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BODY AND ORGAN WEIGHT CHANGES IN MALE WISTAR RATS TREATED WITH SAPONINS EXTRACT OF *Vernonia amygdalina* AND *Vernonia colorata.*

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ABSTRACT

Vernonia amygdalina (VA) and Vernonia colorata (VC) have been reported to have anti-obesity activities attributable to phytochemicals such as saponins. We studied the effect of crude saponin-rich extracts of VA (VACS) and VC (VCCS) on body weight and relative organ weight (ROW) in male Wistar rats fed high-fat diet (HFD). 40 rats were randomly allocated to 8 groups of 5, with average weights of 183g. Group 1; Basal control Diet (BCD), Group 2; HFD only, Group 3 and 4; HFD + 100 mg/kg VACS and HFD + 200 mg/kg VACS respectively, Group 5 and 6; HFD + 100 mg/kg VCCS and HFD + 200 mg/kg VCCS respectively. Group 7; HFD + Orlistat 10mg/kg, Group 8; HFD + Orlistat 10mg/kg + dimethyl sulfoxide (DMSO). All treatments were administered orally for 10 weeks. The HFD-only group had the highest increase in weight. Administration of VCCS and VACS resulted in weight loss relative to the HFD group only. The effect was not dose-dependent but compared favourably with Orlistat (10 mg/kg). 100 mg/kg VACS produced the highest weight reduction of all the saponins -rich extracts treated groups. There was no difference between the ROW of the treatment groups and the BCD group suggesting there was no organ toxicity. crude saponins. Our findings suggest that saponins-rich fractions from VA and VC may be useful in weight management.

Keywords: Obesity, *Vernonia colorata*, *Vernonia amygdalina*, High fat diet.

INTRODUCTION

Saponins are glycosides characterized by their distinctive foaming properties and are naturally occurring in plants (Zhu *et al*., 2019). They are used as soap though studies have shown that they possess anti-obesity and anti-hyperlipidemic effects (Liao *et al*., 2021).

Ijeh *et al*. (2014) reported that saponins extracted from *V. colorata* and *V. amygdalina* exhibited both hypolipidemic activities and body weight regulatory effects in experimental rats. The present study seeks to further compare the effect of concomitant feeding of high fat diet and administrations of crude saponin-rich fractions from VA and VC on body and organ weigh changes in albino rats.

Anti-nutritional phytochemicals such as saponins and tannins have been shown to cause a reduction in body weight of experimental animals either by suppressing the growth of animals, by inhibiting digestion, or by inhibiting the activity of the pancreatic lipase (Shittu *et al*., 2016; Oyeyemi *et al*., 2018).

Though saponins have been shown to possess anti-obesity potentials while previous studies have shown the weight-reducing and anti-obesity potentials of *V. amygdalina* and *V. colorata*, no study has specifically linked these effects to their saponin composition thus warranting this study while we compared the anti-obesity potentials of the two popularly used and often not easily distinguished species of *Vernonia*.

This study aims to determine the effect of the administration of crude extracts of *Vernonia amygdalina* and *V. colorata* on body weight and organ weight changes in male Wistar rats fed a high-fat diet.

MATERIALS AND METHODS

Collection and identification

Fresh leaves of *V. amygdalina* and *V. colorota* were obtained from Orie Ugba market, a local market in Ugba Ibeku, Umuahia North local government area, Abia State, Nigeria. The plant was identified and authenticated by Mr. Ibe Ndukwe of the Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike (MOUAU), Abia State. A voucher specimen was kept at the herbarium in the Department of Physiology and Pharmacology, College of Veterinary Medicine, MOUAU (MOH014). The leaves were thoroughly washed and air-dried at room temperature. The dried leaves were pulverized using a laboratory electric blender. The pulverized sample was stored in air-tight plastic containers until it was required for use.

Preparation of crude saponin

Defatting was done using thirty grams (30 g) of *V. amygdalina and V. colorata* powder, each was weighed and placed in the 250 ml quartz extraction vessel of the microwave-assisted extraction system (fabricated in the Chemical Engineering department, MOUAU, Nigeria), after the addition of 6 ml of distilled water, it was mixed briefly and 180ml of hexane was added and the mixture shaken again. The vessel was inserted in the microwave cavity, fitted with a condenser, and irradiated at 520 MHz for 2 minutes. This was done till 1kg of *V. amygdalina and V. colorata* was defatted. The defatted powder (40g) was mixed with methanol: water (7:3) 160ml and was placed in the fabricated microwave oven and irradiated at 520Mhz for 15mins. This was done till 1 kg of both *V. amygdalina and V. colorata* was extracted. Butanol wash was done with a separating funnel to further purify the crude saponin. Exactly 200 ml of the sample was washed with 60 ml of butanol 4 times. The saponin -rich butanol fraction was washed 3times with 5 % sodium chloride (NaCl) to obtain crude saponin

Preparation of high-fat diet

The high-fat diet was prepared according to the method reported by Mashmoul *et al*. (2017).

The diet was composed of corn starch, casein, beef tallow, sucrose, cellulose, mineral mix, vitamin mix, dl-methionine, and bitartrate at different concentrations. However, corn oil was absent in the high-fat composition (Table 1.0 & 2.0).

Table 1.0 Composition of the High fat diet

Ingredients	High-fat diet (g/kg diet)
Corn starch	150
Casein	200
Beef tallow	400
Corn oil	0
Sucrose	150
Cellulose	50

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Mineral mix	35
Vitamin mix	10
DL-methionine	3
Choline bitartrate	2

Preparation of basal diet.

The preparation of a normal diet was also done according to the method and measurements described by Masmoul *et al*. (2017).

The diet was composed of corn starch, casein, corn oil, cellulose, mineral mix, vitamin mix, DL-methionine, and bitartrate at different concentrations. However, the beef tallow and sucrose were absent in the high-fat composition.

Table 2.0 Composition of the Basal diet

Ingredients Corn starch	Basal diet (g/kg diet) 650
Casein	200
Beef tallow	0
Corn oil	50
Sucrose	0
Cellulose	50
Mineral mix	35
Vitamin mix	10
DL-methionine	3
Choline bitartrate	2

These compositions (high-fat diet and basal diet) were mixed in a container and converted to pellets by extrusion and slicing through an improvised 5 ml syringe and then a 10 ml syringe. The pellets were allowed to dry in an oven at low temperature (35^{o}C) and packaged. The animals were fed accordingly and Lee's index (Lee.,1929) was performed after 7 weeks to ascertain obesity.

Stock preparation and administration

Ten grams (10g) of the methanol extract of *V. amygdalina was* dissolved in 2% dimethyl sulfoxide (DMSO) to form 0.1 m/L stock concentration. The stock concentration was administered to animals according to their body weights. The standard drug (orlistat) was dissolved in dimethyl sulfoxide as well. The volume of standard drug and stock concentration of *V. amygdalina* administered to the animals were obtained using the formula:

$$\text{Volume (ml)} = \frac{Dose\ (mg/kg) \times weight\ of\ rat\ (kg)}{concentration\ of\ stock\ (mg/ml)}$$

Body weight calculation

Changes in body weight: the body weight (g) was recorded for 10 weeks (week 0 and then alternate days for 10 weeks) in each group before giving the food and water. Change in the body weight of animals was calculated on a weekly basis using the formula:

$BW = W_1 - W_0$

Where BW = Body weight

W_1 = final body weight

W_0 = initial body weight

Experimental Design

A total of forty (40) Wistar rats of about 6 – 8 weeks old obtained from the Animal Breeding Unit of the College of Veterinary Medicine, University of Nigeria, Nsukka were used for the study. The animals were housed in well-ventilated stainless-steel cages under standard laboratory conditions and were given commercial rat feed and water *ad libitum.* They were allowed to acclimatize for 2 weeks before the commencement of the experiment. The rats were distributed into 8 groups of 5 animals each and the different fractions of the extract were administered to the experimental groups as follows:

Group I: Basal diet control.
Group II: High-fat diet only.
Group III: High-fat diet + low dose (100 mg/kg bwt) of *Vernonia amygdalina crude* saponin extracts
Group IV: High-fat diet + high dose (200 mg/kg bwt) of *Vernonia amygdalina* crude saponin extracts
Group V: High-fat diet + low dose (100 mg/kg bwt) of *Vernonia colorata* crude saponin extracts
Group VI: High-fat diet + high dose (200 mg/kg bwt) of *Vernonia colorata* crude saponin extracts
Group VII: High-fat diet + anti-obesity drug (Orlistat 10mg/kg bwt)
Group VIII: High fat diet + anti-obesity drug (Orlistat 10 mg/kg bwt + DMSO)

The crude saponin extract was administered orally to the animals based on their body weight throughan oral gavage daily for 10weeks. On the 10^{th} week, the animals were fasted overnight and sacrificed through cervical dislocation, and blood samples were collected via cardiac puncture, while the organs(heart, liver, kidneys and spleen) of the rats were collected for biochemical analysis and histology.

Relative Organ Weight

Following the sacrifice, different organs (heart, liver, kidney, and spleen) were excised from the animal and briefly placed on a filter paper to remove blood. The blood-free organs were weighed and relative organ weight was obtained across the groups.

Relative organ weight = $\frac{W1}{W0}$

Where W_0 = final body weight of the animal before sacrifice

W_1 = weight of excised organ.

Statistical Analysis

Statistical analyseses of the data were carried out with SPSS version 23.0 using One Way Analysis of Variance (ANOVA). The statistically analyzed data were reported as mean ± standard deviation (SD). A significant difference was accepted at a 95 % confidence level of probability ($P < 0.05$).

RESULTS AND DISCUSSION

Results of weekly body weight measurements

The result of the weekly body weight measurements (Fig.1) showed a general increase in weight across all the treatment groups compared to the group that received the basal diet only. Saponins from *V. amygdalina* and *V. colorata* produced weight reduction when compared to the high-fat diet group. The effect was however not dose-dependent but was similar to the results obtained from the standard drug, orlistat 10 mg/kg bwt.

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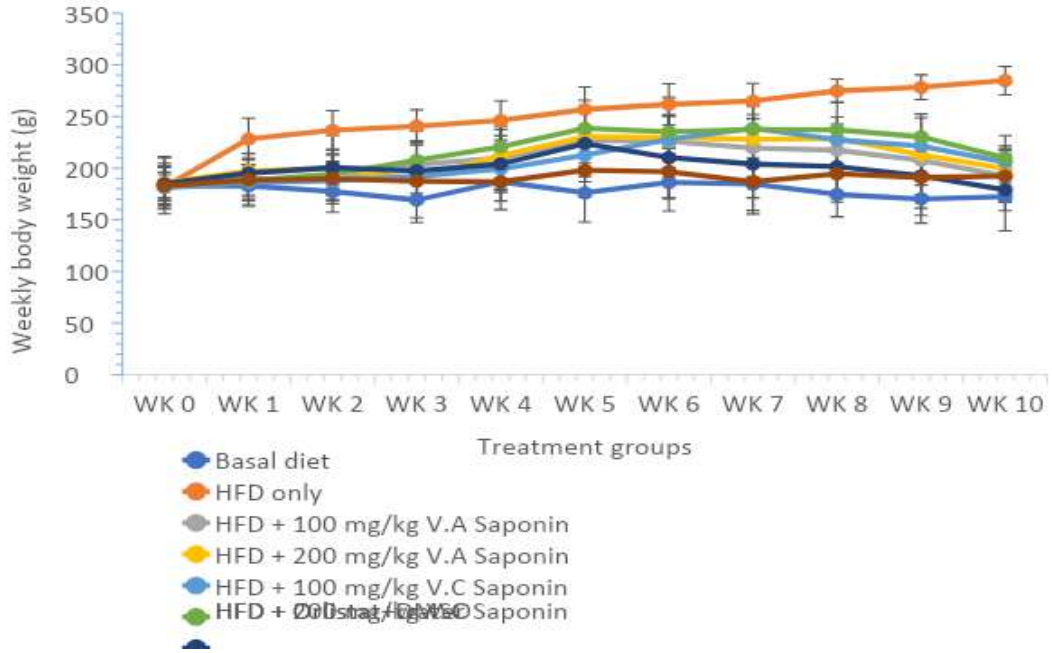

Figure 1: Effects of different concentrations of saponin extract of *V. amygdalina* (VA) and *V. colorata* (VC) on weekly body weight changes of high-fat diet-induced obese rats. The weekly body weight changes were plotted as mean ± standard deviation (n = 5)

Result of body weight gain/loss

The result of the body weight gain/loss (Fig.2) showed general weight loss in the group that was administered basal diet, HFD + Orlistat, while there was an increase in weight in the HFD- only group, there was also a significant decrease in weight of the groups that were co-treated high-fat diet and different doses of saponin extract of *V. amygdalina* and *V. colorata* compared to that of the untreated group (HFD only).

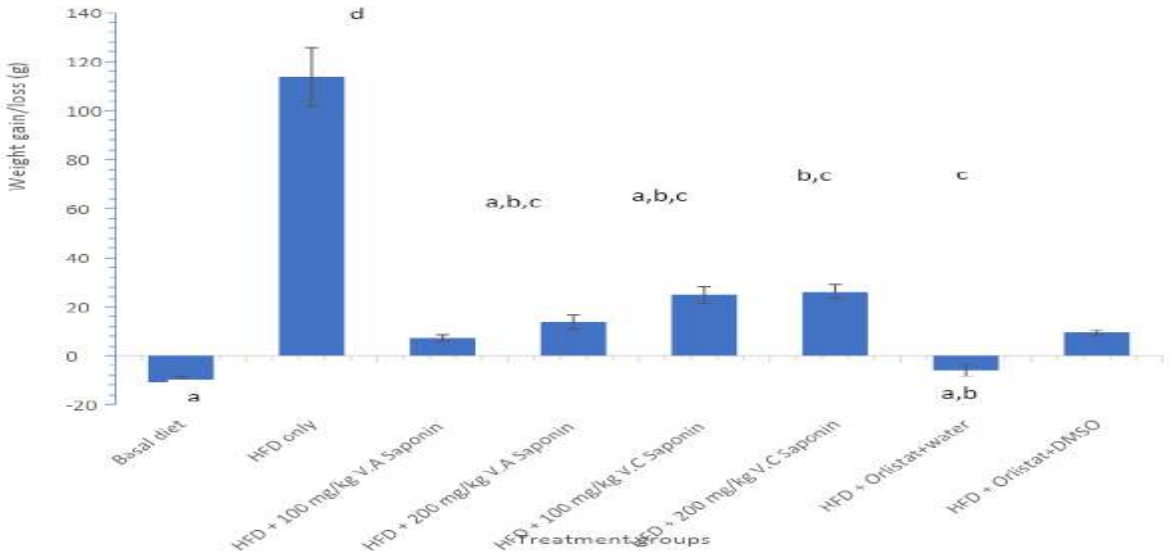

Figure 2: Effects of different concentrations of saponin extract of *V. amygdalina* (VA) and *V. colorata* (VC) on body weight gain/loss of high fat diet-induced obese rats. Bars are presented as mean ± standard deviation (n = 5), and bars with different superscripts letters are significantly ($p<0.05$) different from the paired mean.

Percentage weight gain

The result of the percentage weight gain (Fig.3) showed a high percentage weight gain (67%) in the untreated group (HFD) while there was a negative percentage weight gain (-5%) in the groups that were administered basal diet, HFD + orlistat. Meanwhile, the percentage weight gainwas significantly decreased in groups that were co-treated different doses of saponin extract of *V. amygdalina* and *V. colorata/*

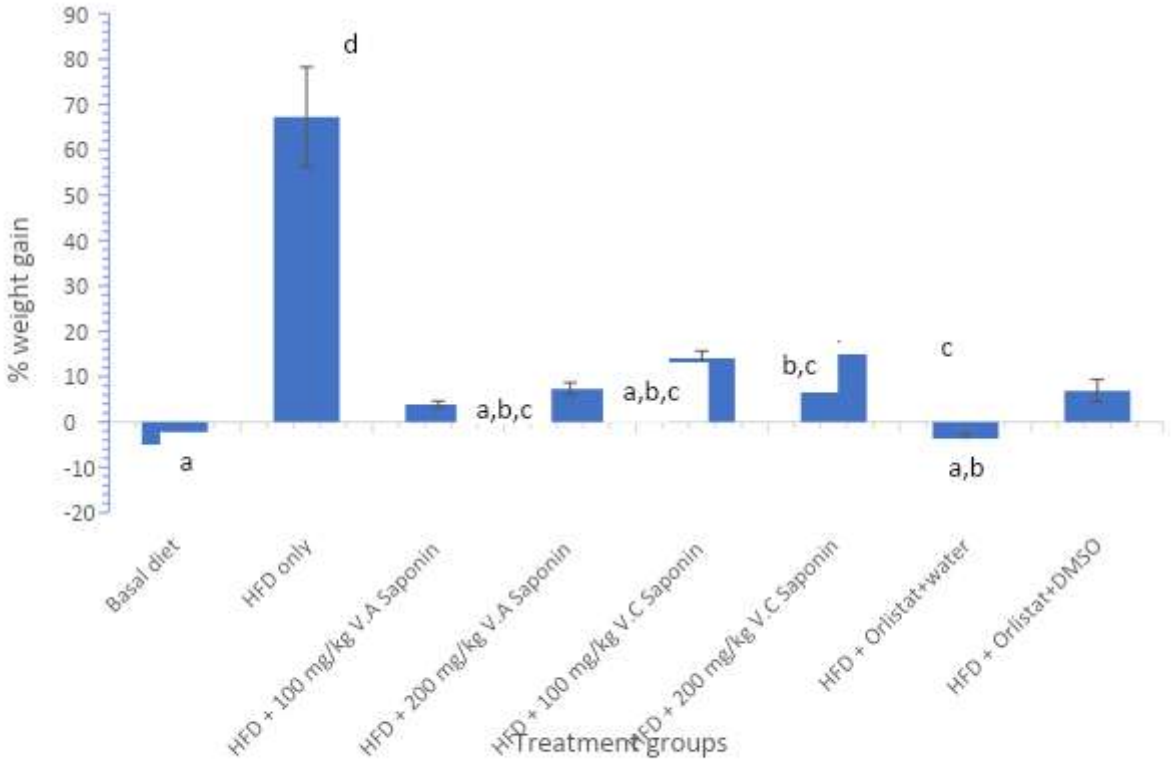

Figure 3: Effects of different concentrations of saponin extract of *Vernonia amygdalina (*VA) and *Vernonia colorata* (VC) on percentage body weight gain/loss of high fat diet-induced body weight gain rats.

Bars are presented as mean ± standard deviation (n = 5); and bars with different superscripts letters are significantly ($p<0.05$) different from paired mean

The result of Lee's Index

The result of Lee's index (Fig.4) showed a slight increase across all the treated groups in week 2 followed by a decrease in week 3. However, the result of Lee's index showed a sharp increase across all the treatment groups in week 8 followed by a sharp reduction in week 9.

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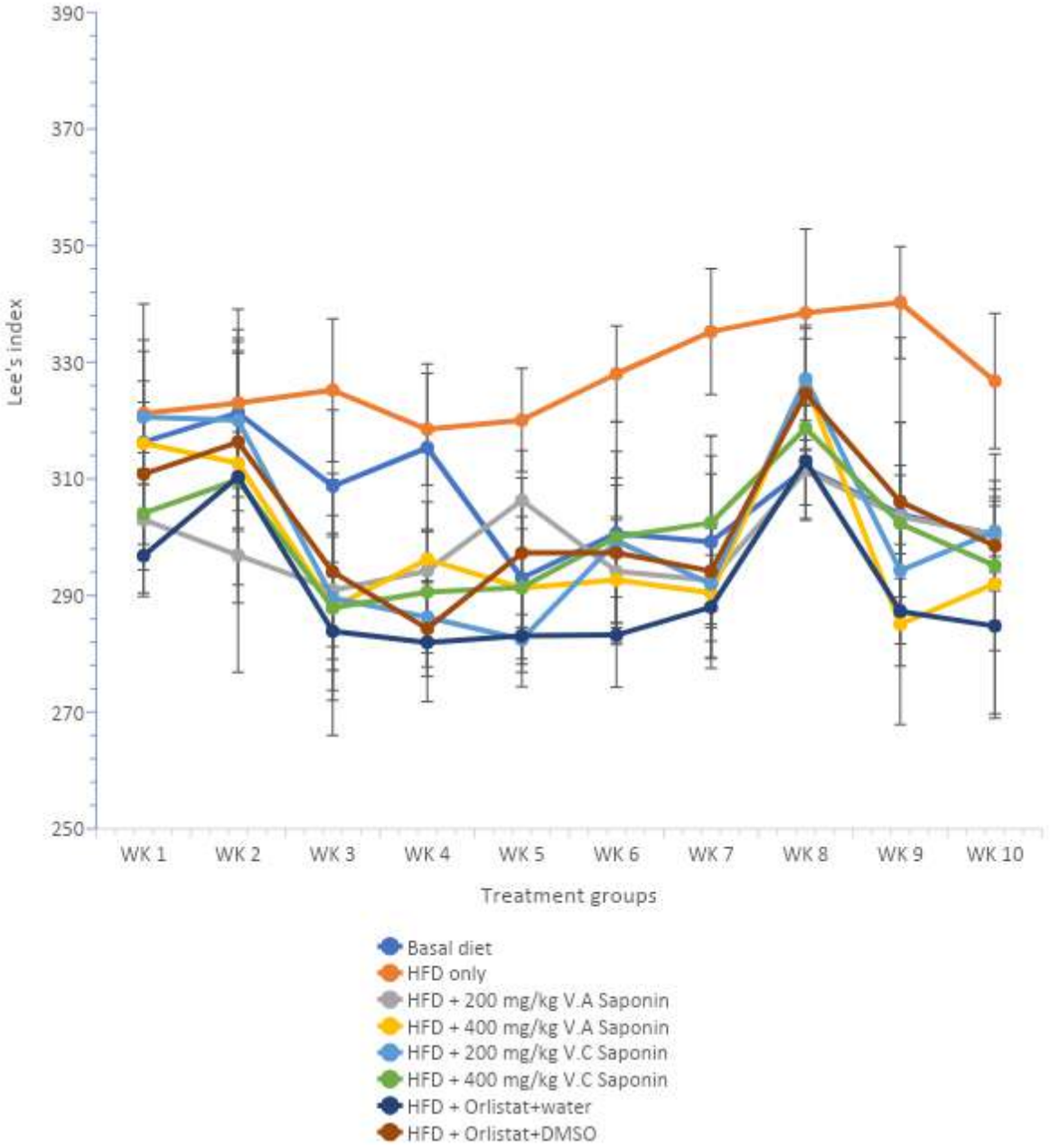


Figure 4: Effects of different concentrations of saponin extract of *Vernonia amygdalina (*VA) and *Vernonia colorata* (VC) on Lee's index of high-fat diet-induced body weight gain in rats

Relative stomach weight.

The result of the relative stomach weight (Fig. 5) showed no significant differences between organ weight in the groups that received HFD + 100 mg/kg *V. amygdalina* saponin, HFD + orlistat + water and HFD + orlistat + DMSO compared to those of the basal diet group.

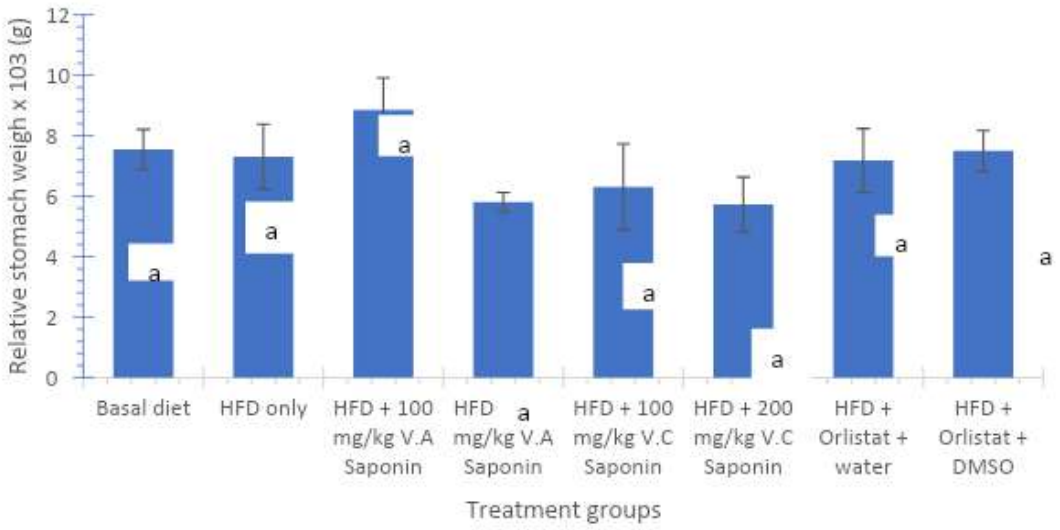
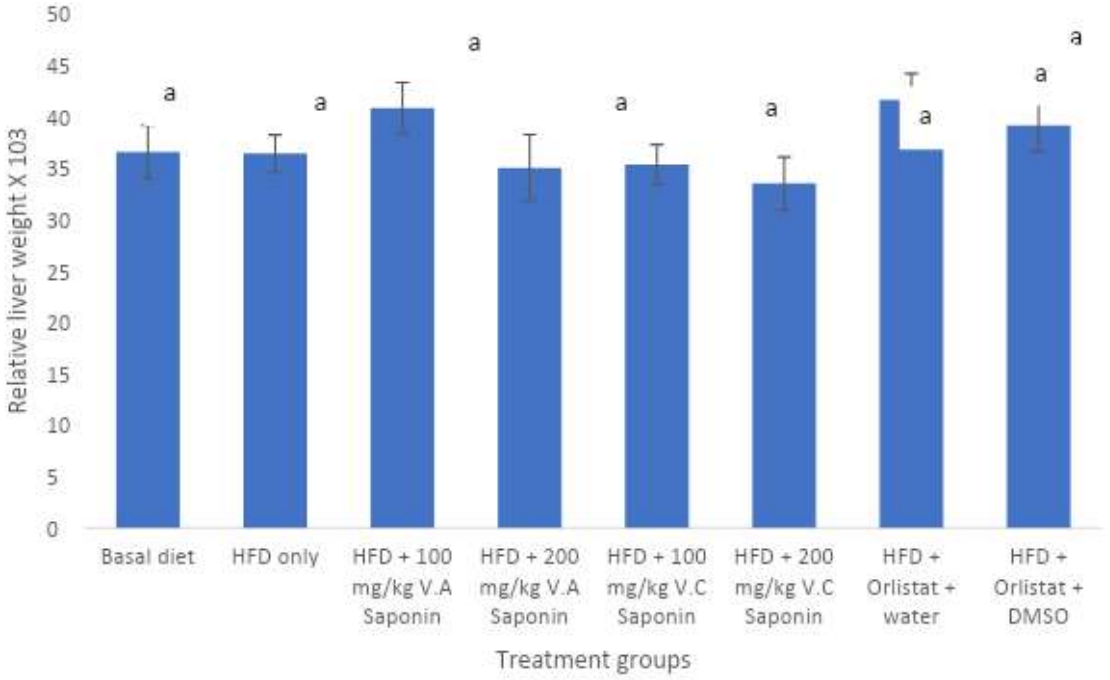


Figure 5: Effects of different doses of crude saponin extract from *Vernonia amygdalina* (VA) and *Vernonia colorata* (VC) on relative stomach weight of high fat diet-induced rats.

Bars are presented as mean ± standard deviation (n = 5), and bars with different superscripts letters are significantly ($p<0.05$) different from the paired mean.

Relative liver weight

The result of the relative liver weight (Fig. 6) showed a marked increase in the liver weight of the groups that received HFD + orlistat dissolved in water and DMSO respectively including the group co-treated HFD and 100 mg/kg bwt V.A saponin. It also indicated reduced organ weight in the groups that co-treated with different concentrations of V.C saponin compared with the basal and untreated groups.



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Relative kidney weight

The result of the relative kidney weight (Fig.7) showed a marked increase in the kidney weight of the groups that received HFD+ orlistat dissolved in water and DMSO respectively. However, the result also indicated significantly (P<0.05) reduced kidney weight in the groups co-treated with different concentrations of V.C saponin and 200 mg/kg bwt V.A saponin compared with the basal and untreated groups.

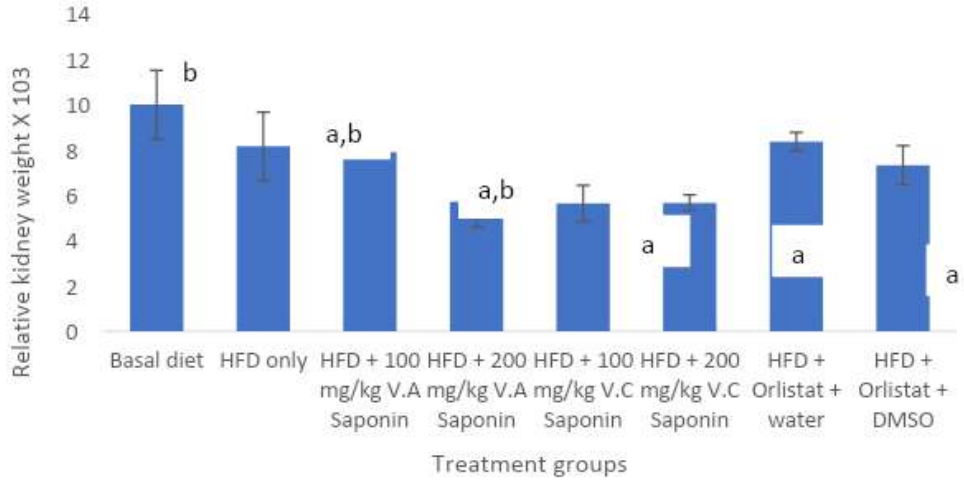

Figure 7: Effects of different concentrations of saponin extract of *Vernonia amygdalina* (VA) and *Vernonia colorata* (VC) on relative kidney weight of high fat diet-induced obese rats. Bars are presented as mean ± standard deviation (n = 5), and bars with different superscripts letters are significantly (P<0.05) different from the paired mean.

Relative heart weight

The result of the relative heart weight (Fig. 8) showed a significant (p<0.05) increase in the heart weight of the groups that received HFD+ 100 mg/kg bwt V.A saponin, HFD + orlistat dissolved in water, and DMSO respectively compared with HFD only. There was also significant (P<0.05) reduced heart weight in the groups co-treated with differentconcentrations of V.C saponin compared with the basal and untreated groups.

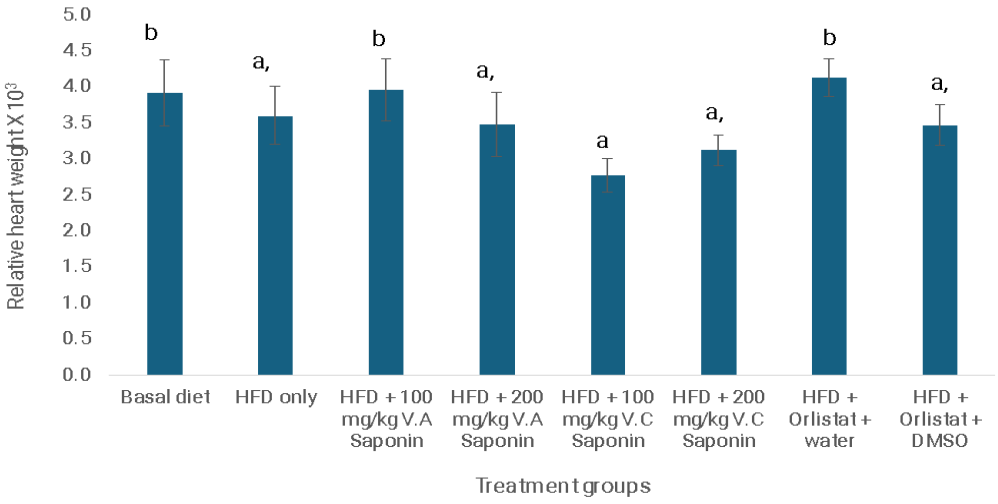

Figure 8: Effects of different concentrations of saponin extract of *Vernonia amygdalina* (VA) and *Vernonia colorata* (VC) on relative heart weight of high fat diet-induced obese rats. Bars are

presented as mean ± standard deviation (n = 5), and bars with different superscripts letters are significantly (P<0.05) different from the paired mean.

Relative spleen weight

The result of the relative spleen weight (Fig. 9) showed a significant (p<0.05) decrease in the mean spleen weight of the groups that received HFD + orlistat dissolved in DMSO and the group co-treated HFD and 200 mg/kg bwt VA saponin and 200 mg/kg VC saponin compared to that of the group that received HFD only. The weight of the groups that were co-treated with HFD + 100 mg/kg bwt VA saponin and HFD + orlistat dissolved in DMSO showed a marked increase compared to that of the HFD-only group.

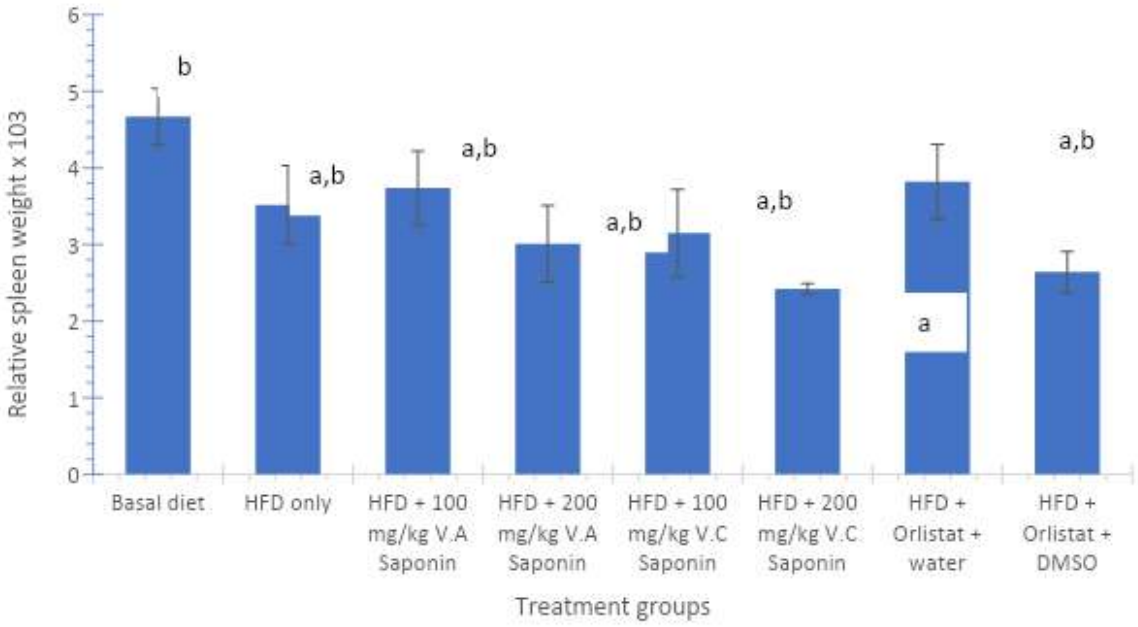

Figure 9: Effects of different concentrations of saponin extract of *V. amygdalina* (VA) and V. colorata (VC) on relative spleen weight of high fat diet-induced obese rats. Bars are presented as mean ± standard deviation (n = 5), and bars with different superscripts letters are significantly (P<0.05) different from the paired mean.

Relative lungs weight

The mean relative lung weight (Fig.10) of the HFD-only group showed a significant (p<0.05) increase compared to that of the basal diet group. However, the result of the relative mean lung weight showed a significant (p<0.05) decrease in the mean weight across all the co-treated groups compared to that of the HFD-only group.

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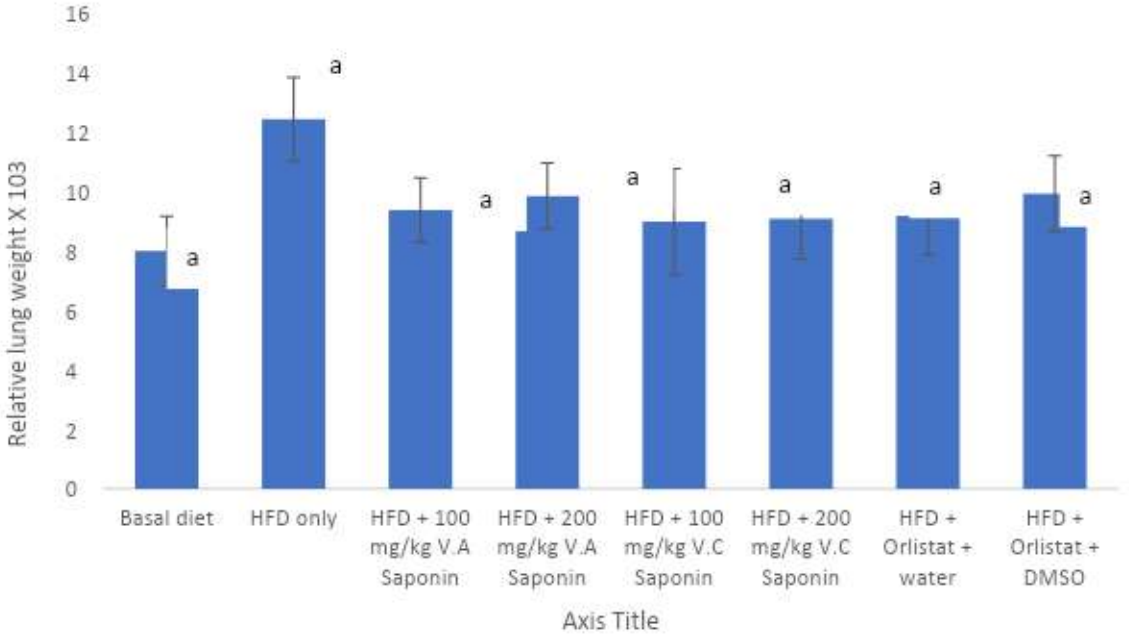

Figure 10: Effects of different concentrations of saponin extract of *Vernonia amygdalina* (VA) and *Vernonia colorata* (VC) on relative lung weight of high fat diet-induced obese rats Bars are presented as mean ± standard deviation (n = 5), and bars with different superscripts letters are significantly (P<0.05) different from paired mean.

DISCUSSION

High-fat diets have been known to play a significant role in the induction of obesity. Obesity is characterized by excessive weight gain when there is increased energy storage because of a lower energy expenditure ratio compared to energy intake (Lee *et al*., 2016).

The study reveals initial weight gain in all animals across all the tested groups. This initial weight gain could be attributed to the disruption of the normal energy balance by the high-fat diets leading to increased positive energy compared to the daily required expenditure resulting in an increase in fatty tissues through the conversion of excess energy into lipids and accumulation of triacylglycerol leading to weight gain (Miyazawa *et al*., 2018). Another mechanism for the weight gain could be the excessive availability of fats for absorption in the intestines following the administration of the high-fat diets leading to increased fat absorption into the circulatory system, hence hyperlipidemia and accumulation of fatty deposits and adipocytes which is implicated in weight gain.

From week 6, there was a marked reduction in the body weight of the animals co-treated with different dosesof crude saponin extract of *V. amygdalina* and *V colorata* with the extracts from *Vernonia amygdalina* producing a superior anti-obesogenic effect compared to *Vernonia colorata*. This weight reduction could be attributed to the anti-obesity properties of saponin since saponins have previously been linked weight lossthrough different mechanisms (Shahriari *et al*., 2021). The result is in agreement with the earlier study reported by Eraga *et al*. (2020), on the benefits of *Vernonia amygdalina*, and *Vernonia colorata* in managing obesity, hence their saponin composition was able to inhibit weight gain in animal models fed with high-fat diets. Saponins have also been found to induce anti-obesity effects in animal models through reduced bioavailability of fats for absorption following intake of fatty foods. Possible mechanisms of action of the saponin extracted from *V. amygdalina* and *V. colorata* include

the formation of micelles with the bile acids thereby limiting the ability of fats to be transported thus, reducing their bioavailability. Reduced absorption of fats or their transportation has been linked to weight reduction in animal models (Lou *et al*., 2018). Another mechanism through which saponins exert their anti-obesity effect is by altering the biological activity of enzymes implicated in the digestion and bioavailability of fats from food. Enzymes such as pancreatic lipase and intestinal and lingual lipases are actively involved in the bioavailability of fats. Probably, the saponins extracted from *V. amygdalina* and *V. colorata* was able to inhibit the activity of these enzymes leading to reduced bioavailability of fats for absorption and storage hence, reduced energy availability. Invariably, prolonged intake of the saponins from the two samples (*V. amygdalina* and *V. colorata*) overtime, restricted the enzymatic activity of these enzymes causing the weight reduction which was first noticed in the latter part of the study (starting from week 6).

The organ weights estimated include the stomach, kidney, liver, heart, spleen, and lungs. The result of the relative organ weights estimation of rats co-treated with high a fat diet with different doses of saponin extract of *V. amygdalina* and *V. colorata* indicated that there was no significant difference in the organ weight across all groups. The internal vital organs are usually the first to be affected by eventualities of metabolic reactions caused by the intake of toxicants. Internal organs play pivotal roles in survival and maintenance of homeostasis such as excretion (kidney), metabolism of xenobiotics (liver), and pumping of blood for the distribution of oxygen to the body (heart). Relative change in organ weight is usually used as a predictor or indicator of organ damage or toxicity and it is characterized by a change in the weight of internal organs without a general change in the weight of the body. An increase in the relative weight of an organ is usually used as a predictor or indicator of pathological condition by toxicants.

The result which showed that the weights of all organs were all within comparable range across all the tested groups could be indicative that the saponin extracts of *V. amygdalina* and *V. colorata* were not toxic to the vital internal organs at the tested cdoses. This agrees with the reports of Udyanee *et al*. (2022), which classified extracts of *V. amygdalina* and *V. colorata* as medicinal and can be used in the management of high-fat diet-induced obesity with results showing similar efficacy with the standard drug orlistat 10 mg/kg bwt.

CONCLUSION

The study showed that the administration of crude saponin extract of *V. amygdalina* and *V. colorata* reduced body weight gain in high-fat diefedmale Wistar rats. This result is further buttressed by the results of the relative organ weights which were all within the acceptable range in the groups co-treated with different concentrations of saponin extract of *V. amygdalina* and *V. colorata.* Our findings suggest that saponin content of *V. amygdalina* and *V. colorata* contribute to the weight reduction potentions and the two plants and that *V. amygdalina* saponins may be more effective than *V. colorata*

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Conflict of interest: The authors declare no conflict of interest.

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