



EFFECT OF DIETARY INCORPORATION OF *VERNONIA CALVOANA* LEAVES ON BODY WEIGHT CHANGES AND SOME LIVER FUNCTION INDICES IN NORMAL ALBINO WISTAR RATS

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ABSTRACT

The effects of *Vernonia calvoana* (VC) diets on body weight changes and some liver function indices in albino Wistar rats were evaluated in this study. A total of twenty four Wistar rats weighing 70 – 100 g were divided into 4 groups of 6 rats each. Group 1 (normal control) received 120g of rat chow. The *V. calvoana* leaves powder were incorporated into pelletized rat chow in percentage ratios: 5/115 %, 15/105 % and 30/90 % to groups 2, 3 and 4 respectively for 30 days. At the end of the experiment, the animals were euthanized with chloroform vapour. Blood was obtained via cardiac puncture and livers were harvested for histological studies. Results showed significant ($p < 0.05$) increase in serum ALT for 30: 90 (group 4) when compared to the control while groups 5/115 % and 15/105 % (groups 2 and 3) showed decreases when compared to the control. The AST levels in 5/115 % and 15/105 % decreased significantly ($p < 0.05$) relative to the control. The ALP activity was high in all the treated groups compared to the control. Serum total protein levels were slightly increased in the treated groups compared to the controls. Morphological changes were observed in the treated groups relative to the controls. We therefore conclude that low level supplementation in the ratio of 5/115 % increased body weight while incorporation at 15/105 % and 30/90 % respectively decreased body weight which suggests probable potential of *V.calvoana* in body weight control.

Keywords: Weight control, *V.calvoana*, hepatic integrity and Wistar rats

INTRODUCTION

Most vegetables are known for their nutraceutical potentials and relevance to mankind as food. *Vernonia* is a member of the Asteraceae family and comprise about one thousand (1,000) species some of which are edible and also used in ethnomedicine. Such species include *Vernonia amygdalina*, *Vernonia colorata*, *Vernonia calvoana* (Ijeh *et al.*, 2016). *V. calvoana* has been reported to be widely distributed in South-Western Cameroun and South-Eastern part of Nigeria alongside its sister species, *V. calvoana* is commonly called 'Ekeke, Adimichi, Uchu-inyin and Nyep-ochu' by the indigenous people of Yakurr, Obubra, Bekwarra and Etung Local Government Areas of Cross River State, respectively. The leaves

are consumed unprocessed in these areas due to its sweet taste; apart from the ethno-medicinal values. However, the leaves are directly incorporated when dried in the diet especially during preparation of native soups, stews and porridges.

Igile *et al.* (2013) reported the use of *V. calvoana* vegetable in the treatment of heart disease, blindness, diabetes, malaria and gastrointestinal disorders based on folk lore claims. Also Focho *et al.*, (2009) reported the ethnomedicinal potentials of *V. calvoana* in the Cameroun region in the management of navel aches and constipation. Toyang and Verpoorte (2013) reported that *V. calvoana* is not well

known and had suggested that further studies be carried out in order to confirm the claimed folkloric uses. The present study was therefore designed to investigate the effect of *V. calvoana* leaves on body weight changes and some liver function indices in normal rats.

MATERIALS AND METHODS

Chemicals

All the chemicals used for this experiment were of analytical grade. Agape diagnostic kits were employed in the determination of biochemical parameters including AST, ALT, and ALP while total protein, total bilirubin and albumin levels were estimated using Randox kits.

Collection, identification and processing of the plant sample

Fresh leaves of *V. calvoana* were purchased from a homestead garden in Ugep, Yakurr Local Government Area of Cross River State. The leaves were transported to Calabar and taken to the Department of Botany, University of Calabar for identification and authentication by a Botanist, Dr. Michael Eko. A voucher

specimen with identification number BCH/VC/2015 was deposited in the Herbarium of the same Department. The leaves were plucked from their stems and thoroughly washed with tap water after which they were shade dried. The dried leaves were blended into powder using a dry Moulinex super blender (LM2070 - 4A, Dubai, United Arab Emirates), then subsequently stored in an airtight container until when required for dietary formulation and proximate estimations.

Experimental animals and design of the study

Twenty-four (24) albino rats of Wistar strain weighing averagely 100g – 170g were used. The rats were purchased from the animal house of the Department of Biochemistry University of Calabar, Cross River State. The rats were weighed and separated into four 4 groups of 6 rats per cage as shown in Table 1 below. The rats were allowed a period of 7 days of acclimatization and fed with normal rat chow and water *ad libitum*.

Table 1: Experimental design

GROUPS	NUMBER OF ANIMALS	ADMINISTERED DIET (IN GRAMMES)
Group 1 (Control)	6	120 g of normal feed
Group 2	6	5 g of sample + 115 g normal feed
Group 3	6	15 g of sample + 105 g normal feed
Group 4	6	30 g of sample + 90 g normal feed

n = 6, sample = dried leaves of *V. calvoana*.

Collection of blood and tissue samples

After the 31days feeding, the animals were fasted twelve (12) hours prior to the time of sacrifice. They were anaesthetized in chloroform vapour and immediately dissected using surgical scissors and forceps. Whole blood was then collected from the heart through cardiac puncture using sterile syringe and needle. The liver was excised from the animals into sterile sample bottles for histopathological assessment. The blood samples were centrifuged at a steady speed of 3000 rpm for 10 minutes using an MSE table centrifuge and the sera samples were carefully separated using Pasteur pipettes into well labeled, dry, plastic

specimen bottles and refrigerated until required for biochemical assays.

Biochemical analyses

Biochemical assay estimations of serum Aspartate aminotransferase, Alanine amino transferase, and Alkaline phosphatase were done using Agape diagnostic kits, Switzerland, according to manufacturer's protocol.

Histology of the Liver

The fixed liver sections were processed for histological examination using haematoxylin and eosin (H and E) basic histological procedures (Drury and Wellington, 1967).

Estimation of proximate analysis

The proximate composition of *V. calvoana* was determined using standard methods of AOAC (2000).

Measurement of weight changes

The initial body weight of each rat was measured and thereafter repeated every two days interval during of the 31 days experimental period. The mean weight for each group was determined and the percentage weight changes were calculated as the ratio of change in weight during the feeding period to the initial weight.

Statistical analysis

Data obtained were expressed as mean \pm SEM (standard error of mean) using Analysis of variance (ANOVA) of statistical package for social sciences (SPSS) software version 17 followed by least significant difference (LSD post hoc test). Differences were considered significant at $p < 0.05$.

Results

The results of the study on the effect of dietary incorporation of *V. calvoana* leaves on body weight changes, liver enzymes, total protein level and histology of the liver as well as the proximate composition of the leaves are presented in figures 1 – 6 and plates 1- 4.

Effect of Vernonia calvoana diets on some serum enzymes activity and total protein concentrations

The diet incorporated at 3: 90 in figure 1 showed significant increase in Alanine aminotransferase (ALT) when compared to the control. Also, at 5/115 and 15/105 incorporation, there was a marked decrease respectively compared to 30/90 incorporation. However, sample 5/115 and 15/105 indicated non-significant decreases respectively when compared to the control (NC). Sample diet 5/115% was also significantly decreased in comparison with NC. Figure 2 indicated significant decrease ($p <$

0.05) in serum Aspartate aminotransferase (AST) activity in groups fed with 5/115 and 15/105 incorporation respectively as compared to the control. However, AST level of 30/90 group was slightly higher than the control.

Results for Alkaline phosphatase activity showed significant difference ($p < 0.05$) with increase in 15/105 fed group when compared to the control in figure 3. Also, there was a significant decrease in ALP for groups fed with 5/115 % and 30/90 % relative to the 15/105 % fed group. Concurrently, ALP activity levels in 5/115 and 30/90 were higher than ALP level in the control though, not significantly. The serum total protein as presented in figure 4 showed no significant difference ($p < 0.05$) in groups fed with 15/105, 5/115 and 30/90 diets as compared to the control. However, a slight increase was observed in the group fed with 5/115 diet relative to the control.

Proximate composition of dried V. calvoana (VC)

The result of the proximate composition of *V. calvoana* showed carbohydrate concentration in large amounts thereby making carbohydrate the most abundant food nutrient in the sample of the plant. Fat, ash and moisture were found in trace amount while the protein and crude fibre content were present in appreciable quantities as shown in figure 5.

Effect of V. calvoana formulated diets on relative body weight changes

The body weight changes of albino Wistar rats whose diets were incorporated with *V. calvoana* indicated increase in body weight of 5:115 supplementation in a manner similar to increases observed in the normal control (NC). However, higher levels of supplementation given as 15: 105 and 30/90 % respectively, significantly showed weight decrease compared to NC and 5/115. There was a decrease in weight in 30/90 supplementation as compared to 15/105 supplementation.

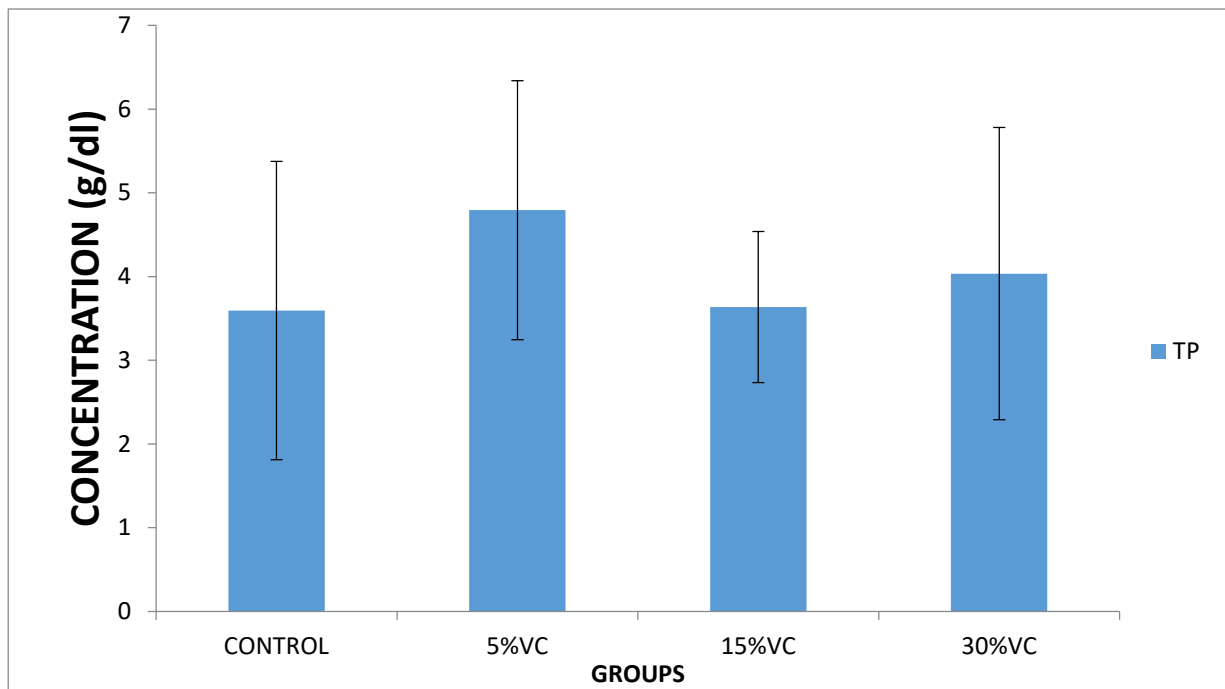


Figure 1: Comparison of total protein concentrations in the different experimental groups. Values are expressed as mean \pm SD. n=6

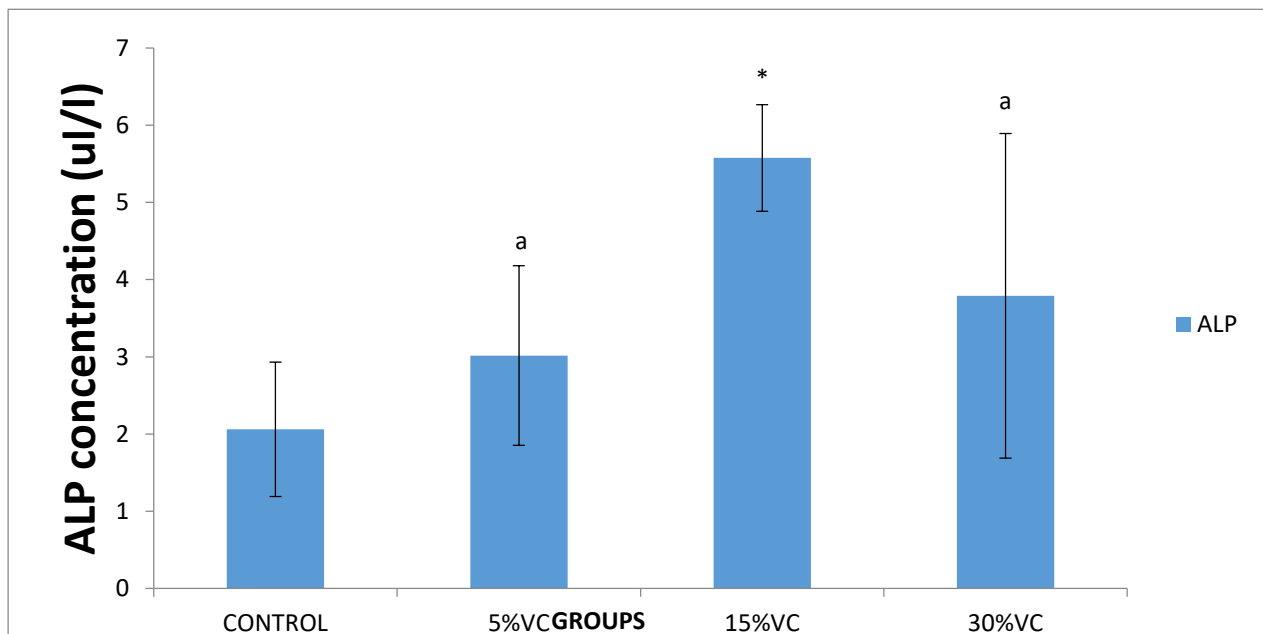


Figure 2: Serum alkaline phosphatase concentrations in the different experimental groups. Values are expressed as mean \pm SD, n = 6

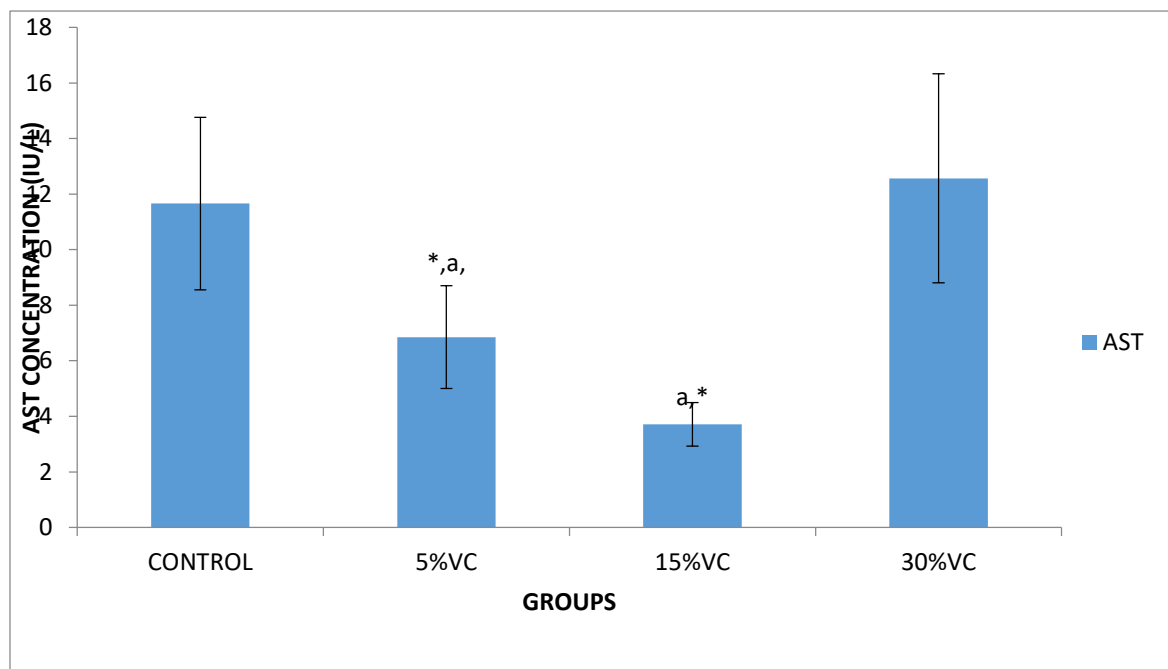


Figure 3: Comparison of aspartate aminotransferase concentrations in the different experimental groups. Values are expressed as mean \pm SD. n=6. *p<0.05vscontrol, ^ap<0.05 vs 30 % VC

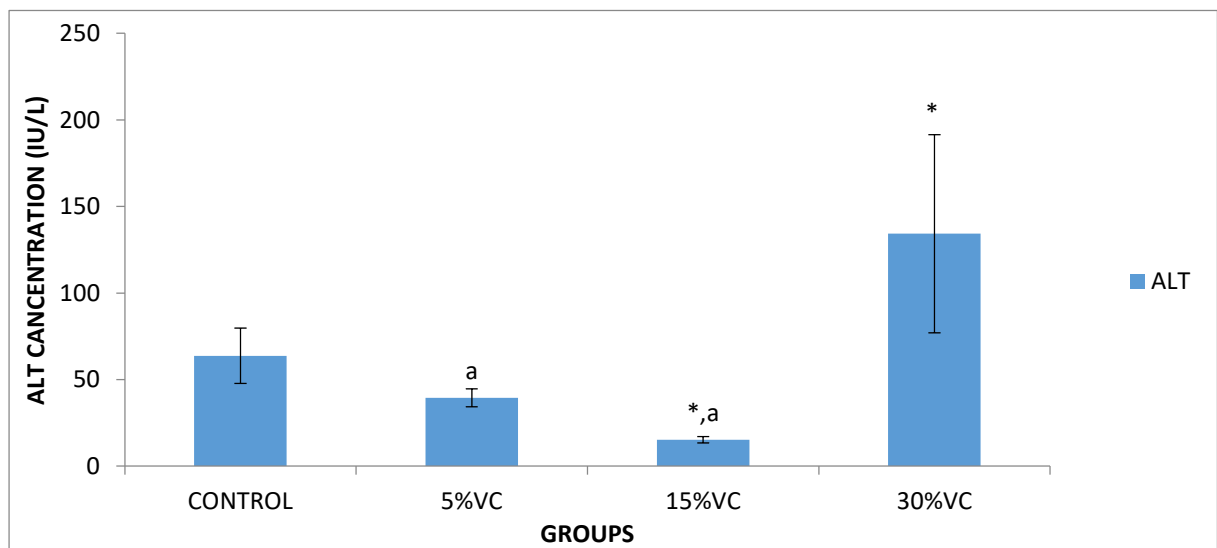


Figure 4: Comparison of alanine aminotransferase concentrations in the different experimental groups. Values are expressed as mean \pm SD. n = 6.*p<0.05vscontrol, ^ap < 0.05 vs 30 % VC

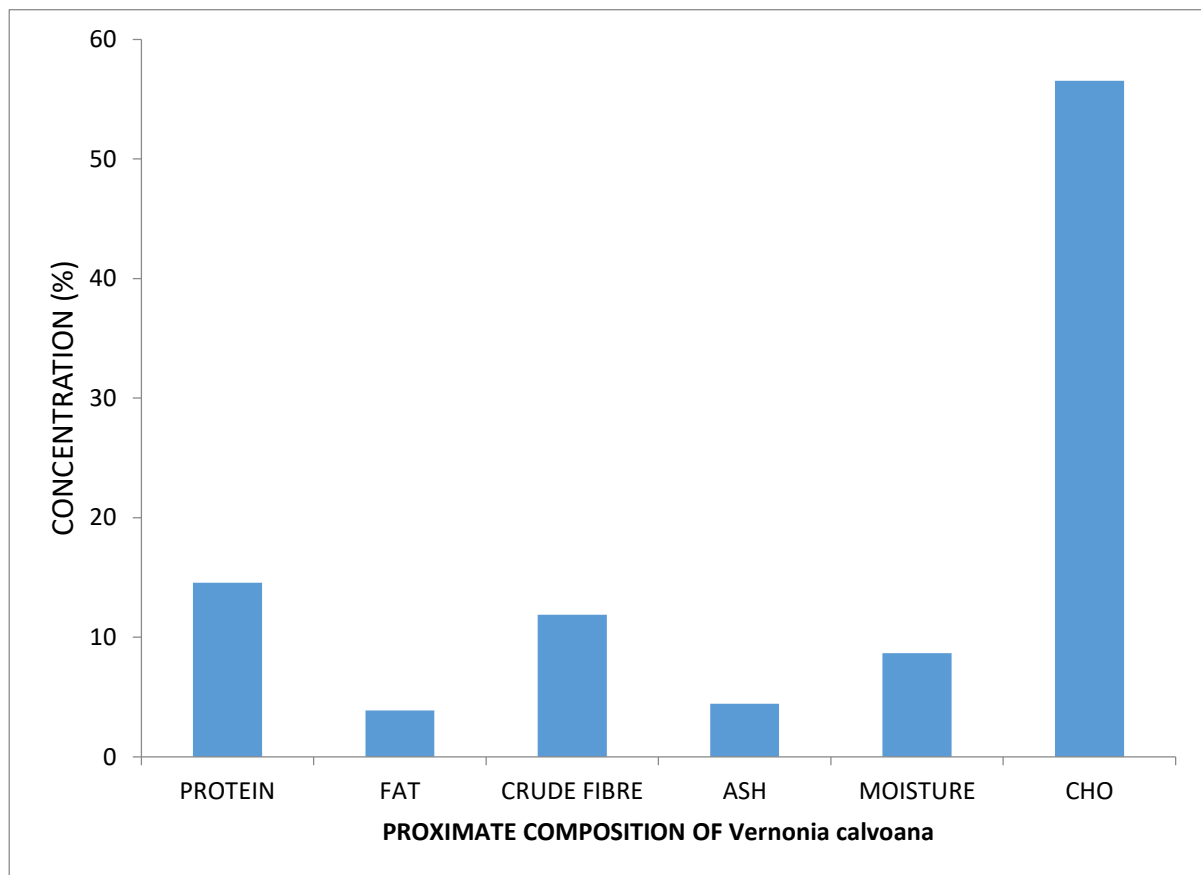
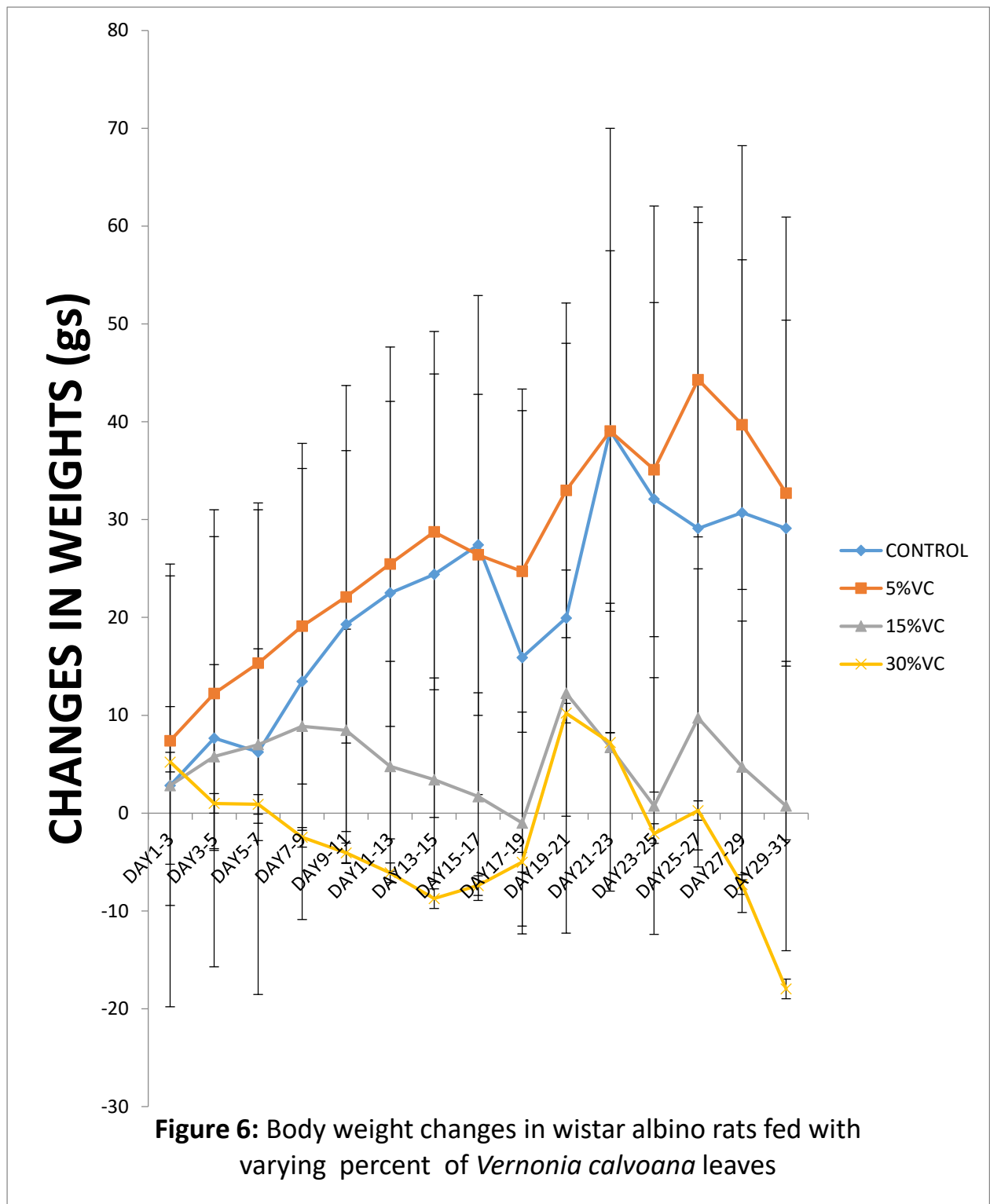


Figure 5: Proximate composition of *V. calvoana* used in the study



Effect of *V. calvoana* diets on the histology of the liver

Group 1 (Normal control): The section showed a hepatic lobule with its central vein. Its sinusoids radiates from the central vein in its normal hexagonal pattern. The hepatocytes presented with multi-nuclei. There was no necrotic degeneration of tissues or cells. Group 2 (5/115 %): The section also presented a hepatic lobule with sclerotic vessels. The sinusoids radiate out in hexagonal pattern but appeared dilated (wider than normal). Degeneration of the hepatocytes and necrosis of some of the nuclei was also observed. Group 3 (15/105 %): The section presented a hepatic lobule with its enlarged central vein. Partly, sinusoids radiates out from within and partly not. The hepatocytes were well demonstrated but with less nuclei compared to group 4 section but nuclei appeared enlarged. There may be hypertrophy of the hepatocytes. Group 4 (30/90 %): The section showed a hepatic lobule with its central vein. The sinusoids were dilated compared to that of group 1. There was a degeneration of the hepatocytes and necrosis of some of the nuclei while in some of the persisting nuclei, they appeared hypertrophic.

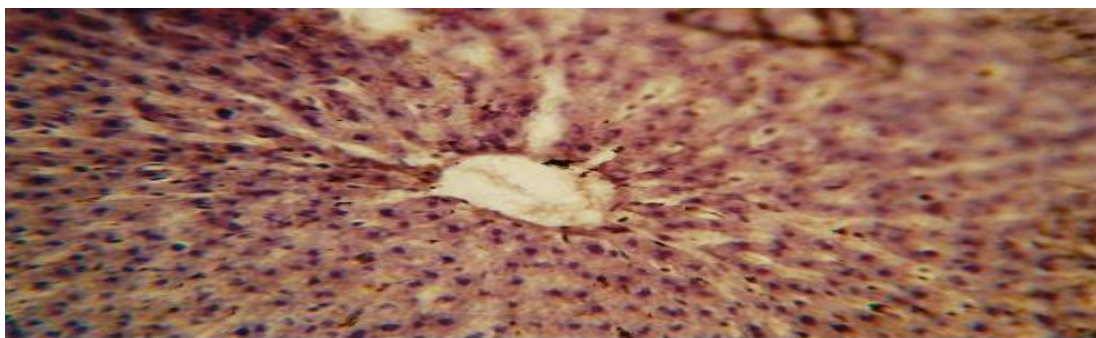


Plate 1: Photomicrograph of section of the liver of Normal Control fed group stained with H & E. Magnification x 400

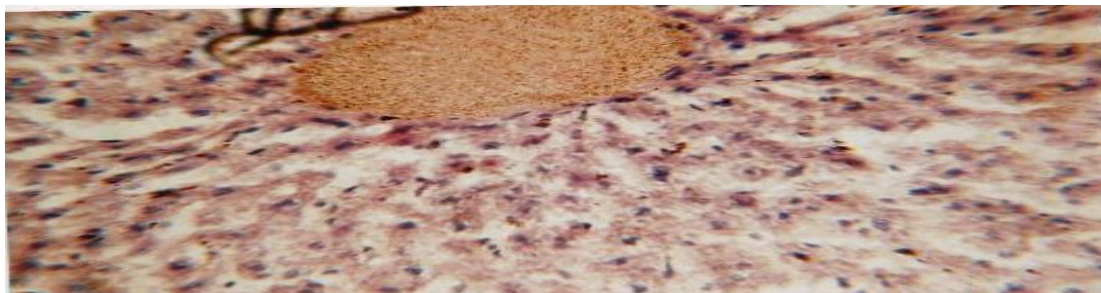


Plate 2: Photomicrograph of section of the liver of 5/115% fed group stained with H & E. Magnification x 400

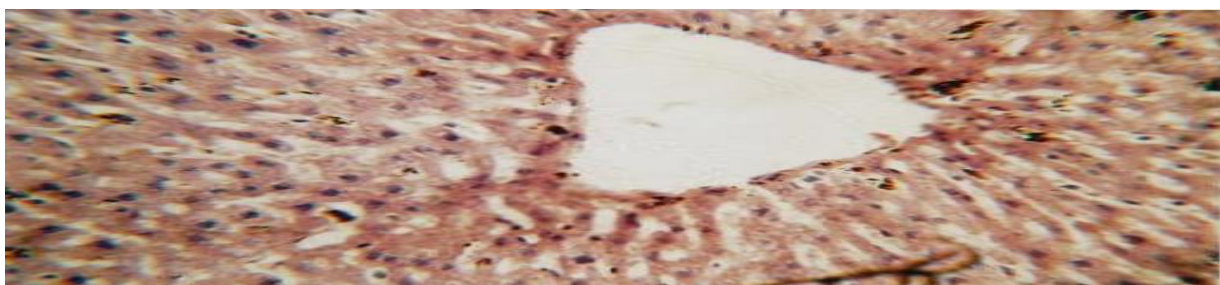


Plate 3: Photomicrograph of section of the liver of 15/105% fed group stained with H & E. Magnification x 400



Plate 4: Photomicrograph of section of the liver of 30/90% fed group stained with H & E. Magnification x 400

DISCUSSION AND CONCLUSION

Slight increases were observed in the concentration activity of total protein in all the experimental groups. Kenneth (1990) suggests that total protein levels may be significantly decreased as a result of malnutrition, malabsorption, nephrotic syndrome, amongst others. Also, its significant increase may be potentiated by conditions such as chronic infections, liver dysfunction and dehydration. However, in this study, there were no marked changes in any of the experimental groups. Only sample diet 5/115 % showed a mild difference compared to 30 / 80 % and 15 / 105 groups respectively. These mild differences may be due to minor inflammation of the liver. This finding has highlighted that the vegetable is rich in protein from the proximate composition data, potentiating protein biosynthesis in the liver. Therefore, *V. calvoana* diets at 15/105 could be more beneficial for consumption.

Alanine transaminase (ALT) is more specific to the liver and is the main biomarker in the investigation of liver injury. In this study, ALT results indicated significant increase in the group fed with 30/90% relative to the normal control group while group 15/105 showed a significant decrease against the NC fed group. However, the group 5/115 group showed a mild difference in reduction to the Normal Control. Ijeh and Obidoa (2010) reported elevated levels of ALT in albino rats fed high amount of *V. colorata*, which has a close taxonomic relationship with *V. calvoana*. The significant increase in ALT levels could be suggestive of liver damage.

The AST showed significant reduction in groups 2 and 3 respectively (5/115% and 15/105%)

relative to the normal control. Both groups also reduced significantly against group 4 (30 / 90 %) while the same group 4 (30 / 90 %) experienced a mild increase relative to the normal control (NC). It can therefore be inferred that VC incorporated diets at low doses (5 / 115 % and 15 / 105 %) had little or no effect on the liver to potentiate the presence of AST enzyme activity in blood serum, in levels that will lead to possible liver tissue damage. However, the mild increase in the high dose diet (30 / 90 %) group suggests mild inflammatory action on liver tissues. This finding is similar to the report of Rosalki and Wilkriion (1976) that emphasized that changes in serum AST activity will vary depending on the severity, type and stage of liver damage. All the groups showed increases in ALP activity with a significant increase in group 3 relative to the normal control. It can be inferred that significant increase in serum ALP level can potentiate hepatobilliary obstruction which ultimately leads to cholestasis. This is true from the submission of Tietz (1987) which presents ALP to be a cholestasis marker being a membrane bound enzyme.

The histopathological studies on the liver revealed changes in the architecture of the experimental groups (2, 3 and 4) compared to the normal control section which presented a normal liver morphology. These morphological changes in the experimental groups especially the 30 / 90 % group explains the significant increase in serum ALT enzyme activity level and other enzymes whose serum concentrations were altered.

The carbohydrate concentration was shown to be highly abundant indicating that the vegetable is a good food source of energy. The sweet taste

left on the tongue after consumption may not be unconnected with the monosaccharide and disaccharide composition of carbohydrates as suggested by Igile *et al.* (2013). The high crude fibre content in the vegetable could aid in digestibility via intestinal motility thereby preventing constipation. Furthermore, its moisture content was observed to be in appreciable quantity indicating the susceptibility of the vegetable to spoilage and therefore needs regular and appropriate preservation. The fat composition of the vegetable was found to be low, giving possible clue to the reduction in body weight following increased percentage incorporation. This corroborated the report by Gordon and Kessel. (2002) that low fat foods tend to reduce the level of cholesterol and obesity. The vegetable is therefore suitable when consumed in adequate amounts and could help in body weight control. The ash content of the vegetable indicates the presence of inorganic elements like magnesium, potassium and calcium. We therefore concluded that dried *V. calvoana* incorporated diet is capable of probable protective effect on the integrity of the liver.

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