Scientia Africana, Vol. 23 (No. 2), April, 2024. Pp127-146
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

ANTI-DYSLIPIDEMIC AND CARDIO-PROTECTIVE EFFECTS OF DIETARY *VERNONIA AMYGDALINA* LEAVES IN MONOSODIUM GLUTAMATE INTOXICATED HIGH FAT DIET FED WISTAR RATS

***[1]Ubah, E.E., [2]Ijeh, I.I., [3]Oguamanam, K., C., [4]Obike, C., A., and [5]Egbuonu, A., C.**
[1,2,3,4,5]Department of Biochemistry, College of Natural Sciences,
Michael Okpara University of Agriculture, Umudike, Abia State.
***Corresponding author E-mail:**chucksdeville@gmail.com;ijeh.ifeoma@mouau.edu.ng

Received: 09-01-2024
Accepted: 26-03-2024

https://dx.doi.org/10.4314/sa.v23i2.13

Journal Homepage: http://www.scientia-african.uniportjournal.info
Publisher: Faculty of Science, University of Port Harcourt.

ABSTRACT

The study evaluated the proximate analysis, anti-dyslipidemic and cardioprotective effects of dietary incorporated Vernonia amygdalina (VA) leaves in Monosodium glutamate (MSG) intoxicated High fat diet (HFD) fed Wistar rats. The proximate analysis of the leaves revealed the highest ash and carbohydrate content in the control (basal) diet, highest protein, crude fibre and moisture content in the 10% VA incorporated HFD and the highest crude fat in the HFD only group. Dietary incorporation of VA in HFD reversed the trend of hypercholesterolemia and hypertriglyceridemia observed in experimental animals after chronic MSG, HFD and MSG + HFD administration. At the end of week 8, HFD only group produced a higher level of total cholesterol and triglyceride when compared to the basal control diet group and the MSG only group while the 10% VA HFD group produced the greatest reduction in total cholesterol and triglyceride from week 9 – week 12. Possible mechanisms include the presence of crude fibers which binds to bile acids reducing the absorption of cholesterol. Histopathology studies of the myocardial tissues at the end of the study revealed in the MSG + HFD group signs of a marked diffused edema of the myocardium which was absent in the MSG only and HFD only groups while 5% and 10% VA incorporated HFD and the standard drug Orlistat 10 mg/kg failed to reverse the observed toxicity. Conclusion: Dietary VA reversed lipid derangement observed but failed to reverse myocardial histopathologies after chronic MSG + HFD administration and could be used as an adjunct for the treatment of obesity.

INTRODUCTION

Obesity is defined as the excess of body fat or adipose tissue (AT) resulting from excessive intake of nutrients and/or decreased energy expenditure (Aitlhadj et al., 2011). It is characterized by the combined result of satiety center dysfunction at the cerebral level, imbalance of intake and use of energy, and genetic variations (Cheung & Li.,2012) manifesting itself in an abnormal, excessive increase or accumulation of energy in the form of fat in AT (Cohen & Spiegelman., 2016).

Africa and Nigeria are not left out in the recent surge in the scourge of obesity as studies reveal an increase in the number of obese and overweight individuals. Bashir et al. (2022) reported rates of central obesity in Nigeria to be 39 % which translates to an estimated 46.8 million centrally obese adult

Nigerians higher than the most recent range for Africa of 13.6% to 31% by the World Health Organization (WHO) (WHO., 2022).

Herbal Medicines used for the management of metabolic syndrome including obesity, diabetes, and its complications have come a long way down the years. Akpoveso et al. (2023) reviewed extensively the role of antioxidants in the management of diabetic complications through upregulation of endogenous antioxidants via various mechanisms. Natural phytochemicals have been found useful in the treatment of obesity by converting white adipose tissues (WAT) which stores unhealthy fat to brown adipose tissues (BAT) in a process of adipocyte differentiation known as browning which helps to release excess stored energy as heat (El Hadi et al., 2019). Examples include rutin, naringenin, luteolin and quercetin as they all promote browning of WAT and prevent obesity. Genistein; a polyphenolic isoflavone found in soy beans and fava beans and myricetin, a flavone present in berries , onions, grapes and red wines both prevent obesity by elevating uncoupling protein -1 (UCP-1) expression, peroxisome proliferator - activated receptor gamma co-activator 1-alpha (PGC-1a), and positive regulatory domain containing protein 16 (PRDM-16) , a transcription coregulator that controls the development of brown adipocytes in brown adipose tissue through **peroxisome proliferator-activated receptors** (PPAR-gamma) as an alternative mechanism. Other natural phytochemicals prevent obesity by reducing appetite or inducing satiety as an alternative mechanism. Inhibition of lipase enzyme activity by phytochemicals is also useful in reducing obesity (Tucci et al., 2010). The inhibition of pancreatic phospholipase enzyme which triggers the breakdown of triglycerides into free fatty acids and monoglycerides in the intestinal lumen is useful in treating obesity. The plant-derived flavonoid luteolin is a strong phospholipase inhibitor. Also, epigallocatechin -3,5-digallate has significant phospholipase inhibition activity while some phytochemicals also possess regulatory activity on adipocyte proliferation and differentiation. Other mechanisms include the regulation of fat metabolism by targeting important steps in the catabolism of fats reducing the circulation of free fatty acids (Khalilpourfarshbafi et al., 2019).

Side effects and the high cost of existing anti-obesity drugs highlight the urgent need for the discovery of newer molecules mostly from phytochemicals to serve as adjuvants in the effective management of obesity and its comorbidities.

Africa is home to the perennial shrub *Vernonia amygdalina* Del., sometimes known as bitter leaf, a member of the Asteraceae family. The plant is used in traditional medicine as a febrifuge, digestive tonic, laxative, anti-helminth, anti-malarial, and topical wound therapy and an anti-inflammatory agent (Ijeh & Ejike.,2010).

Egedigwe et al. (2016) studied the anti-obesity potentials of aqueous and methanol extracts of *Vernonia amygdalina* leaves in high fat diet (HFD) fed rats. Dietary incorporation of *Vernonia amygdalina* for six weeks lowered serum triacylglycerol and LDL-cholesterol levels, and increased HDL-cholesterol levels (Egedigwe & Ijeh, 2010).

Nwanjo, (2005) previously reported on the aqueous extract of leaves of the same plant which reduced triacylglycerol levels and normalized cholesterol concentrations in the serum of diabetic rats. Other reported medicinal uses of the leaves of the plant include antioxidant, hypoglycaemic, anti-microbial, anti-helmintic and anti-cancer/tumor properties.

According to Krisanits et al. (2020), a high-fat diet is one in which fats make up at least 35% of all calories consumed. The high fat diet induces a metabolic profile characterized by obesity and insulin resistance (Zhong et al., 2020).

Increased consumption of processed meals; higher in fat content, which are simple to

Scientia Africana, Vol. 23 (No. 2), April, 2024. Pp127-146
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

purchase and typically cheaper when socioeconomic considerations like reduced family income are considered may be responsible for the current obesity crisis in Africa. (Laar et al., 2022).

The current obesity problem may also be caused by a dual assault from HFD and monosodium glutamate (MSG) toxicity; however, relatively few studies have examined the effect of HFD and MSG double toxicity in the general population.

Meals are sometimes added with MSG as a flavour enhancer as it produces an umami flavor to enhance the meaty, savory flavor of the food. The Chinese restaurant syndrome, which is characterized by neuro-excitotoxicity and obesity (Gobatto et al., 2020) is one of the documented negative effects of MSG consumption in both humans and experimental animals.

Repeated MSG consumption has been linked in pre-clinical trials to asthma, cancer-induced obesity, diabetes, and oxidative stress. MSG consumption is also linked to neurotoxic side effects as well as hepatotoxic, cardiotoxicity, genotoxic, reproductive, renal, and other toxicities (Kazmi et al., 2017). Additionally, consuming MSG has been related to conditions like Parkinson's, Alzheimer's, addiction, brain injury, anxiety, stroke, depression, and epilepsy (Bera et al., 2017).

MATERIALS AND METHOD

Collection of Plant Materials

Fresh leaves of *Vernonia amygdalina* were purchased from Ubani local market in, Umuahia, Abia State, Nigeria. The plant was identified by Prof. G. C. Osuagwu of the Department of Plant Science and Biotechnology, College of Natural Sciences Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. Voucher specimens were deposited at the herbarium (FHI 28786-*Vernonia amygdalina*). All leaves were collected between August and November 2021.

The fresh leaves were separated from the stalk, washed, and air-dried for three to four days to attain a constant weight at room temperature (26-28^{o}C) and then pulverized, crushed into fine powder, and weighed using an electric blender, and stored in an airtight plastic container.

Experimental Design and Procedure

The study was carried out with thirty-six age matched male Wistar rats obtained from the Veterinary Department of the University of Nigeria, Nsukka. They were acclimatized in the animal house of the department of Veterinary Medicine of Michael Okpara University of Agriculture, Umudike, Abia state, Nigeria for two weeks before the commencement of the experiment. Two main types of diets were used: High fat diets (HFDs) and Control rat chow (Normal diet), formulated as shown in Tables 3.0 and 3.1 for inducing obesity in the rats. All feeding stuffs were sourced locally from Jocan Agro Feeds Ltd, Umuahia, Abia state, Nigeria.

Table 3.0: High Fat Diet Composition

Ingredients	(g/ 1000 g)	(g /100 g)
Cellulose	100	10.0
Sucrose	100	10.0
Beef tallow	250	25.0
Corn starch	200	20.0
Fish meal	240	24.0
Vitamins	40	4.0
Groundnut oil	70	7.0
Total	1000.00	100.00

Table 3.1: Basal (Control) Diet Composition

Ingredients	(g /1000 kg)	(g /100 g)
Cellulose	100g	10.0
Sucrose	100	10.0
Corn starch	450	45.0
Fish meal	240	24.0
Vitamins	40	4.0
Groundnut oil	70	7.0
Total	1000.00	100.00

Table 3.2: 5% *Vernonia amygdalina* Incorporated High Fat Diet Composition

Ingredients	(g /1000 kg)	(g /100 g)
Cellulose	100	10.0
Sucrose	100	10.0
Beef tallow	250	25.0
Corn starch	200	20.0
Fish meal	240	24.0
Vitamins	40	4.0
Groundnut oil	70	7.0
Total	1000.00	100.00
Weighing Out	50	5
Balance	950	95.0
Vernonia amygdalina powdered leaves added	50	5
Total	1000.00	100.00

Table 3.3: **10% *Vernonia amygdalina* Incorporated High Fat Diet Composition**

Ingredients	(g /1000 kg)	(g /100 g)
Cellulose	100	10.0
Sucrose	100	10.0
Beef tallow	250	25.0
Corn starch	200	20.0
Fish meal	240	24.0
Vitamins	40	4.0
Groundnut oil	70	7.0
Total	1000.00	100.00
Weighing Out	100	10
Balance	900	90.0
Vernonia amygdalina powdered leaves added	100	10
Total	1000.00	100.00

Scientia Africana, Vol. 23 (No. 2), April, 2024. Pp127-146
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

The basal (control) diet consisted of all the listed feed stuff except the beef tallow. This was done by mixing these feedstuffs after weighing them in a bowl. The diets were then converted to pellets by extrusion through an improvised device made by neatly slicing the end of a 5 ml syringe as described by Ijeh et al. (2013). After pelleting, it was dried in an oven at a temperature of 35º C. The experimental animals were assessed for obesity using Lee's index (Hariri &Thibault., 2010, Lee ., 1929) .

The beef tallow was obtained from the Ubakala slaughterhouse, Umuahia south local government area, Umuahia, Abia state, Nigeria. Fat from the intestines of slaughtered cows were melted in an oven to extract lipids from them which were subsequently used in the formulation of the High fat diets.

Monosodium glutamate was prepared from stock; Ajinomoto ® brand sourced from the local spice market in Umuahia, Abia state, Nigeria. The High fat diet was further incorporated with 5% and 10 % *Vernonia amygdalina.*

Standard drug Orlistat 10 mg/kg was prepared from Xenical® brand of Orlistat manufactured by CHEPLAPHARM Arzneimittel GmbH Ziegelhof 24,17489 Greifswald, Germany.

After twelve weeks of feeding, blood was collected by retro-orbital sinus bleeding based on the institutional guidelines on the safe handling of experimental animals of the Veterinary Department of Michael Okpara University of Agriculture, Umudike, Abia state, Nigeria.

Housing, adaptation, and feeding of experimental animals.

Vital grower's mash supplied by Vital Feed Limited, Lagos, Nigeria and clean tap water were given to the animals *ad libitum* during the acclimatization period. The experimental animals, after two weeks acclimatization period were divided into six groups of six rats each. Pelleted diets and clean tap water were provided to all the animals in their respective separate cages. The experimental animals were randomly selected into the following groups:

Experimental grouping of MSG, HFD and *Vernonia amygdalina* incorporated HFD.

Group A: Rats in this group received basal control diet comprising normal rat chow for 12 weeks *ad libitum*

Group B: Rats in this group received high fat diet for 12 weeks *ad libitum*

Group C: Rats in this group received monosodium glutamate (MSG 8000 mg/kg) and basal control diet for 12 weeks *ad libitum*

Group D: Rats in this group received monosodium glutamate (MSG 8000 mg/kg) and high fat diet for 12 weeks *ad libitum* + 5% *Vernonia amygdalina* incorporated high fat diet from week 9 to week 12.

Group E: Rats in this group received monosodium glutamate (MSG 8000 mg/kg) and high fat diet for 12 weeks *ad libitum* + 10% *Vernonia amygdalina* incorporated high fat diet from week 9 to week 12.

Group F: Rats in this group received monosodium glutamate (MSG 8000 mg/kg) and high fat diet for 12 weeks *ad libitum* + Orlistat 10 mg/kg from week 9 to week 12.

Induction phase: MSG 8000 mg/kg for initial eight (8) weeks.

Treatment phase: 5%, 10% *Vernonia amygdalina* and Orlistat 10 mg/kg for final four (4) weeks.

Collection of Blood Samples

After twelve (12) weeks of feeding the rats concomitantly in various groups with HFD, MSG and 5% and 10% *Vernonia amygdalina* incorporated HFD respectively as required, the animals were sacrificed. Blood samples were collected by retro-orbital bleeding from the eyes. The serum was allowed to separate and used for the analysis of various parameters including Cholesterol, HDL-cholesterol, triacylglycerol.

Proximate Analysis of Feed Samples, HFDs, Control Diet and *Vernonia amygdalina* Incorporated HFDs.

The proximate composition of the feed samples was determined using the recommended methods of the Association of Official Analytical Chemists (DeVries et al., 1999) to determine the moisture, ash, crude fiber, crude fat and protein content.

Crude Fibre Determination

The crude fibre was determined by sequential extraction of defatted feed samples with 1.25% H_2SO_4 and 1.25 % NAOH.

$$\%Fibre = \frac{X_1 - X_2}{W} \times 100$$

Where X_1= weight of de-fatted feed samples before ashing;

X_2= weight of de-fatted feed samples after ashing;

W= weight of actual feed samples before de-fatting

Crude Fat Determination

The soxhlet fat extraction procedure was used to determine the crude fat.

$$\% \ Fat = \frac{M_2 - M_1}{W} \times 100$$

Where W= weight of bitter leaf sample; M_1 = Weight of empty flask; M_2 = weight of flask + fat

Determining Moisture Content

The moisture content was determined using the direct heat method:

$$\% \ Moisture \ = \frac{M_{INITIAL} - M_{INITIAL}}{M_{INITIAL}} \times 100$$

Determination of Ash

The feed samples were weighed after evaporation to dryness using the oven at 105ºC in a porcelain dish. The feed samples were transferred into pre-heated muffle furnace at 550ºc.

They were cooled in desiccators and reweighed.

$$\% \text{ Ash of sample} = \frac{(C - A)}{(B - A)} \times 100$$

Where A = weight of dish

B = weight of dish + sample

Scientia Africana, Vol. 23 (No. 2), April, 2024. Pp127-146
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

C = weight of dish + ash

Protein Determination

The protein was determined by the digestion of feed samples using Kjedahl method:

$$\%\,\text{Total Nitrogen} = \frac{100(\text{VA - VB}) \times NA \times 0.01401 \times 100}{W \times 10}$$

%Protein = F factor x % Nitrogen

But F factor = 6.25

VA = volume (ml) of the standard acid used in sample titration

VB = volume (ml) of the standard acid used in the blank titration

NA = normality of the acid (HCl)

W = weight in grams of the respective samples

Determination of Carbohydrate

The carbohydrate was calculated by difference rather than analyzed directly.

Carbohydrate = 100 – (weight in grams [protein+ fat + water + ash +fibre] in 100g of food) (AOAC., 2023)

Lipid Profile Assay

This was determined at the end of week 8 of the study using a Lipid Profile testing Machine and test strips from Osang Healthcare Co. Ltd, 132, Anyangcheondong-ro, Dongan-gu, Anyang-si ,Gyeonggi-do, Korea (14040) while the serum analysis was done after sacrificing the animals at the end of the study period.

Component lipids were estimated using standard commercial test kits (RANDOX laboratories, Ltd,55 Diamond Road, Crumlin, County, Antrim, BT29 4QY United Kingdom).

Cholesterol determination

The determination of serum concentrations of cholesterol was carried out using enzymatic colorimetric endpoint method as described by Allain et al. (1974).

Triacylglycerol estimation

This was determined after enzymatic hydrolysis with lipases as described by Tietz, (1990) .

High Density Lipoprotein (HDL) cholesterol determination

Low density lipoproteins (LDL) and Very low density lipoproteins (VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungistic acid in the presence of the magnesium ions as describe by Gidez, (1982).

Low Density Lipoprotein (LDL)- cholesterol estimation

LDL-cholesterol estimation was estimated from the values of total cholesterol, triglyceride and HDL-cholesterol using the formula described by Friedewald et al. (1972) below:

LDL-cholesterol = Total cholesterol – Triglyceride - HDL cholesterol

Statistical Analyses

Data were analyzed by using Statistical package for social sciences (SPSS version 20) (IBM SPSS Inc, Chicago, IL) software. All values were expressed as the mean value ± Standard deviation (SD) and the level of significance was calculated by one way analysis of variance (ANOVA). Duncan Multiple Range Test complemented with student's T test was used for comparison of the means of the various groups. A probability level of less than 5% ($p<0.05$) was considered statistically significantly different between the test and control groups as well as among test groups for measured values.

RESULTS

Results of the Proximate Composition of Various Diets

Table 4.1: The proximate composition ± SEM

Parameter	High fat diet	10 % VA	5 % VA	Normal pellet
Ash %	7.17 ± 0.16^{a}	7.40 ± 0.24^{a}	8.36 ± 0.13^{b}	9.46 ± 0.47^{c}
Protein %	19.34 ± 0.23^{c}	22.38 ± 0.15^{d}	16.73 ± 0.10^{b}	15.71 ± 0.17^{a}
Crude fat %	45.23 ± 0.82^{d}	37.23 ± 0.75^{b}	42.87 ± 0.81^{c}	15.64 ± 0.33^{a}
Crude fibre %	0.06 ± 0.00^{a}	1.91 ± 0.00^{d}	1.16 ± 0.00^{c}	0.09 ± 0.00^{b}
Moisture %	4.98 ± 0.08^{a}	14.93 ± 0.32^{c}	7.27 ± 0.16^{b}	6.34 ± 0.99^{ab}
Carbohydrate %	23.21 ± 0.82^{b}	16.14 ± 0.97^{a}	23.61 ± 0.68^{b}	52.76 ± 1.01^{c}

The different superscripts (^{abc}) are significant ($p<0.05$) across the row (horizontally)

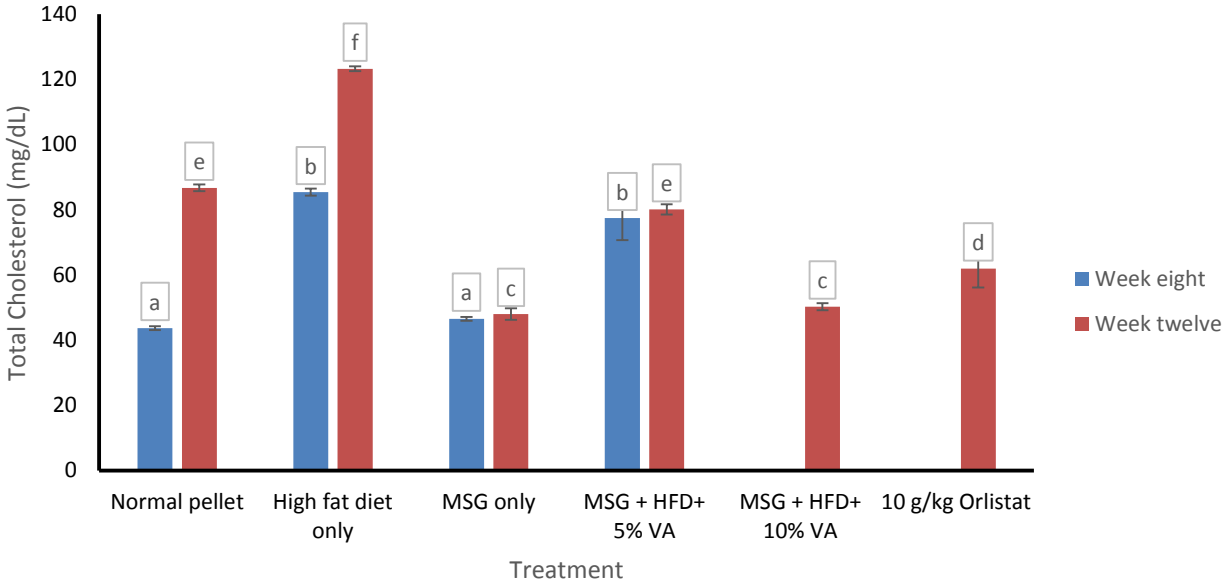

Figure 1: The effects of HFD, MSG, HFD+MSG and *Vernonia amygdalina* incorporated HFD on Total cholesterol of Rats at the end of Week 8 and 12.

The different superscripts (^{abc}) are statistically significant ($p<0.05$)

Scientia Africana, Vol. 23 (No. 2), April, 2024. Pp127-146
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

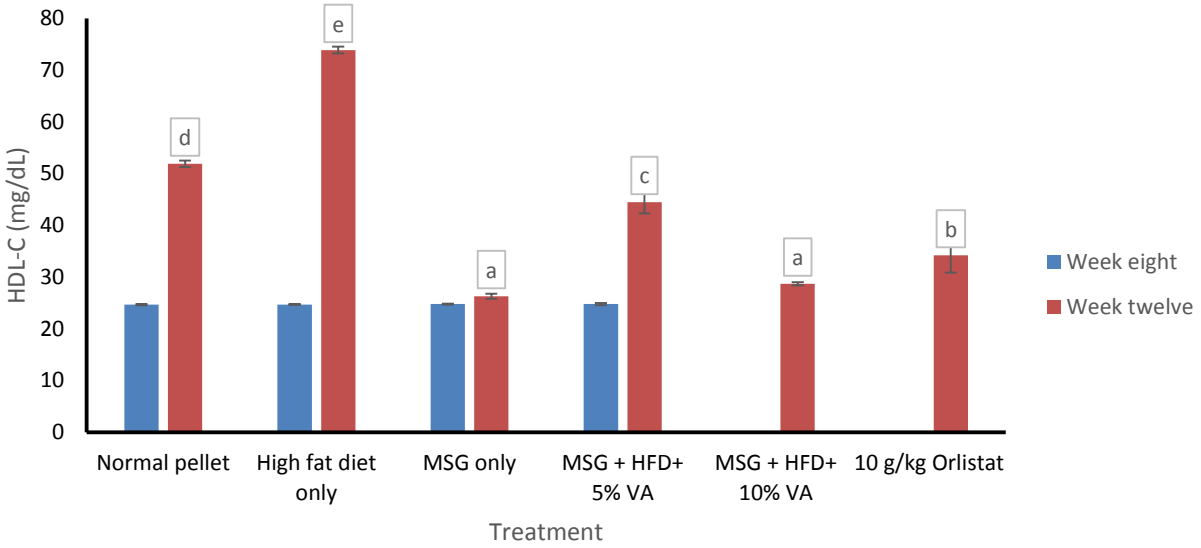

Figure 2: The effects of HFD, MSG, HFD+MSG and *Vernonia amygdalina* incorporated HFD on High Density Lipoprotein-Cholesterol of Rats at the end of Week 8 and 12.

The different superscripts (^{abc}) are statistically significant ($p<0.05$)

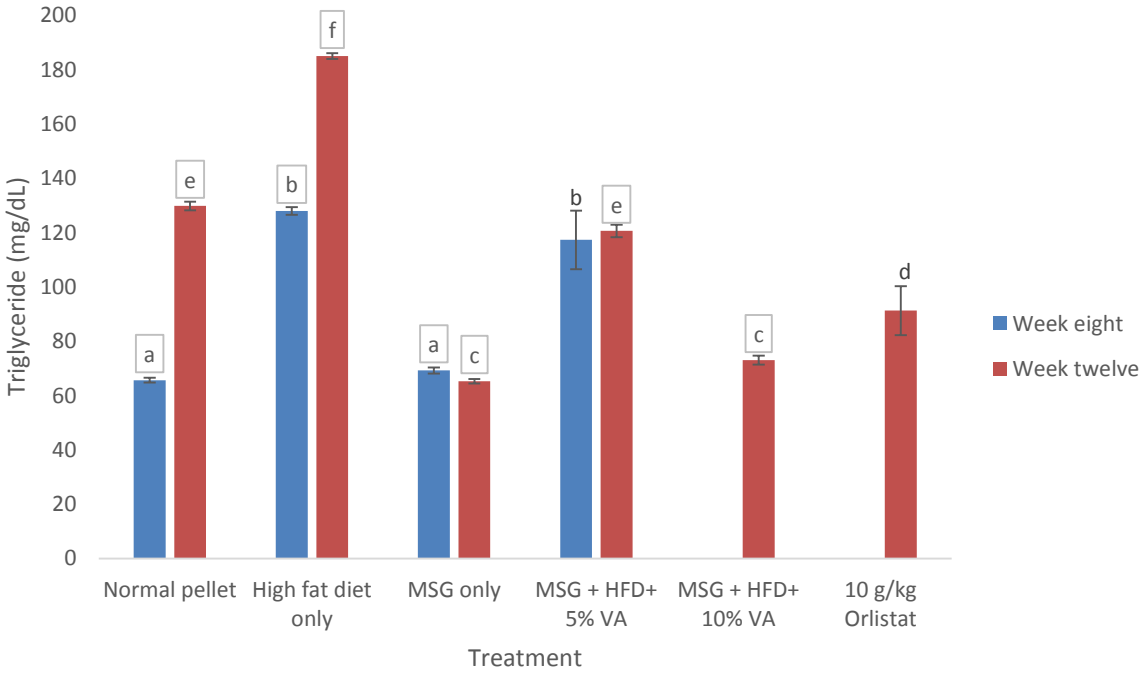

Figure 3: The effects of HFD, MSG, HFD+MSG and *Vernonia amygdalina* incorporated HFD on Triglycerides of Rats at the end of Week 8 and 12.

The different superscripts (^{abc}) are statistically significant ($p<0.05$)

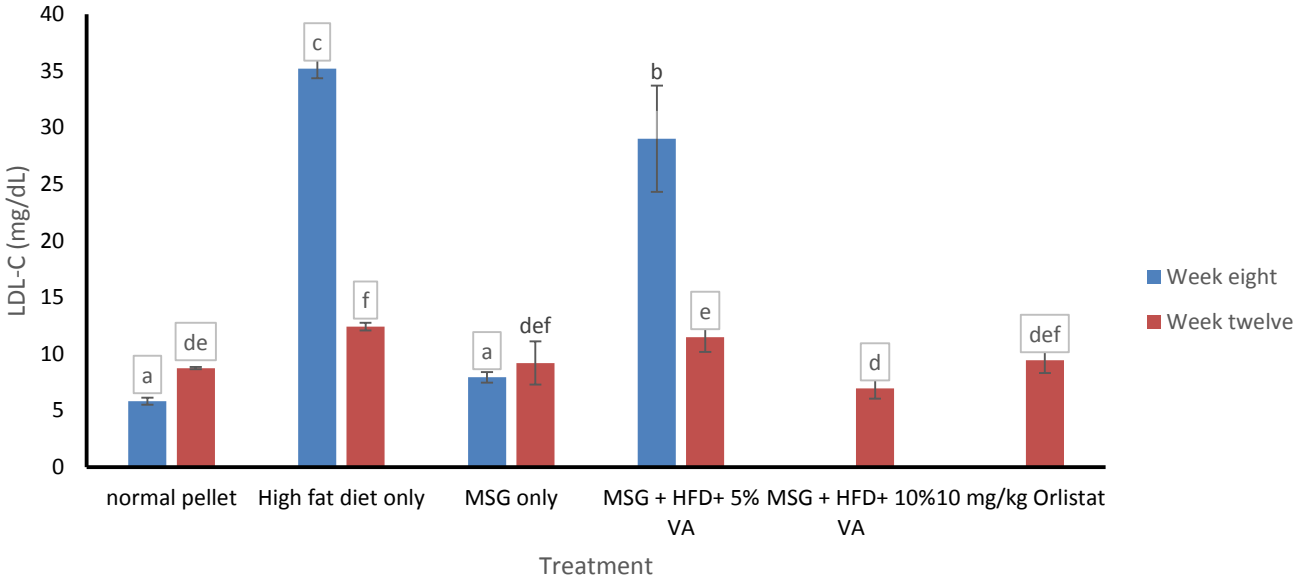

Figure 4: The effects of HFD, MSG, HFD+MSG and *Vernonia amygdalina* incorporated HFD on Low Density Lipoprotein-Cholesterol of Rats at the end of Week 8 and 12.

The different superscripts (^{abc}) are statistically significant ($p<0.05$)

Histopathology Results

Plate 1: Photomicrograph of sections of heart of rat fed basal diet only.

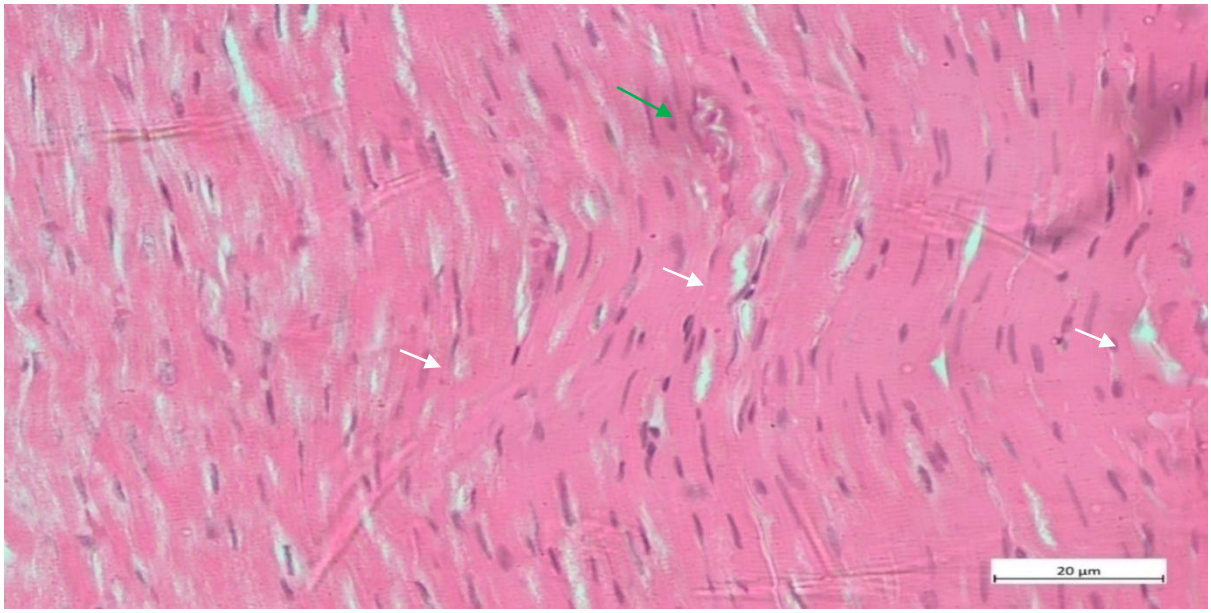

Plate 1: Section of the heart presented in this group showed the normal myocardial histo-architecture. Cardiomyocytic nuclei (White arrow); Capillaries (green arrow) with a Myocardial histopathology showing no damage. H & E., X400

Scientia Africana, Vol. 23 (No. 2), April, 2024. Pp127-146
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

Plate 2: Photomicrograph of sections of heart of rat fed high fat diet only

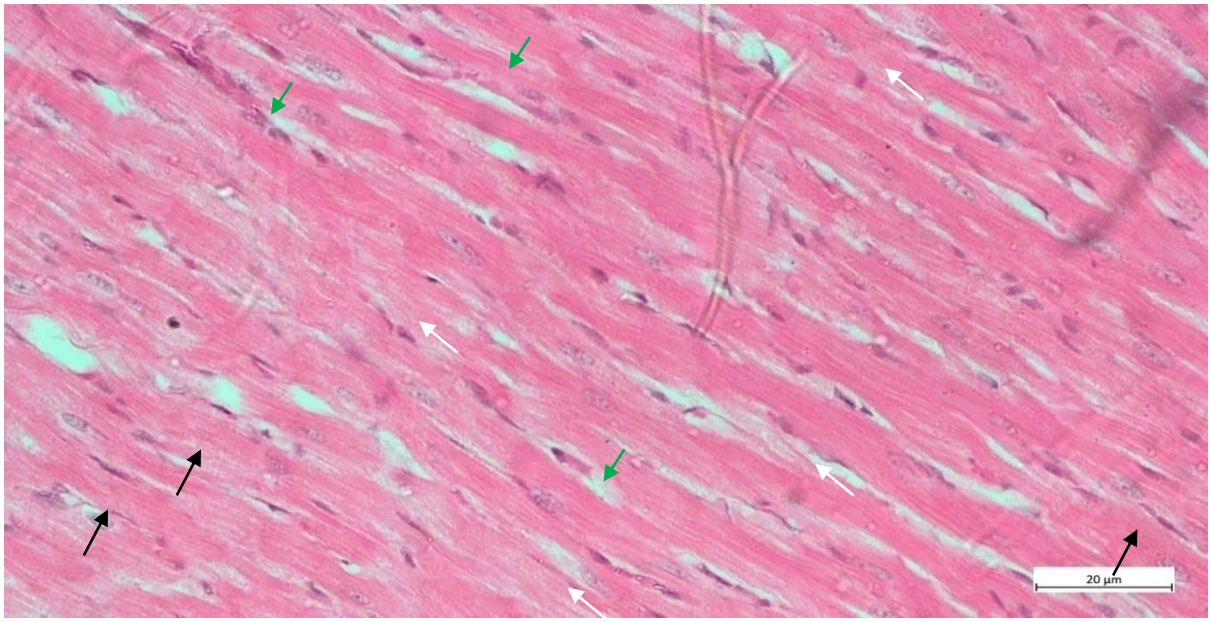

Plate 2; Sections of the heart presented in this group showed the normal myocardial histo-architecture. Cardiomyocytic nuclei (White arrow); Pericyte (black arrow); Capillaries (green arrow) with a Myocardial histopathology showing no damage. H & E., X400

Plate 3: Photomicrograph of sections of heart of rat fed MSG only.

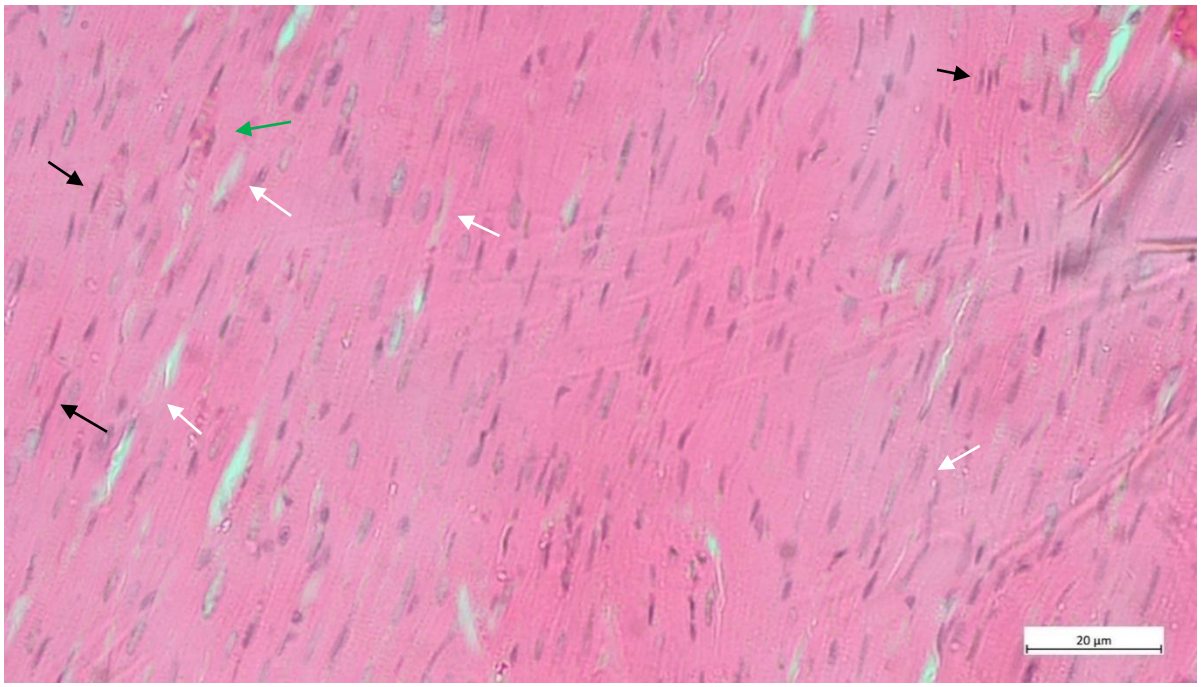

Plate 3; sections of the heart presented in this group showed the normal myocardial histo-architecture. Cardiomyocytic nuclei (White arrow); Pericyte (black arrow); Capillaries (green arrow) with a Myocardial histopathology showing no damage. H & E., X400

Plate 4: Photomicrograph of sections of heart of rat fed MSG + HFD for 8 weeks and 5% *Vernonia amygdalina* incorporated HFD for 4 weeks.

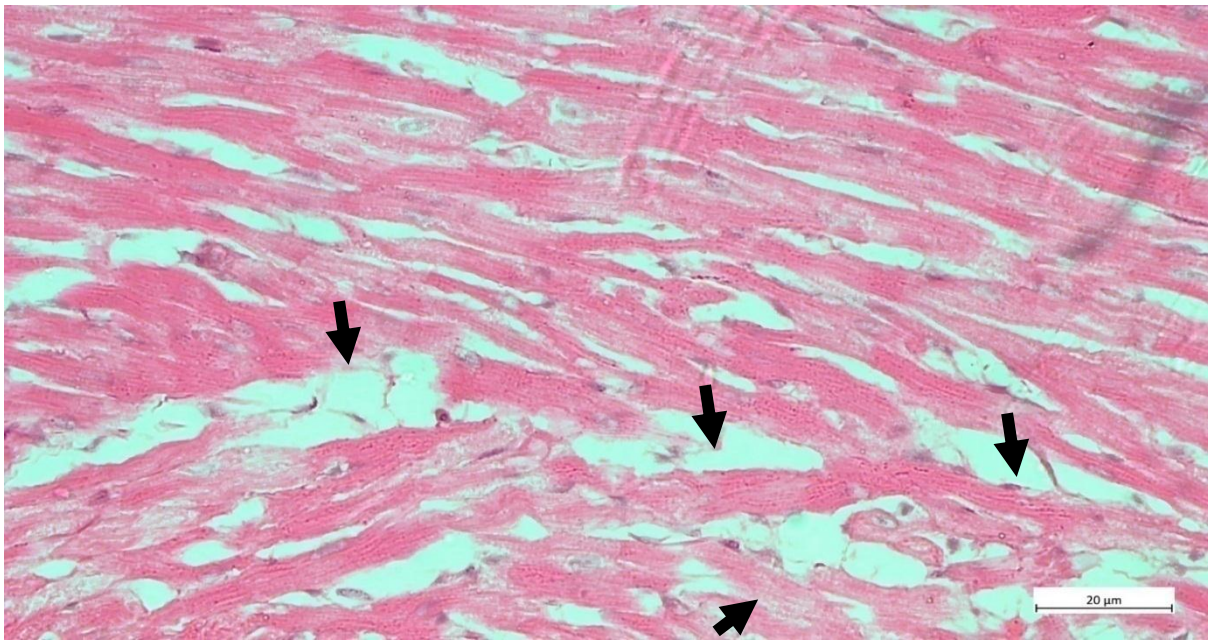

Plate 4; Sections of the heart presented in this group showed a marked diffused edema of the myocardium (arrow) with a myocardial histopathology showing mild – interstitial edema and focal necrosis. H & E., X400.

Plate 5: Photomicrograph of sections of heart of rat fed MSG + HFD for 8 weeks and 10 % *Vernonia amygdalina* incorporated HFD for 4 weeks.

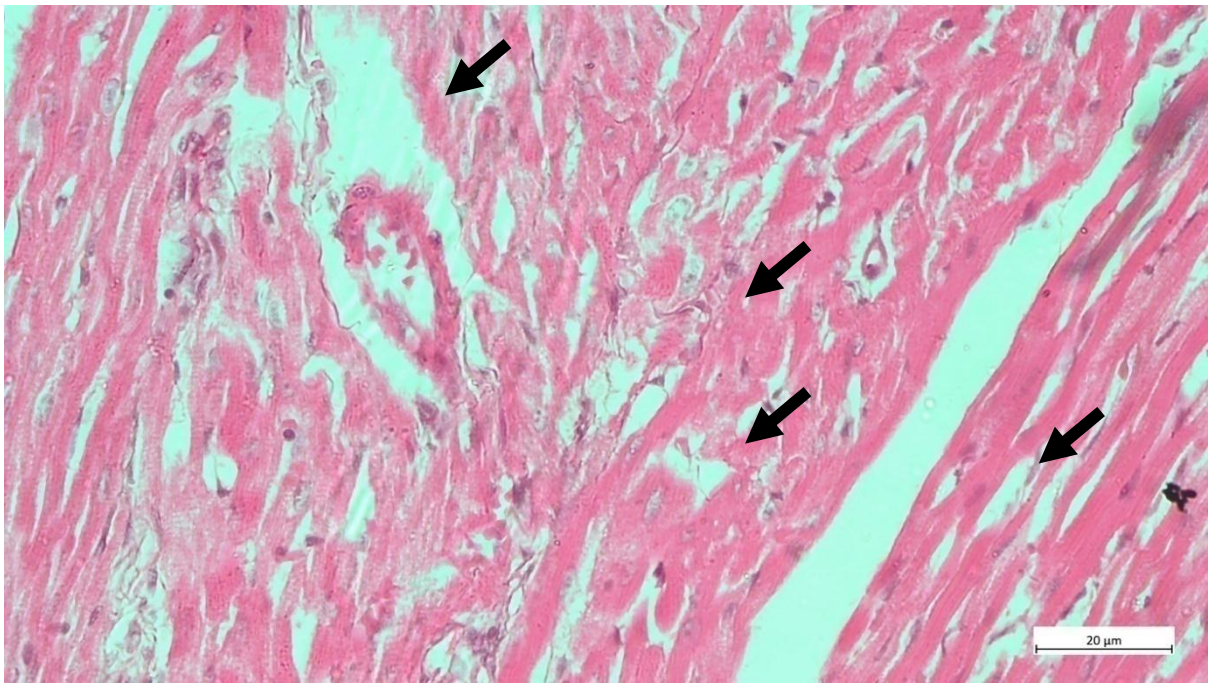

Plate 5; Sections of the heart presented in this group showed a marked diffused edema of the myocardium (arrow) mild – interstitial edema and focal necrosis. H & E., X400

Scientia Africana, Vol. 23 (No. 2), April, 2024. Pp127-146
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

Plate 6: Photomicrograph of sections of heart of rat fed MSG + HFD for 8 weeks and Orlistat 10mg/kg + HFD for 4 weeks.

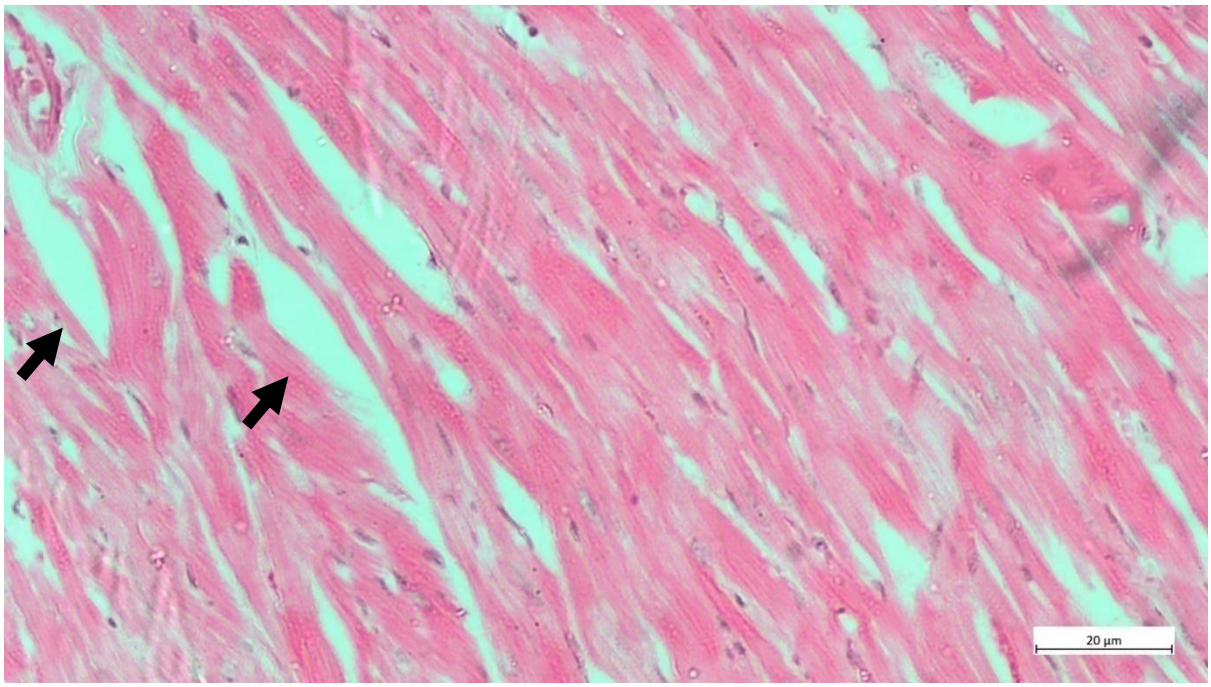

Plate 6; Sections of the heart presented in this group with mild multifocal areas of myocardial edema (arrow), mild – interstitial edema and focal necrosis.H & E., X400

DISCUSSION

Proximate analysis of diets used in the study

The ash content of the compounded diets was highest in the control (basal) diet which was different from all the other compounded diets suggesting a richer mineral content. The high fat diet incorporated with *Vernonia amygdalina* (5% and 10%) had a higher ash content compared to HFD only. This result suggests that *Vernonia amygdalina* did contribute to the ash content of the HFD incorporated *Vernonia amygdalina*. This finding was contrary to the results reported by Egedigwe et al. (2016) on the modulatory effects of dietary supplementation of *Vernonia amygdalina* on HFD-induced obesity in Wistar rats where the authors reported a lower ash content.

Protein content of the compounded feeds was highest in the 10% *Vernonia amygdalina* incorporated high fat diet which was different from the HFD only group. The high protein content in the 10% incorporated high fat diet could help provide amino acids necessary for various biological processes.

The highest crude fat was observed in the HFD only group which was higher than all the other formulated diets. The incorporation of 5% and 10% *Vernonia amygdalina* in HFD reduced the crude fat content of these diets.

The dietary crude fiber content was highest in the 10% *Vernonia amygdalina* incorporated HFD when compared to the other diets. The high crude fiber content suggests its potential to lower serum cholesterol and reduce the risk of coronary artery disease. Pereira et al. (2004) reported that the consumption of dietary fibers from cereals and fruits was inversely associated with the risk of coronary heart disease while the possible mechanisms involved include the improvement of profiles of blood lipid (Jenkins et al., 2002).

The moisture content of the 10% *Vernonia amygdalina* incorporated high fat diet was higher than all other formulated diets. The

high moisture content could negatively affect packaging, keeping quality, nutrients provided and the rate of microbial spoilage. This was however avoided during the period of experimentation as the formulated feeds were used up immediately after preparation to avoid spoilage.

Finally, the basal (control) diet recorded the highest carbohydrate content than the other diets

Taken together, the proximate composition analysis of the formulated diets showed the presence of sufficient amounts of crude fat in the HFD and could be sufficient in the induction of obesity in experimental animals while dietary incorporation of *Vernonia amygdalina* added extra fiber content that could be beneficial against cardiovascular diseases.

Effect of HFD, MSG and *Vernonia amgydalina* incorporated with HFD on Lipid profile.

At the end of week 8, HFD only group produced a higher level of total cholesterol when compared to the basal (control) diet group and the MSG only group while it was similar to the MSG + HFD group. The high amount of total cholesterol may be attributed to the fact that consumption of dietary cholesterol is known to increase serum total cholesterol, LDL cholesterol, VLDL cholesterol and decrease HDL cholesterol (Kapourchali et al., 2016). There was however no additive effect on total cholesterol in the MSG + HFD group after eight weeks as the value obtained for the 5%,10% *Vernonia amygdalina* incorporated HFD group and the 10 mg/kg Orlistat group was less than the total cholesterol level obtained for HFD only group at the end of week 12.

Saponins in leafy vegetables including *Vernonia amygdalina* have been reported to have hypocholesterolemic effects (Afrose et al., 2009). They bind to cholesterol and make it unable to be reabsorbed into the systemic circulation and are excreted from the body. The inhibition of the intestinal lipid absorption, and the inhibition of adipogenesis are some examples of their widely reported effects (del Hierro et al., 2018).

Total cholesterol of the HFD only group showed an increase when compared to all the other treatment groups from week 9 to the end of week 12. This rise is similar to other reports by various authors on the effect of chronic HFD on total cholesterol (Othman et al., 2021). The 10% *Vernonia amygdalina* incorporated HFD group produced a reduction in total cholesterol from week 9 – week 12 when compared to the orlistat 10 mg/kg group and the 5% *Vernonia amygdalina* incorporated HFD group. The results also show the efficacy of short-term *Vernonia amygdalina* incorporation in reversing hypercholesterolemia with results greater than what was obtained from the standard drug Orlistat 10 mg/kg. Possible mechanisms include the presence of saponins that are able to inhibit the intestinal lipid absorption, and the inhibition of adipogenesis (Kapourchali et al.,2016).

MSG only group did not produce any increase in total cholesterol levels.

Taken together, these results confirm the effect of HFD on total cholesterol and the efficacy of dietary incorporation of *Vernonia amygdalina* in reversing total cholesterol even after short-term administration. The results also show that the concomitant administration of chronic MSG + HFD did not have any additive effect on total cholesterol concentration.

At the end of week 8, there was no difference between the basal (control) diet group and all other treatment groups for HDL-C. At the end of week 12, there was a substantial increase in the HDL-C across all the groups.

The highest value of HDL-C was seen in the HFD group between end of week 8 - week 12. HFD only group was higher than all the other groups. The 5 % *Vernonia amygdalina* incorporated HFD group produced an increase in HDL-C compared to the 10 %

Scientia Africana, Vol. 23 (No. 2), April, 2024. Pp127-146
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

Vernonia amygdalina incorporated HFD group and Orlistat 10mg/kg group after 8 weeks of MSG + HFD co-administration. This result however could not be verified as there were similar increases in the HDL-C levels of the basal (control) diet group and HFD only group but was however higher compared to the MSG only group.

These results however allude to the toxicity of MSG administration as there was no observable increase in the HDL-C at the end of week 12 when compared to the other treatment groups.

HFD only group showed the highest triglyceride concentration which was different from basal (control) diet group and the MSG only group but not the MSG + HFD group at the end of week 8. There was no additive effect in triglyceride concentration in the MSG +HFD group as the value was less than the HFD only group at the end of week 8. At the end of week 12, triglyceride concentration of the HFD group only was higher than the basal (control) diet group, MSG only group, 5% and 10% *Vernonia amygdalina* incorporated HFD groups and the Orlistat 10mg/kg group. The 10 % *Vernonia amygdalina* incorporated HFD group showed a reduction in the triglyceride concentration when compared to the 5 % *Vernonia amygdalina* incorporated HFD group and the Orlistat group.

The results reestablish the efficacy of *Vernonia amygdalina* incorporation in reversing chronic hypertriglyceridemia. MSG only group did not alter the triglyceride concentration as there was a decrease in triglyceride from week 9 – end of week 12.

Taken together, these results show the hypolipidemic efficacy of dietary incorporation of *Vernonia amygdalina* and its possible benefit in managing obesity.

In keeping with earlier results, the HFD only group produced a higher amount of LDL-C when compared to all the other treatment groups at the end of week 8 and this trend continued till the end of the study period. There was no observable additive effect on LDL-C from MSG + HFD chronic administration after 8 weeks as the value was lower than that obtained for HFD only group and MSG only group.

At the end of week 12, the 10 % *Vernonia amygdalina* incorporated HFD group produced a higher reduction in LDL-C when compared to the 5 % *Vernonia amygdalina* incorporated HFD group and the Orlistat 10 mg/kg group and the effect was dose dependent. Elevated LDL-C is associated with metabolic diseases (Sun et al., 2022).

Taken together, the results of the lipid profile of the various treatment groups confirm the efficacy of HFD as a model in inducing hypercholesterolemia and hypertriglyceridemia, it also shows the benefit of short-term dietary incorporation of *Vernonia amygdalina* in reversing the trend while also confirming the absence of any additive mechanism in long term co-administration of MSG + HFD in the induction of obesity.

Microscopic examination of the heart tissues of rat fed basal control diet revealed the presence of a normal myocardial histo-architecture with no evidence of age-related signs of cardiotoxicity at the end of the study period. The same results were observed for the MSG only group and the HFD only groups with no signs of toxicity. The 5 % and 10% *Vernonia amygdalina* incorporated HFD group after MSG + HFD showed signs of a marked diffused edema of the myocardium and focal necrosis. This toxicity was similar to that observed in the standard drug Orlistat 10 mg/kg group. From the results, it could be inferred that there was an additive effect of toxicity on myocardium after chronic MSG+HFD intoxication as seen with the marked diffused edema and mild multifocal areas of myocardial edema which were all absent from the MSG only group and the HFD diet only group at the end of the study period while the various treatments (5 %,10% *Vernonia amygdalina* incorporated HFD group and Orlistat group 10 mg/kg after MSG

+ HFD) failed to reverse the trend. The result is similar to those published by (Othman et al., 2022) where Orlistat 10 mg/kg was administered for six weeks (6) after six weeks induction of obesity with HFD. The authors reported that histology results of the cardiomyocyte showed the normal group with a normal architecture of cardiomyocyte cells which were lined in homogenous pattern while the Obese group and the Obese + Orlistat 10 mg/kg groups exhibited significant increases in cardiomyocyte diameter when compared to the normal group. Cardiomyocyte oxidative stress, short duration of treatment (four weeks) with Orlistat 10 mg/kg , and the release of various pro-inflammatory factors and activation of caspase pro-apototic factors could be responsible for the observation in this present study (Eid et al., 2021) while more studies will be needed to characterize the signaling pathways in MSG +HFD -induced cardiomyocyte toxicity.

Taken together, the results reveal the capacity of chronic MSG +HFD administration to inflict mild toxicity on the myocardium and the failure of short term *Vernonia amygdalina* dietary incorporation to reverse the trend.

CONCLUSION

The study results revealed that the proximate composition of the formulated diets had enough crude fat in the high fat diets that could be sufficient in the induction of obesity while the dietary incorporation of *Vernonia amygdalina* in HFD showed anti-obesity activities similar to the standard drug orlistat 10 mg/kg while there was no apparent additive effect after concomitant administration of HFD +MSG. Hypercholesterolemia and hypertriglyceridemia were observed after chronic MSG , HFD, MSG and HFD concomitant administration while dietary incorporated *Vernonia amygdalina* reversed the trend with results similar to what was obtained with the standard drug Orlistat 10 mg/kg. Histology results of the myocardium at the end of the study period revealed mild toxicities that dietary incorporated *Vernonia amygdalina* and the standard drug Orlistat 10 mg /kg failed to reverse the trend. From the foregoing, leaves of *Vernonia amygdalina* could be incorporated into diets as an adjuvant in the management of some components of metabolic syndrome including hypercholesterolemia, hypertriglyceridemia and insulin resistance because of its availability and affordability.

Acknowledgments: **Mr. George Ubah, Mrs. Victoria Aliba, Mr. Ikechukwu Aliba, Mr. Gregory Ubah and Mr. Stanley Ossai and Mrs.Vera Ubah all provided equal funding for the project,** Dr. Chukwunonso Ezeasor interpreted the histological slides, Dr. Daniel C. Ifenkwe assisted with handling the animals, Mr. Agbakwuru Isaiah Okezie preserved and prepared the histological slides**,**Dr. Onoja, Samuel Okwudili assisted with the statistical analyses.

Authors' contributions : Professor Ifeoma Irene Ijeh designed the study, supervised the experiment and read the literature review, Dr. Anthony Cemaluk C. Egbuonu assisted with the design and also read the literature review, Mr. Emeka Emmanuel Ubah carried out the experiment and compiled the writeup, and Mrs Obike Chiemeziem Adanma and Dr. Kelechi Callistus Oguamanam proofread the work before submission.

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

Afrose, S., Hossain, M.S., Maki, T. and Tsujii, H. (2009). Karaya root saponin exerts a hypocholesterolemic response in rats fed a high-cholesterol diet. *Nutrition research*, *29*(5), pp.350-354. https://doi.org/10.1016/j.nutres.2009.05.008

Aitlhadj, L., Ávila, D.S., Benedetto, A., Aschner, M. and Stürzenbaum, S.R. (2011). Environmental exposure, obesity, and Parkinson's disease: lessons from fat and old worms. *Environmental health perspectives*, *119*(1), pp.20-28.

Scientia Africana, Vol. 23 (No. 2), April, 2024. Pp127-146
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

Akpoveso, O.O.P., Ubah, E.E. and Obasanmi, G. (2023). Antioxidant phytochemicals as potential therapy for diabetic complications. *Antioxidants*, *12*(1), p.123.https://doi.org/10.3390/antiox12010123

Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W.F.P.C. and Fu, P.C. (1974). Enzymatic determination of total serum cholesterol. *Clinical chemistry*, *20*(4), pp.470-475.

AOAC INTERNATIONAL [Internet]. (cited 2023 Jun 22). Official Methods of Analysis Program. Available from: https://www.aoac.org/scientific-solutions/standards-and-official-methods/

Araujo, T.R., Freitas, I.N., Vettorazzi, J.F., Batista, T.M., Santos-Silva, J.C., Bonfleur, M.L., Balbo, S.L., Boschero, A.C., Carneiro, E.M. and Ribeiro, R.A. (2017). Benefits of L-alanine or L-arginine supplementation against adiposity and glucose intolerance in monosodium glutamate-induced obesity. *European journal of nutrition*, *56*, pp.2069-2080. https://doi.org/10.1007/s00394-016-1245-6

Bashir, M.A., Yahaya, A.I., Muhammad, M., Yusuf, A.H. and Mukhtar, I.G. (2022). Prevalence of central obesity in Nigeria: a systematic review and meta-analysis. *Public Health*, *206*, pp.87-93.https://doi.org/10.1016/j.puhe.2022.02.020

Bera, T.K., Kar, S.K., Yadav, P.K., Mukherjee, P., Yadav, S. and Joshi, B. (2017). Effects of monosodium glutamate on human health: A systematic review. *World journal of pharmaceutical sciences*, pp.139-144.

Cheung, B.M. and Li, C. (2012). Diabetes and hypertension: is there a common metabolic pathway? *Current atherosclerosis reports*, *14*, pp.160-166. https://doi.org/10.1007/s11883-012-0227-2

Cohen, P. and Spiegelman, B.M. (2016). Cell biology of fat storage. *Molecular biology of the cell*, *27*(16), pp.2523-2527.https://doi.org/10.1091/mbc.e15-10-0749

delHierro, J.N., Herrera, T., Fornari, T., Reglero, G. and Martin, D. (2018). The gastrointestinal behavior of saponins and its significance for their bioavailability and bioactivities. *Journal of Functional Foods*, *40*, pp.484-497. https://doi.org/10.1016/j.jff.2017.11.032

DeVries, J.W., Prosky, L., Li, B. and Cho, S. (1999). A historical perspective on defining dietary fiber. *Cereal foods world*, *44*, pp.367-369.

Egbuonu, A.C.C., Obidoa, O., Ezeokonkwo, C.A., Ejikeme, P.M. and Ezeanyika, L.U.S. (2010). Some biochemical effects of sub-acute oral administration of L-arginine on monosodium glutamate-fed Wistar albino rats 1: Body weight changes, serum cholesterol, creatinine, and sodium ion concentrations. *Toxicological & Environmental Chemistry*, *92*(7), pp.1331-1337. https://doi.org/10.1080/02772240903450645

Egedigwe, C.A. and Ijeh, I.I. (2010). Body and organ weight changes following dietary incorporation of Vernoniacolorata and Vernonia amygdalina Del in Albino rats. *Niger. J. Nutri. Sci*, *31*(2), pp.58-61.

Egedigwe, C.A., Ijeh, I.I., Okafor, P.N. and Ejike, C.E., 2016. Aqueous and methanol extracts of Vernonia amygdalina leaves exert their anti-obesity effects through the modulation of appetite-regulatory hormones. *Pharmaceutical biology*, *54*(12), pp.3232-3236. DOI: 10.1080/13880209.2016.1216135

Eid, B.G. and El-Shitany, N.A., 2021. Captopril downregulates expression of Bax/cytochrome C/caspase-3 apoptotic pathway, reduces inflammation, and oxidative stress in cisplatin-induced acute hepatic injury. *Biomedicine & Pharmacotherapy*, *139*, p.111670.

El Hadi, H., Di Vincenzo, A., Vettor, R. and Rossato, M. (2019). Food ingredients involved in white-to-brown adipose

tissue conversion and in calorie burning. *Frontiers in Physiology*, *9*, p.1954.https://doi.org/10.3389/fphys.2018.01954

Friedewald, W.T., Levy, R.I. and Fredrickson, D.S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry*, *18*(6), pp.499-502. PMID: 4337382.

Gidez, L.I., Miller, G.J., Burstein, M., Slagle, S. and Eder, H.A. (1982). Separation and quantitation of subclasses of human plasma high density lipoproteins by a simple precipitation procedure. *Journal of lipid Research*, *23*(8), pp.1206-1223. https://doi.org/10.1016/S0022-2275(20)38059-7

Gobatto, C.A., Mello, M.A., Souza, C.T. and Ribeiro, I.A. (2002). The monosodium glutamate (MSG) obese rat as a model for the study of exercise in obesity. *Research communications in molecular pathology and pharmacology*, *111*(1-4), pp.89-101. PMID: 14632317

Hajihasani, M.M., Soheili, V., Zirak, M.R., Sahebkar, A. and Shakeri, A. (2020). Natural products as safeguards against monosodium glutamate-induced toxicity. *Iranian Journal of Basic Medical Sciences*, *23*(4), p.416. https://doi.org/10.22038/ijbms.2020.43060.10123

Hariri, N. and Thibault, L., 2010. High-fat diet-induced obesity in animal models. *Nutrition research reviews*, *23*(2), pp.270-299.

He, K., Du, S., Xun, P., Sharma, S., Wang, H., Zhai, F. and Popkin, B. (2011). Consumption of monosodium glutamate in relation to incidence of overweight in Chinese adults: China Health and Nutrition Survey (CHNS). *The American journal of clinical nutrition*, *93*(6), pp.1328-1336.**DOI :** 10.3945/ajcn.110.008870

Ijeh, I.I. and Ejike, C.E.C.C. (2011). Current perspectives on the medicinal potentials of Vernonia amygdalina Del. *Journal of medicinal plants research*, *5*(7), pp.1051-1061.

Ijeh, I.I., Amadi, I.P. and Ejike, C.E. (2013). Improvement of glucose tolerance in rats fed with diets containing Vernonia amygdalina leaves. *Biokemistri*, *25*(1), pp.1-5.

Ishak, K., Baptista, A., Bianchi, L., Callea, F., De Groote, J., Gudat, F., Denk, H., Desmet, V., Korb, G., MacSween, R.N. and Phillips, M.J. (1995). Histological grading and staging of chronic hepatitis. *Journal of hepatology*, *22*(6), pp.696-699.https://doi.org/10.1016/0168-8278(95)80226-6

Jenkins, D.J., Kendall, C.W., Vuksan, V., Vidgen, E., Parker, T., Faulkner, D., Mehling, C.C., Garsetti, M., Testolin, G., Cunnane, S.C. and Ryan, M.A. (2002). Soluble fiber intake at a dose approved by the US Food and Drug Administration for a claim of health benefits: serum lipid risk factors for cardiovascular disease assessed in a randomized controlled crossover trial. *The American journal of clinical nutrition*, *75*(5), pp.834-839. https://doi.org/10.1093/ajcn/75.5.834

Kapourchali, F.R., Surendiran, G., Goulet, A. and Moghadasian, M.H. (2016). The role of dietary cholesterol in lipoprotein metabolism and related metabolic abnormalities: a mini-review. *Critical reviews in food science and nutrition*, *56*(14), pp.2408-2415. https://doi.org/10.1080/10408398.2013.842887

Kazmi, Z., Fatima, I., Perveen, S. and Malik, S.S., 2017. Monosodium glutamate: Review on clinical reports. *International Journal of food properties*, *20*(sup2), pp.1807-1815.

Keenan, J.M., Pins, J.J., Frazel, C., Moran, A. and Turnquist, L. (2002). Oat ingestion reduces systolic and diastolic blood pressure in patients with mild or borderline hypertension: a pilot trial. *J*

Scientia Africana, Vol. 23 (No. 2), April, 2024. Pp127-146
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

FamPract, *51*(4), p.369. PMID:11978262

Khalilpourfarshbafi, M., Gholami, K., Murugan, D.D., Abdul Sattar, M.Z. and Abdullah, N.A. (2019). Differential effects of dietary flavonoids on adipogenesis. *European journal of nutrition*, *58*, pp.5-25. https://doi.org/10.1007/s00394-018-1663-8

Krisanits, B., Randise, J.F., Burton, C.E., Findlay, V.J. and Turner, D.P. (2020). Pubertal mammary development as a "susceptibility window" for breast cancer disparity. *Advances in cancer research*, *146*, pp.57-82.https://doi.org/10.1016/bs.acr.2020.01.004

Laar, A.K., Addo, P., Aryeetey, R., Agyemang, C., Zotor, F., Asiki, G., Rampalli, K.K., Amevinya, G.S., Tandoh, A., Nanema, S. and Adjei, A.P., 2022. Perspective: Food environment research priorities for Africa—Lessons from the Africa food environment research network. *Advances in Nutrition*, *13*(3), pp.739-747.

Lee, M.O. (1929). Determination of the surface area of the white rat with its application to the expression of metabolic results. *American Journal of Physiology-Legacy Content*, *89*(1), pp.24-33.https://doi.org/10.1152/ajplegacy.1929.89.1.24

Nwanjo, H.U. (2005). Efficacy of aqueous leaf extract of Vernonia amygdalina on plasma lipoprotein and oxidative status in diabetic rat models. *Nigerian Journal of Physiological Sciences*, *20*(1), pp.39-42.

Othman, Z.A., Zakaria, Z., Suleiman, J.B., Ghazali, W.S.W. and Mohamed, M., (2021). Anti-atherogenic effects of orlistat on obesity-induced vascular oxidative stress rat model. *Antioxidants*, *10*(2), p.251.https://doi.org/10.3390/antiox10020251

Othman, Z.A., Zakaria, Z., Suleiman, J.B., Mustaffa, K.M.F., Jalil, N.A.C., Wan Ghazali, W.S., Zulkipli, N.N. and Mohamed, M., 2022. Orlistat Mitigates Oxidative Stress-Linked Myocardial Damage via NF-κβ-and Caspase-Dependent Activities in Obese Rats. *International Journal of Molecular Sciences*, *23*(18), p.10266.

Pereira, M.A., Jacobs Jr, D.R., Pins, J.J., Raatz, S.K., Gross, M.D., Slavin, J.L. and Seaquist, E.R. (2002). Effect of whole grains on insulin sensitivity in overweight hyperinsulinemic adults. *The American journal of clinical nutrition*, *75*(5), pp.848-855. **DOI** 10.1093/ajcn/75.5.848

Pereira, M.A., O'Reilly, E., Augustsson, K., Fraser, G.E., Goldbourt, U., Heitmann, B.L., Hallmans, G., Knekt, P., Liu, S., Pietinen, P. and Spiegelman, D. (2004). Dietary fiber and risk of coronary heart disease: a pooled analysis of cohort studies. *Archives of internal medicine*, *164*(4), pp.370-376.

Sun, C., Wang, Z., Hu, L., Zhang, X., Chen, J., Yu, Z., Liu, L. and Wu, M., 2022. Targets of statins intervention in LDL-C metabolism: Gut microbiota. *Frontiers in Cardiovascular Medicine*, *9*, p.972603.

Tietz NW. Clinical guide to laboratory tests, WB Saunders Company. Phila USA. (1990);554:556.

Tucci, S.A., Boyland, E.J. and Halford, J.C. (2010). The role of lipid and carbohydrate digestive enzyme inhibitors in the management of obesity: a review of current and emerging therapeutic agents. *Diabetes, metabolic syndrome and obesity: targets and therapy*, pp.125-143. DOI: 10.2147/dmsott.s7005

Tung, B.T., Rodríguez-Bies, E., Ballesteros-Simarro, M., Motilva, V., Navas, P. and López-Lluch, G. (2014). Modulation of endogenous antioxidant activity by resveratrol and exercise in mouse liver is age dependent. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, *69*(4), pp.398-

409.https://doi.org/10.1093/gerona/glt102

WHO | Regional Office for Africa [Internet]. (2022 Oct 24). Obesity rising in Africa, WHO analysis finds. Available from: https://www.afro.who.int/news/obesity-rising-africa-who-analysis-finds

Zhong, F., Zhou, X., Xu, J. and Gao, L. (2020). Rodent models of nonalcoholic fatty liver disease. *Digestion*, *101*(5), pp.522-535. https://doi.org/10.1159/000501851

Zhou, W., Rahimnejad, S., Lu, K., Wang, L. and Liu, W. (2019). Effects of berberine on growth, liver histology, and expression of lipid-related genes in blunt snout bream (Megalobramaamblycephala) fed high-fat diets. *Fish Physiology and Biochemistry*, *45*, pp.83-91. https://doi.org/10.1007/s10695-018-0536-7