been very satisfactory to patients, warranting a search for alternative therapeutic options that are natural, safe and affordable. This study therefore investigated the anti-obesity potentials of aqueous and methanol extracts of *Vernonia amygdalina* Del (AEVA and MEVA respectively) in a rat model in which obesity was induced using a high-fat diet.

Materials and Methods: Forty two Wistar rats were randomised into 7 groups of 6 rats each. One group served as the Normal Control group and obesity was induced in the other 6 groups. One of the 6 groups each served as Positive Control and Negative Control while the 4 test groups were designated AEVA100, AEVA500, MEVA50 and MEVA200, respectively. The study lasted for 12 weeks after which standard protocols were followed for all analyses and determinations.

Results: The results show that both AEVA and MEVA at the tested concentrations resulted in significant ($P < 0.05$) weight loss (without affecting internal organs negatively), and significant ($P < 0.05$) improvement in some metabolic markers of obesity in the test rats compared to the negative control rats. MEVA 200 had the greatest anti-obesity effect while MEVA 50 was the least effective. All the test extracts compared well with Orlistat used as the positive control drug on all counts.

Conclusion: The observed weight-loss benefits of AEVA and MEVA are attributable to the rich milieu of phytochemicals found in *Vernonia amygdalina* Del. Further studies to unlock the mechanisms through which the observed weight loss is mediated are warranted.

Key words: High-fat diet, Obesity, Phytochemicals, *Vernonia amygdalina* extracts, Weight loss

Introduction

Obesity has assumed epidemic proportions globally and developing countries such as Nigeria are currently thought to be experiencing large increases in the obesity prevalence owing largely to improving economic conditions and the negative life-style modifications that come with it, particularly in hitherto economically disadvantaged societies (Ejike and Ijeh, 2012). According to the World Health Organisation, as at 2010, the prevalence of overweight and obesity in sub-Saharan Africa exceeded 60% and 70% in men and women, respectively, while in Nigeria the figures stood at 29% and 45% respectively (Ono *et al.*, 2012). This rising prevalence, coupled with the limited resources available to countries in sub-Saharan Africa demands that effective management options for tackling obesity are developed. Moreover, these countries are currently faced with chronic diseases in conjunction with communicable diseases – a double burden which is inimical to development.

Traditionally, obesity is managed by lifestyle modifications and in severe cases, pharmacological therapy. Lifestyle modifications aim at reducing energy intake while increasing its expenditure. Pharmacological therapy currently involves the use of medications such as sibutramine [an appetite suppressant (Tziomalos *et al.*, 2009)] and orlistat [which inhibits pancreatic lipase (Drew *et al.*, 2007)]. Unfortunately, it is difficult to get subjects to religiously abide by lifestyle modifications while drug treatment often results in weight rebound once the drug is withdrawn. Furthermore, drug-use is expensive, and comes with a plethora of side effects such as elevated blood pressure, constipation, xerostomia, and insomnia (Karamadoukis *et al.*, 2009). Consequently, natural products are now being explored for their potential in providing cheap, effective and safe anti-obesity drugs. Natural products from plants that can induce weight loss have attracted considerable attention recently (Raylam *et al.*, 2008) and one of such plants is *Vernonia amygdalina* Del.

Vernonia amygdalina Del. (Compositae) (VA) popularly called bitter leaf is a 2-10 m high shrub with rough barks which grows predominantly in sub-Saharan Africa. Its leaves are dark green, etiolated, elliptic in shape and have a characteristic odour and bitter taste. VA leaves are used as culinary vegetable across Africa. Its parts are used in ethno-medicinal treatment of several diseases including diabetes, malaria, worm infestation, etc. (Ijeh and Ejike, 2011). The use of VA as slimming bitters is yet to be properly investigated despite the increasing global market for bitters. Given that previous studies on other health benefits of the use of VA have consistently shown weight loss in various animal models fed diets incorporated with VA or its extracts (Ijeh and Obidoa, 2004; Ijeh and Adedokun, 2006; Egedigwe and Ijeh, 2010; Ijeh *et al.*, 2013; Ijeh *et al.*, 2014), this study investigated the anti-obesity potentials of aqueous and methanol extracts of VA in a rat model in which obesity was induced via feeding on a high-fat diet.

Materials and Methods

Preparation of the VA Extract

Fresh mature leaves of *Vernonia amygdalina* Del. were harvested from the Forestry Research Institute, Ahiaeke, Abia State, Nigeria. The leaves were identified and authenticated by IK Ndukwe, a plant taxonomist at the Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike. A voucher specimen (FHI 28786-VA) was deposited at the herbarium. The leaves were shade-dried to a constant weight, milled to fine powder using an electric blender (Q-Link, Model QBL, Taiwan) and stored in air tight containers. The extract was prepared by soaking the powdered leaves in distilled water in one case, and methanol in another, and allowing it to stand for 48 hours with occasional shaking. Thereafter, the mixtures were filtered using a Whatman No 1 filter paper and concentrated using a rotary evaporator. The extracts, designated as aqueous extract of VA (AEVA) and methanol extract of VA (MEVA) respectively, were then reconstituted in 2% DMSO in normal saline to get the stock concentrations used in the study.

Preparation of High Fat Diet (HFD)

The basal diet and the HFD were prepared from basic feed materials, following standard protocol. The HFD was designed such that 35% of the total energy in the diet came from fats. The feed components were thoroughly mixed in a bowl and then made into pellets using an improvised extrusion apparatus. After extrusion, the pelleted diets were dried in the oven at 35 °C to a constant weight, allowed to cool, then stored at -4 °C until needed. The compositions of both diets are shown in Table 1.

Table 1: Basal and High fat diets composition

Component	High Fat Diet (g/100g)	Basal Diet (g/100g)
Maize	38.9	66.7
Groundnut Cake	13.4	8.9
Egg yolk Powder	5.8	0.0
Crayfish	2.2	2.9
Vitamin/Mineral	2.0	1.1
Bone meal	2.0	1.1
Non-Nutritive Cellulose	0.4	0.2
Palm kernel oil	7.0	0.0
Palm oil	7.0	0.0
Corn starch	21.5	19.2

Animal Treatment and Feeding

Forty two male Wistar rats weighing 110-140 g were purchased from a private breeder at the University of Nigeria, Nsukka, and acclimatised to the animal house for 2 weeks. Thereafter, they were randomised into seven groups of six rats each (as shown in Table 2) and housed in stainless steel cages with a plastic base under humid tropical conditions. The rats had access to feed and water *ad libitum*.

The experiment lasted for twelve weeks after which the rats were weighed to note their final weights, following a 12 hour fast. They were then euthanized humanely. The rats were bled exhaustively by cardiac puncture and the blood collected in appropriate tubes. The sera from the blood were separated from cells by centrifugation after clotting and were thereafter placed in labelled tubes to be used for subsequent analyses. Each rat carcass was quickly dissected and their livers, hearts, spleens and kidneys excised, and weighed.

Table 2: Protocol for grouping the animals and treatments administered

Group	Normal Control	AEVA 100	AEVA 500	MEVA 50	MEVA 200	Negative Control	Positive Control
Diet	Basal Diet	HFD	HFD	HFD	HFD	HFD	HFD
Treatment	Distilled water	50 mg/kg bw AEVA	200 mg/kg bw AEVA	50 mg/kg bw MEVA	200 mg/kg bw MEVA	Distilled water	20 mg/kg bw Orlistat

Treatments were given *per os*. Orlistat was purchased from a reputable commercial pharmaceutical vendor. AEVA, MEVA and orlistat were each dissolved in 2% DMSO. AEVA, MEVA and HFD represent Aqueous extract of *Vernonia amygdalina*, Methanol extract of *Vernonia amygdalina* and High-fat diet respectively.

Body Weight Gain, Organ Relative Weights, and Feed Intake Estimation

Body weight gain was calculated as the difference between the initial body weight and body weight of rats in each group at the end of each week. The relative weights of the selected organs were calculated as the weight of the organ divided by the final weight of the rat from which it was excised. The feed intake of the rats was calculated as the difference between the feed supplied the rats daily and their daily leftover.

Biochemical Analyses

Serum concentrations of triacylglycerol (TAG), total cholesterol (TChol), and high density lipoprotein cholesterol (HDL-Chol) were determined using the enzymatic colorimetric methods described by Tietz (1990), Allain *et al.*, (1974) and Lopes-Virella *et al.*, (1977). Very low density lipoprotein cholesterol (VLDL-Chol) was estimated using the formula: VLDL cholesterol = Triacylglycerol concentration (mg/dL)/5, while low density lipoprotein cholesterol (LDL-Chol) was estimated by difference (Friedewald *et al.*, 1972). Fasting blood glucose concentrations were determined using an automated blood glucose monitor (Accu-Check Advantage, Roche Diagnostics GmbH, Mannheim, Germany).

Statistical Analyses

The data generated were subjected to descriptive statistical tests and the data represented as means \pm standard deviations within the respective groups. To test for significant differences between the groups, the One Way ANOVA test was employed with the significant threshold fixed at $P < 0.05$. The IBM-SPSS version 20.0 (IBM Corp. Atlanta, GA) software was used for all data analyses. The results are presented in Figures and a Table.

Results

The rats in all the groups were appropriately randomised so they all had statistically similar weights at the start of the experiment. Both concentrations of AEVA and MEVA resulted in significant ($P < 0.01$) weight loss at all the time points in the study. At the end of the study, rats in the AEVA 100 and AEVA 500 groups had mean weights that were similar to that of rats in the normal control group, significantly ($P < 0.001$) lower than the negative control group, but significantly ($P < 0.05$) higher than the positive control group. Rats in the MEVA 200 (but not MEVA 50) group had statistically similar ($P > 0.05$) mean weights with those of rats in the normal control and positive control groups at the end of the study (Figure 1).

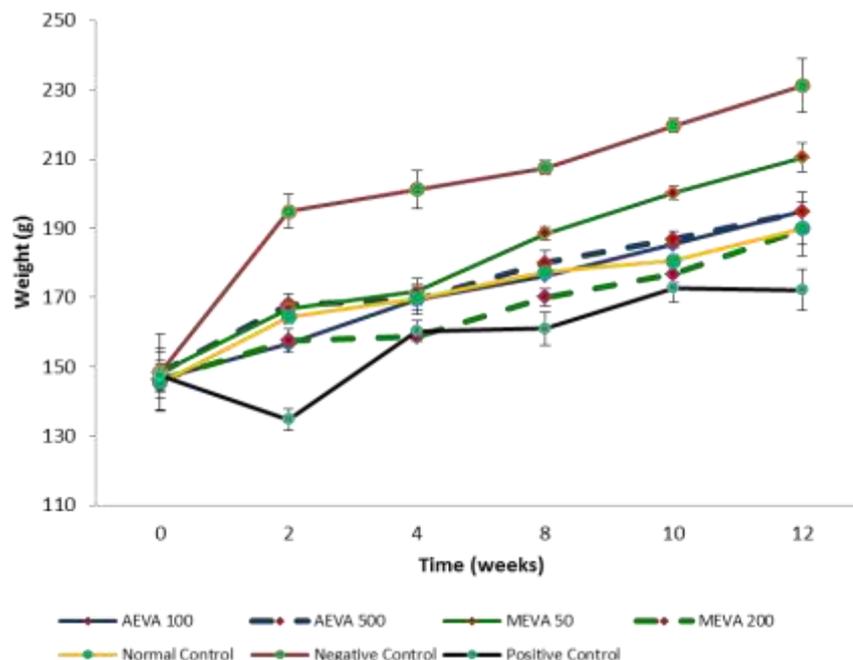


Figure 1: Effects of AEVA and MEVA on weight gain/loss in the test rats

The loss in weight seen in the test groups may not have affected internal organs adversely as seen in Figures 2-5. Other than rats in the AEVA 500 groups (where the relative liver weight was significantly ($P < 0.01$) lower than that of the negative control group), the relative liver weights of the test animals were statistically similar ($P > 0.05$) to those of the normal control and the positive control groups (Figure 2). The relative heart weights were also statistically similar ($P > 0.05$) except for the AEVA 500

and MEVA 200 groups that had significantly ($P < 0.05$) higher values relative to the negative control (Figure 3). From Figures 4 and 5 it is seen that the relative weights of the spleens and kidneys of the rats in all the test groups were statistically similar ($P > 0.05$) to those of the control groups. Apparently the test rats lost some of their fat mass, muscle mass, or both.

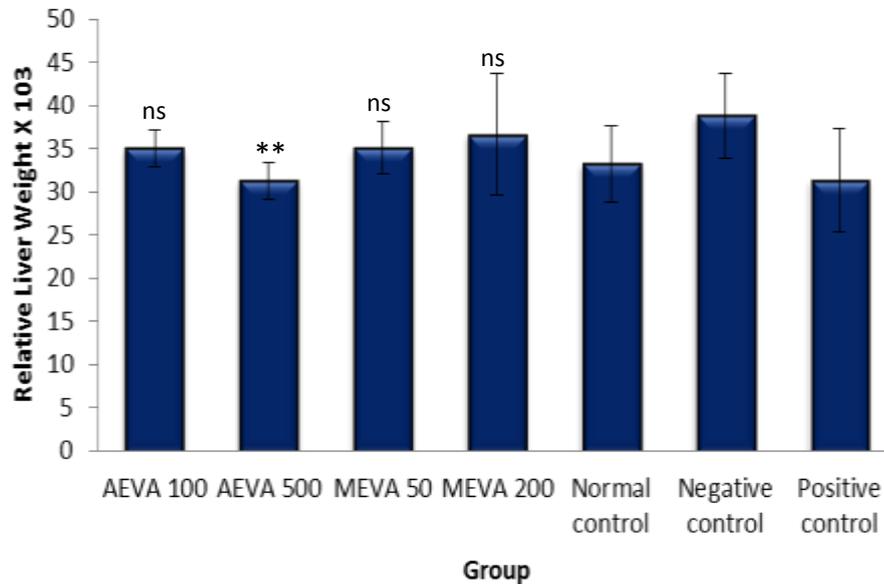


Figure 2: Relative liver weights of rats in the different groups
Comparisons are made with reference to the negative control group.

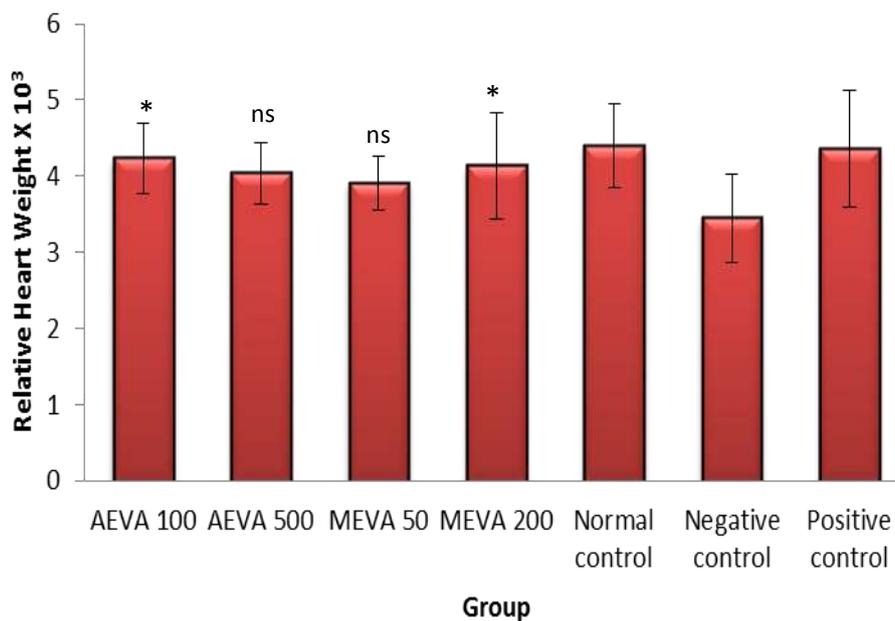


Figure 3: Relative heart weights of rats in the different groups
Comparisons are made with reference to the negative control group.

It appears the feed intake of the rats may have played a role in the observed weight loss. At the second week all the rats on the HFD ate significantly less than their counterparts in the normal control group. This pattern continued till week 6 (except for the MEVA 50 group). At the end of the study however, rats in the negative control group had readjusted to the diet and were consuming quantities of feed that were found to be statistically similar ($P > 0.05$) to those of the normal control group. All the rats in the test groups consumed significantly ($P < 0.01$) less feed relative to their negative control counterparts. Their feed consumptions (except for the MEVA 50 group) were statistically similar ($P > 0.05$) to that of rats in the positive control group (Figure 6). It appears therefore that appetite modulation may be central to the observed weight loss in the test groups.

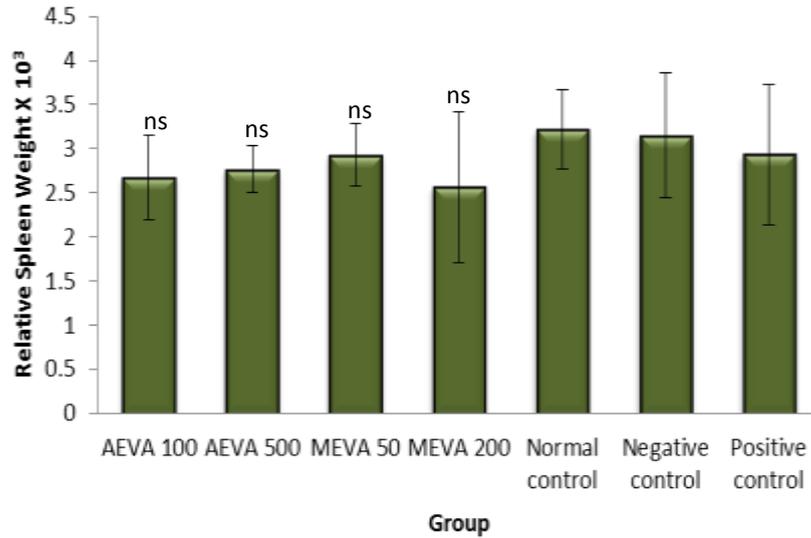


Figure 4: Relative spleen weights of rats in the different groups
Comparisons are made with reference to the negative control group.

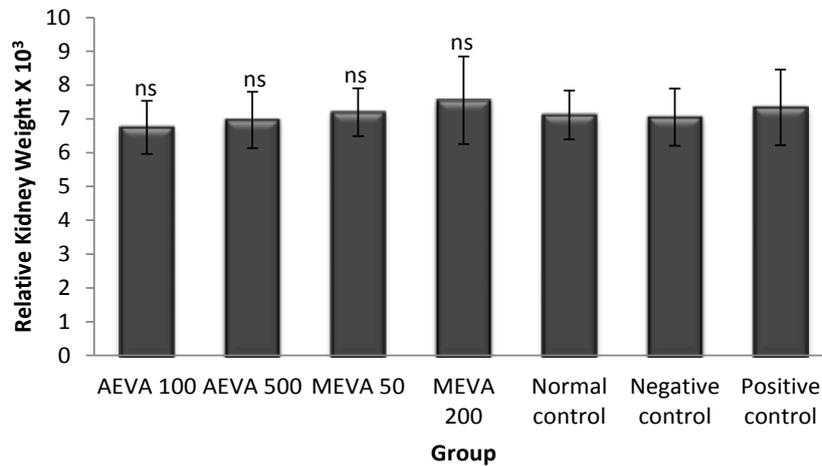


Figure 5: Relative kidney weights of rats in the different groups
Comparisons are made with reference to the negative control group.

Clearly, both AEVA and MEVA positively affected the lipid profiles of the test rats in a dose-dependent manner (Table 3). All the extracts resulted in the significant lowering of TAG, TChol, LDL-Chol and VLDL-Chol concentrations, and elevation of HDL-Chol concentrations in the sera of test rats to values that were comparable to both the positive and normal control rats. Conversely, the blood glucose concentrations of all the rats placed on the HFD, irrespective of treatment were significantly ($P < 0.001$) lower than that of the normal control rats. Nonetheless, the values obtained for the test rats were similar ($P > 0.05$) to that of the positive control. Furthermore, the mean blood glucose concentration for the MEVA 200 group was significantly ($P < 0.05$) lower than that of the negative control. The methanol extract at high dose was therefore capable of reversing the hyperglycaemia occasioned by the HFD.

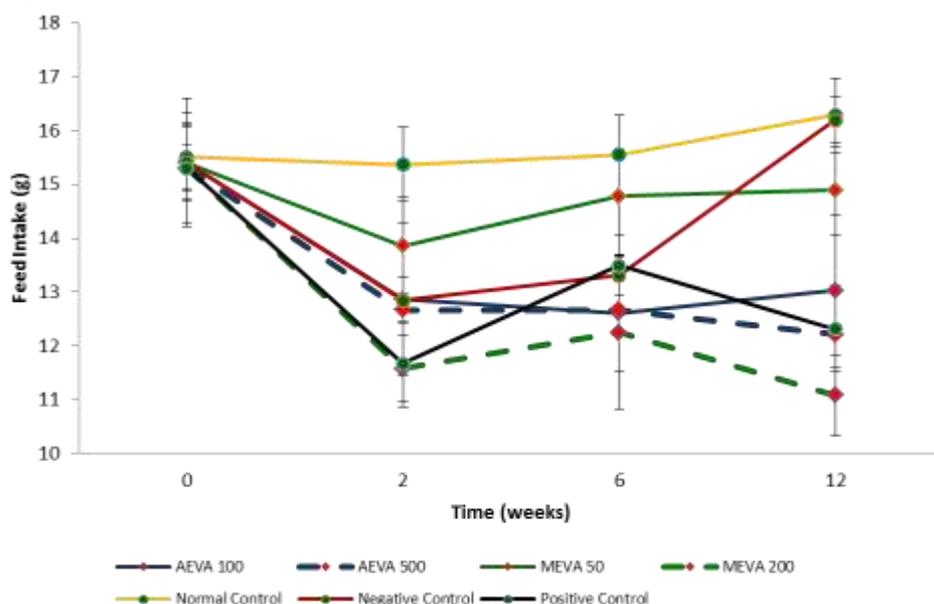


Figure 6: Feed intake of rats treated with AEVA and MEVA for twelve weeks

Table 3: Blood glucose concentrations and lipid panel of rats at the end of the study

	TAG	Total-Chol	HDL-Chol	LDL-Chol	VLDL-Chol	FBG
Normal control	57.4 ± 10.1	195.8 ± 36.3	67.5 ± 10.1	106.3 ± 49.4	11.5 ± 2.0	82.2 ± 18.4
Positive control	52.6 ± 26.9	123.3 ± 17.4	66.0 ± 5.1	46.7 ± 22.6	10.5 ± 5.4	72.2 ± 18.1
Negative control	199.6 ± 34.8	274.1 ± 35.1	54.9 ± 10.9	181.2 ± 32.5	37.9 ± 9.5	97.3 ± 24.2
MEVA 50	55.1 ± 10.9	164.7 ± 39.0	56.0 ± 3.0	90.7 ± 38.5	11.0 ± 2.2	108.2 ± 19.8
<i>P</i>	< 0.001	< 0.001	0.062	< 0.001	< 0.001	0.346
MEVA 200	43.4 ± 7.3	145.5 ± 31.6	62.9 ± 2.9	64.1 ± 32.2	8.7 ± 1.5	87.2 ± 10.3
<i>P</i>	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.109
AEVA 100	89.5 ± 13.2	151.3 ± 5.8	72.8 ± 2.9	71.0 ± 10.7	17.9 ± 2.6	79.8 ± 22.6
<i>P</i>	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.163
AEVA 500	50.7 ± 10.8	144.3 ± 37.6	62.4 ± 9.6	66.7 ± 40.9	10.1 ± 2.2	82.2 ± 10.8
<i>P</i>	< 0.001	< 0.001	0.005	< 0.001	< 0.001	0.163

P values are for comparison with the negative control group. AEVA, MEVA, TAG, Chol, HDL, LDL, VLDL and FBG represent Aqueous extract of *Vernonia amygdalina*, Methanol extract of *Vernonia amygdalina*, triacylglycerols, cholesterol, high density lipoprotein, low density lipoprotein, very low density lipoprotein and fasting blood glucose respectively

Discussion

Obesity is characterised by an increase in either adipose tissue cell number (hyperplasia) or cell size (hypertrophy), or both. It is currently a global epidemic requiring urgent attention, yet it is preventable (Wilborn *et al.*, 2005). Unfortunately, conventional medicines have side effects which raise serious concerns. For example, Orlistat (which is currently approved and sold globally, and which was used as positive control drug in this experiment) is known to cause disturbances in the gastrointestinal tract, and to interfere with the absorption of lipid soluble drugs and vitamins, thereby lowering their effectiveness (Li and Cheung, 2009). Without prejudice to the above, conventional medicines are usually costly, and also often result in weight rebound especially when the medicines are discontinued even for a short time (Abdollahi and Afshar-Imani, 2003). Consequently, owing to these concerns, many people have sought succour in herbal preparations which are thought to be safer in the management of obesity (Yun, 2010; Jadeja *et al.*, 2011). *Vernonia amygdalina* Del. is one plant that holds a lot of promise in this direction, hence this study.

The tested concentrations of the extracts of the leaves of VA (AEVA and MEVA) caused significant weight loss in the test rats. The observed weight loss may have been due to loss of adipose/fat tissues as internal organs were not adversely affected. Yamamoto *et al.* (2000) had reported that edible herbs often caused a reduction in body weight via reduction in fat mass. The observed weight loss may have arisen from a variety of pathways. Phytochemicals and preparations from medicinal plants are known to enhance satiety/suppress appetite, increase metabolic efficiency (especially by decreasing lipogenesis and increasing lipolysis), increase thermogenesis, negatively modulate enzymes involved in the absorption and digestion of lipids, and decrease pre-adipocyte differentiation and proliferation (Hasani-Ranibar *et al.*, 2009; McCrory *et al.*, 2010; Zhang *et al.*, 2014). The data on feed intake presented here however suggests that the observed weight loss may have been due to appetite modulation. Though the exact mechanism(s) are yet to be illuminated, it is plausible that both AEVA and MEVA increase satiety or reduce appetite, thus lowering feed consumption. This is one major finding of this study.

The second important finding is that both AEVA and MEVA clearly modulated metabolic obesity especially with respect to serum lipids. The dyslipidemia that arises due to obesity is one of the links between obesity and its sequelae of metabolic

abnormalities. Natural compounds that are able to result in significant weight loss as well as reverse obesity-related dyslipidemia are therefore very advantageous (Goto *et al.*, 2013). This finding is in consonance with earlier reports of favourable serum lipid modulation in rats fed VA-incorporated diets (Egedigwe and Ijeh, 2010). The observed hypolipidemic effect is suggestive of a possible effect on enzymes involved in lipid homeostasis, chief among which is the pancreatic lipase. This is noteworthy, especially given that the control drug is known to inhibit the said enzyme and some plant extracts are known to act in this manner (Bustanji *et al.*, 2011). Though the differences in serum blood glucose concentrations were not statistically significant, it is worth noting that the extracts, especially AEVA, reduced the serum blood glucose concentrations to the level observed in the normal rats. The lack of statistical significance is likely due to the absence of marked elevations in serum blood glucose concentrations as a result of the high-fat diet. This observed reduction (albeit modest) is of interest as it further ensures that precursors for endogenous lipid synthesis are not available in the test rats. This finding agrees with reports of beneficial blood glucose modulation by VA (Ijeh *et al.*, 2013).

Thirdly, MEVA 200 appears to have had the greatest impact while MEVA 50 had the least, though only AEVA 500 appeared to significantly ameliorate fatty liver (Fig 2). *Vernonia amygdalina* Del. is known to be rich in phytochemicals including terpenes, coumarins, phenolic acids, lignans, and xanthenes (Ijeh and Ejike, 2011). These phytochemicals in VA are apparently responsible for the observed weight loss and beneficial metabolic profile in the test rats. A few examples illustrate this assertion. Terpene trilactones from *Ginkgo biloba* L. possess hypolipidaemic and antiobesity activities as they are able to inhibit pancreatic lipase (Bustanji *et al.*, 2011). The coumarin, esculetin, from *Fraxinus rhynchophylla* is reported to be active in inhibiting early stage adipogenic differentiation thereby being useful in obesity management (Shin *et al.*, 2010) and in preventing HFD-induced hepatic steatosis (Um *et al.*, 2013). Naturally occurring flavonoids and phenols inhibit adipogenesis *in vitro* (Hsu and Yen, 2007) and have inhibitory effects on obesity (Hsu and Yen, 2008). Flaxseed lignans have been reported to show beneficial effects on lipid metabolism in diet-induced obesity in mice (Fukumitsu *et al.*, 2008). They also cause significant loss in weight and beneficial reduction in fat accumulation (Park and Velasquez, 2012). Xanthenes from *Garcinia mangostana* have been shown to be useful in preventing and treating obesity (Liu *et al.*, 2015). It is very plausible therefore that a synergistic interrelationship between these active phytochemicals may be responsible for the observed effects. In the absence of hard data, this would remain rather speculative.

Finally, it is interesting to note that all extracts compared considerably well with the standard drug, Orlistat. This clearly stands VA out as a very useful plant especially in the search for active phytochemicals that can be developed into an effective, cheap, and natural anti-obesity therapeutic. This is particularly interesting as significant toxicity has not been reported in studies evaluating the use of VA in the management of any metabolic derangement, and the leaves are eaten raw or processed and used as culinary vegetables in many cultures. The identification of the specific mechanism(s) of action of these extracts is obviously warranted and further studies are underway to elucidate that.

In conclusion, the anti-obesity potentials of an aqueous and a methanol extract of *Vernonia amygdalina* Del. were studied in high-fat diet-induced obesity of rats. Both extracts resulted in significant weight loss and significant improvement in some metabolic markers of obesity in the test rats compared to the negative control rats. The extracts closely mimicked the action of the standard drug, Orlistat. The observed effects are likely as a result of the rich chest of phytochemicals present in VA. An investigation into the mechanism(s) of action of the extracts is nonetheless warranted, and is ongoing in our laboratory.

Acknowledgments

The authors would like to acknowledge research support from TETFund Nigeria 2012/2013 grant administered through the MOUAU-DURA which supported this study.

Declaration: The authors have no real or potential conflicts of interest to declare. CAE was responsible for the experiments, and participated in study design, data analyses and revision of the initial manuscript. CECCE participated in study design, analysed the data, plotted the charts/graphs, and wrote the manuscript. III conceived and designed the study, and participated in revising the initial manuscript. UH participated in study design and revising of the initial manuscript. GIO and VUA participated in study design. All authors made significant contributions to the project supervision, and read and approved the eventual manuscript.

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