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RESEARCH ARTICLE

VERNONIA AMYGDALINA LEAVES INCORPORATION INTO HIGH FAT DIET EXHIBITED HYPOGLYCEMIC, HEPATOPROTECTIVE, AND WEIGHT LOWERING EFFECTS IN MONOSODIUM GLUTAMATE INTOXICATED WISTAR RATS

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Abstract

Background and aims: Insulin resistance (IR) and obesity (Ob) are hallmarks of metabolic syndrome while high-fat diet (HFD) and monosodium glutamate (MSG) consumption have been implicated in both IR and Ob. In current study, hypoglycemic, hepatoprotective, and weight-reducing effects of dietary *Vernonia amygdalina* (VA) in MSG-intoxicated HFD fed wistar rats was investigated.
Methods: Male wistar rats (36) were randomly allocated into six groups of six rats each, each weighing between 150 and 250 grams on average. The animals were given 5%, 10% dietary incorporated VA (VAHFD), and Orlistat 10 mg/kg for 4 weeks after being treated orally with MSG 8000 mg/kg for the first 8 weeks while on HFD. Changes in the experimental animals' weight, liver histology, total protein and albumin concentrations, and fasting blood glucose concentrations were all noted, and the data was analyzed for statistical significance using appropriate techniques.
Results: Peak fasting blood glucose concentration was recorded for the MSG-only group at the end of the study period while 10% VAHFD was greater than Orlistat 10 mg/kg in reducing it. Hepatotoxicity was observed in rats fed the HFD or HFD + MSG while the 10% VAHFD was comparable to Orlistat 10 mg/kg in protecting the liver both of which were greater than the 5% VAHFD. The Orlistat group showed a peak reduction in weight gain from week 9 to the end of week 12, followed by the 5% VAHFD > 10% VAHFD.
Conclusions: Following co-intoxication with MSG + HFD, dietary VAHFD was effective in managing hyperglycemia, hepatotoxicity, and weight gain, however, there was no additive effect on toxicity from MSG + HFD co-intoxication on the various studied parameters.
Keywords: Hepatotoxicity, hyperglycaemia, monosodium glutamate, *Vernonia amygdalina*, weight gain.

INTRODUCTION

In sub-Saharan Africa, the onset of the dietary shift is thought to have been influenced by the rise in the middle class, fast industrialization, economic expansion, inequality, and an environment that encourages obesity. Diet-related non-communicable diseases (DR-NCD) have also become more common[1].
The modern diet know to possess a high-fat diet (HFD) and refined sugar with low fibre content and containing a large proportion of processed food contributes majorly to weight gain, obesity and possible insulin resistance and type II diabetes mellitus (T2DM) development[2]. The easy availability of hypercaloric foods and a greater percentage of the population adopting a sedentary lifestyle or the decline of physical exercise brought on by increased urbanization, the evolution of transportation patterns in many nations, or the absence of robust regulations in critical areas like the environment, health, agriculture, and urban planning that would encourage healthier living all contribute to the exacerbation of the problem of obesity which if unchecked will predispose many Africans to develop insulin resistance[2].
Insulin resistance (IR) provides the framework for a shared knowledge of chronic metabolic diseases, such as diabetes, hypertension, cancer, and nonalcoholic fatty liver disease. It is also essential to the development and course of many illnesses.

In order to maintain blood sugar stability, hyperinsulinemia (HI) commonly co-exists with insulin resistance (IR), a complex pathophysiological disease. It is distinguished by a reduction in sensitivity, and an inability to suppress peripheral glucose elimination and block glucose synthesis[3]. Physiologically, any abnormality that results in disruptions to the route of signaling via insulin, like errors in insulin receptors, changes within the interior setting (Oxygen deprivation, lipotoxicity, swelling and immune response), abnormalities in the liver's metabolic role and other organs, causes the development of IR in the host[4]. Lipotoxicity results in abnormalities to the functionality of many metabolic pathways, in peripheral organs and adipose tissues alike, similar to the pancreas, liver, heart and muscles. Within the muscle and the liver, elevated measures of circulating fats and physiological changes in utilization of fatty acids and intracellular signaling have been connected to IR[5] while different pathways like a novel protein kinase C pathway and the c-Jun N-terminal kinases (JNK-1) pathway participate in the process via which lipotoxicity results in IR in organs and tissues that aren't fat while mitochondrial dysfunction, through primarily enhanced oxidative stress, endoplasmic reticulum stress also plays a significant role in the development of IR. IR is an essential part of metabolic syndrome in addition to obesity and dyslipidemia (Adult Treatment Panel III)[6]. Insulin produced within the human body, have a wide range of effects on the metabolism of fats and proteins, transport of iron and amino acids, cell cycle expansion, cell differentiation and nitric oxide synthesis.

Herbal Medicines for the management of IR have come a long way as physicians used them to control diabetes before the discovery of insulin and the invention of chemical drugs. Some of them promote GLUT4 translocation in various cell lines by inducing phosphoinositide-3-kinase (PI3K) activity and phosphorylation of insulin receptor substrate-1 (IRS-1) and protein kinase B (Akt)[8], Others exert a beneficial effect on insulin sensitivity by activating AMP-activated protein kinase (AMPK)[9] or suppress endoplasmic recticulum (ER) adipose tissue-mediated activation of inflammasomes under stress, resulting in less secretion of inflammatory cytokines and subsequent insulin resistance[10]. High-fat diet is widely used to induce Non-Alcohol Fatty Liver Disease (NAFLD)/Non-Alcoholic Steato-Hepatitis (NASH) in various experimental animals[11,12]. A high-fat liquid diet consisting of (71% of energy from fat, 10% from carbohydrates and 18% from protein) was fed to Sprague Dawley rats *ad libitum*, after 3 weeks, the rats developed lobular steatosis, mitochondria, and mononuclear inflammation with elevated plasma insulin while the high-fat diet induced a metabolic profile characterized by obesity and insulin resistance[13].

Ogasawara *et al*.,[14] reported that gold thio-glucose plus High-Fat Diet induced dysmetabolism with hyperphagia, obesity with increased abdominal adiposity, insulin resistance, and consequent steatohepatitis with hepatocyte ballooning; mallory–denk bodies, pericellular fibrosis as seen in adult NASH in experimental mice. The sodium salt of the naturally occurring monosodium glutamate (MSG) consists of L-glutamic acid and is used as a flavor enhancer by the food industry. MSG is known to have some adverse effects in humans and experimental animals which include the Chinese restaurant syndrome characterized by neuro-excitotoxicity[15], and obesity[16]. 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, genotoxic, reproductive, renal, and other toxicities. Additionally, consuming MSG has been related to conditions like Parkinson's, Alzheimer's, addiction, brain injury, anxiety, stroke, depression, and epilepsy[17]. Chronic administration of MSG (4000 mg/kg body weight) caused oxidative damages in hepatic and cardiac tissues in experimental animals[18].

Pongking *et al.*,[19] studied the effect of a combination of monosodium glutamate and High fat and High fructose (HFHF) diets in hamsters and concluded that the consumption of both MSG and HFHF diet increased the risk of kidney injury while inducing gut dysbiosis and increasing the amount of P-cresol sulfate in hamsters. Nahok *et al.*,[20] reported significant changes in the liver of MSG-treated rats after short and long-term consumption of MSG. Onaolapo *et al.*,[21] reported a negative effects of low doses of MSG on histological parameters in mice leading to cell death. While MSG is commonly used in the diet, its safety and systemic side effects needs to be fully studied[22]. Yang *et al.*,[23] extensively reviewed data on the safety of MSG consumption in human health and reported that the salt showed negative consequences on people, including cancer, immune system dysfunction, metabolic syndrome, and others while the use of phytochemicals from plants could help mitigate the deleterious effect.

V. amygdalina Del. (*Asteraceae*), also known as bitter leaf in Africa and Asia, is a popular medicinal herb used in several illnesses, such as the healing of wounds, malaria and hyertension, and diabetes mellitus[24,25]. In addition to being green, the leaves have a distinctive taste and smell. The plant has been domesticated in numerous regions of west Africa[26]. Other authors have reported hypolipidemic activities of the leaves of the plant in various high-fat diet (HFD) obese animal models while this study investigated the benefit of dietary incorporation of *V. amygdalina* leaves in monosodium glutamate (MSG) intoxicated HFD-fed rats. Djeujo *et al.*,[27] studied the leaves and roots of *V. amygdalina* used as aqueous decoctions to prevent diabetes. The authors came to the conclusion that whereas luteolin was more prevalent in the leaves of *V. amygdalina*, vernodalol was more prevalent in the roots. The leaf extracts were linked to the luteolin-dependent antioxidant and antidiabetic benefits, while the root extracts were linked to the vernodalol-dependent anti-proliferative activity.

Hajhasani *et al*.,[28] suggested the use of various natural products as safeguards against MSG-induced toxicity with no information on the benefits of *V. amygdalina* leaves. Banerjee *et al.*,[29] considered an overview of

worldwide flavour enhancer MSG and High lipid diets (HLD). They focused on the combined effects of MSG and HLD and discovered that both induced the generation of (ROS) thereby altering redox balance to result in systemic change. MSG mixed with HLD consumption led to dyslipidemia in rats and provoked anomalies related to the liver and heart caused by changes in redox balance, immune response and predetermined cell death, activating Nuclear factor – Kappa B (NF-KB) and mitochondrial caspase-mediated pathway, unfortunately, there isn't much data on long-term use of MSG + HFD related abnormalities in the system.

Taken together, there is strong evidence of the deleterious effect of the consumption of HFDs and MSG while this study will evaluate the possibility of any additive effect on co-administration of both. This study will aim to identify the effects of various concentrations of short-term dietary incorporation of *V. amygdalina* leaves on glucose levels, liver organ histopathology, and weight changes in MSG and high-fat diet co-intoxicated animal models.

MATERIALS AND METHODS

Collection of plant materials

Fresh *V. amygdalina* leaves were acquired from the Ubani local market in Umuahia, Abia State, Nigeria. Prof. G. C. Osuagwu from the Department of Plant Science and Biotechnology at the Michael Okpara University of Agriculture in Umudike, Abia State, Nigeria, identified the plant. Voucher specimens were put in 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 (26-28ºC) and then pulverized, crushed into fine powder, and weighed using an electric blender, and stored in airtight plastic containers.

Table 1: 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	100

Experimental design and procedure

The investigation was conducted using mature male wistar rats (150-250 g) procured from the University of Nigeria's Veterinary Department in Nsukka, Enugu State, Nigeria. They were acclimatized in the animal home of the Department of Veterinary Medicine at Michael Okpara University of Agriculture in Umudike, Abia State, Nigeria, for two weeks before the experiment began. Two types of diets were used: high-fat diets (HFDs) and control rat chow diets, as illustrated in Table 1 and Table 2, respectively. All feeding supplies were obtained locally from Jocan Agro Feeds Ltd, Umuahia, Abia State, Nigeria.

Table 2: Basal (control) diet composition.

Ingredients	(g/1000 kg)	(g/100 g)
Cellulose	100	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	100

The basal (control) diet consisted of all the listed feed items except the beef tallow. This was done by mixing these feed items 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.*,[30]. After pelleting, it was dried in an oven at a temperature of 35^{o}C. The beef tallow was obtained from the Ubakala slaughterhouse, Umuahia South L.G.A, Umuahia, Abia state, Nigeria. A substantial number of intestines of slaughtered cows were melted in an oven to extract the oils which was subsequently used in the formulation of the High-fat diets. Monosodium glutamate was prepared from stock; Ajinomoto ® brand. The high-fat diet was further incorporated with 5% and 10% *V. amygdalina*.

After twelve weeks of feeding, the animals were sacrificed with anesthesia, blood samples were collected by retro-orbital bleeding from the eyes based on the institutional guidelines on the safe handling of experimental animals with ethical approval obtained from the ethical committee, Veterinary Medicine of Michael Okpara University of Agriculture, Umudike, Abia state, Nigeria.

Housing, adaptation, and feeding of experimental animals.

During the acclimatization period, the animals received unlimited food with Vital Grower's Mash from Vital Feed Limited in Nigeria and clean tap water. Following the two-week acclimatization phase, the experimental animals were separated into six groups of six rats each. All of the animals in their typical cages received pelleted food and clean tap water. The experimental animals were randomly allocated to the following groups:

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

Group A: Rats in this group received a 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% *V. 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% *V. 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% *V. amygdalina*,and Orlistat 10 mg/kg orally for the 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% *V. amygdalina* incorporated HFD respectively as required, the animals were sacrificed with anesthesia, Blood samples were collected by retro-orbital bleeding from the eyes.

Table 3: *V. amygdalina* (5%) 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	100
Weighing Out	50	5
Balance	950	95.0
V. amygdalina powdered leaves added	50	5
Total	1000	100

Table 4: ***V. amygdalina* (10%) 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
V. amygdalina powdered leaves added	100	10
Total	1000.00	100.00

Body weight calculation

Change in body weight: The body weight (g) was recorded on week 0 (and then on alternate days for 12 weeks in each group) before giving the food and water. Change in body weight (weekly) was calculated using the formula:

Body weight = Final body weight – Initial body weight.

Determination of fasting blood glucose concentration

After an overnight fast, the experimental animals were tested for fasting blood glucose levels. This was performed using an Accu-Check active glucometer and test strips supplied by Roche Diagnostics GmbH, San Hofer Strasse Mannheim Germany.

Principle: The principle was based on the reaction of glucose in the blood (from the tail end of the rats) with glucose dehydrogenase enzyme (on the test strip) resulting in a colour change. The intensity of this colour gives the blood glucose concentrations (mg/dl) as converted by the glucometer[31].

Determination of serum albumin

The serum albumin content of the sample was determined using Bromocresol Green according to Doumas *et al.*,[32] as recently reported by[33]. Three test tubes labeled Reagent, Standard, and Sample were provided. Into the Reagent test tube was pipetted 0.01 mL of distilled water. The volume, 0.01 mL of standard and sample was pipetted into Standard and Sample test tubes appropriately. A known volume (3.0 mL) of 3, 3′,5,5- tetrabromo-m-cresosulphonephthelein (BCG) into each of the three test tubes. The contents of the test tubes were incubated at 25ºC for 3 minutes and absorbance was taken against reagent blank at 630 nm. The albumin concentration in the sample was calculated from the following formula:

$$\frac{\Delta Asample}{\Delta Astandard} \times Cstandard = Csample$$

Determination of serum total protein

The rat's total protein content was determined using Peterson's modifications of the Micro-Lowry method[34] using a protein assay kit. This method is based on the principle that Cu^{2+} complexes into the peptide bonds of the proteins functional groups. The development of a

Cu^{2+} protein complex takes two peptide links and produces a violet-coloured chelate product which is measured at 540 nm by absorption spectroscopy.

Histological studies of the liver

Tissue preparation and staining

The liver was promptly excised and dabbed with filter paper to remove blood and other liquid. The organ was weighed using a Sartorius top-loading balance and fixed in buffered 10% formalin preparatory for histological studies

Sections of the liver from each group were collected in a sterile universal container containing 10% formal saline solution based on the method described by[35].

Statistical analysis

Data were analyzed by using Statistical Package for the 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 the 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 AND DISCUSSION

The effects of HFD, MSG, HFD+MSG, and *V. amygdalina* incorporated HFD on fasting blood glucose

Glucose level increased significantly ($p<0.05$) in the MSG group compared to the basal control diet group, high-fat diet group, 5% VAHFD and 10% VAHFD groups and the Orlistat 10 mg/kg group at the end of week 12.

The effects of HFD, MSG, HFD+MSG, and *V. amygdalina* incorporated HFD on body and organ weight.

At the end of the induction phase (week 8), there was a significant ($p<0.05$) increase in the body weight of rats in the MSG group compared to the basal control diet group, high-fat diet only group, and MSG + HFD group. At the end of the treatment phase (week 12), the Orlistat treatment group had a significant ($p<0.05$) reduction in weight gain compared with the basal control diet group, the high-fat diet only group, the MSG group, 5% VAHFD, and 10% VAHFD. Also, a significant ($p<0.05$) increase in weight gain was seen in the MSG-only group when compared to the basal control diet group, high fat diet-only group, 5% VAHFD, 10% VAHFD, and Orlistat treatment group at week 12.

The effects of HFD, MSG, HFD+MSG, and *V. amygdalina* incorporated HFD on Adiposity Index

At the end of week 8, there was a non-significant ($p>0.05$) increase in the adiposity index of the high-fat diet-only group compared with the basal control diet group, MSG group,5% VAHFD and 10% VAHFD groups and the Orlistat 10 mg/kg. At the end of week 12, the orlistat group showed a significant ($p<0.05$) decrease in adiposity index compared to the high-fat diet-only group, basal control diet group, MSG group, 5% VAHFD, and 10% VAHFD groups.

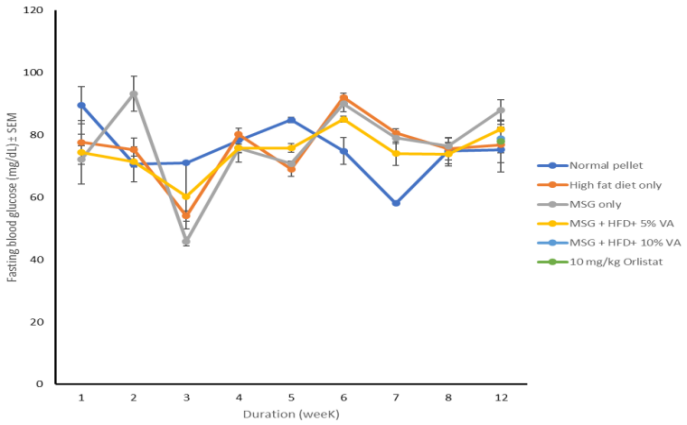

Figure 1: Fasting blood glucose levels in different experimental groups.

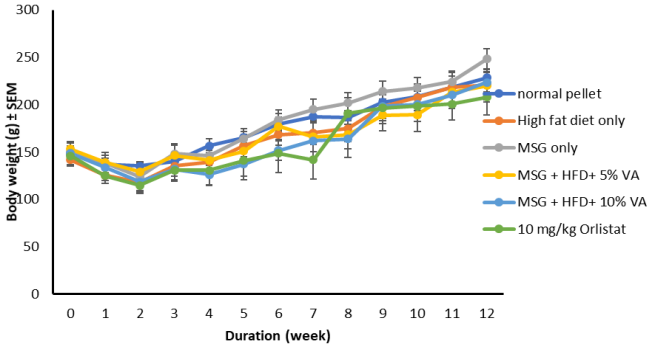

Figure 2: The effects of HFD, MSG, HFD+MSG, and *V. amygdalina* incorporated HFD on body weight.

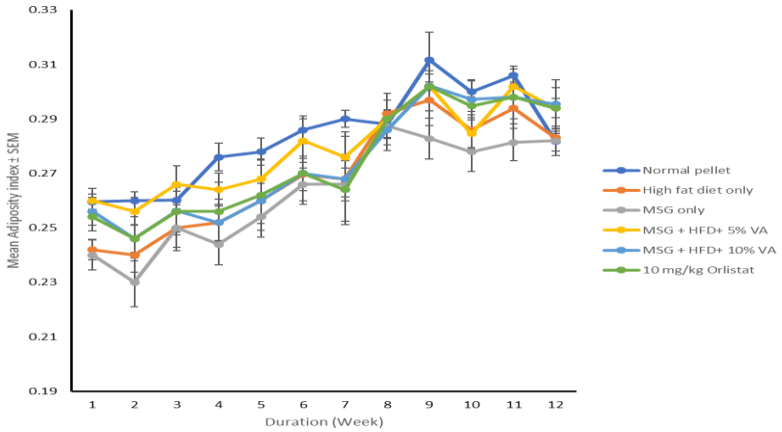

Figure 3: Adiposity index of different experimental groups.

Table 5: The effects of HFD, MSG, HFD+MSG, and *V. amygdalina* incorporated HFD on serum protein.

Group	Total protein (g/dL)	Albumin (g/dL)
Normal pellet	6.88±0.24	3.30 ± 0.12^{b}
High fat diet only	6.78±0.47	3.50 ± 0.12^{bc}
MSG only	6.45±0.26	3.70 ± 0.06^{c}
MSG + HFD+ 5% VA	9.52±3.57	3.42 ± 0.07^{bc}
MSG + HFD+ 10% VA	5.94±0.17	3.26 ± 0.05^{b}
10 mg/kg Orlistat	5.36±0.34	2.66 ± 0.11^{a}

Values are mean±SD, n = 6. The different superscripts (^{abc}) are significant ($p<0.05$) across the Column (vertically)

Effects of HFD, MSG, HFD+MSG, and *Vernonia amygdalina* incorporated HFD on serum protein

Table 5 describes the effects of HFD, MSG, HFD+MSG and *V. amygdalina* incorporated HFD on serum protein. The first column describes the total protein. There was a non significant ($p>0.05$) increase in total protein concentration in the 5% VAHFD group compared with the basal control diet group, High fat diet only group, MSG only group,10% VAHFD and Orlistat 10 mg/kg group respectively. The second column shows the Albumin concentration of the various groups. The Orlistat 10 mg/kg group showed a significant ($p<0.05$) decrease in albumin concentration compared to the basal control diet group, High-fat diet only group, MSG only group, 5% VAHFD group, and 10% VAHFD respectively.

Histopathology results of the liver

Sections of the liver presented in this group showed mild multifocal aggregations of mononuclear inflammatory leukocytes around the portal triads (arrow) (Figure 4). Central vein (V); Portal triad (P). Rats in this group had a histopathological score of two (2) characterized by peri-septal hepatitis and portal inflammation based on the histopathological scoring system described by Ishak *et al*.,[36].

Sections of the liver presented in this group showed mild multifocal aggregations of mononuclear inflammatory leukocytes around the portal triads as well as multifocal areas of lytic necrosis (Figure 5). Central vein (V); Portal triad (P). Rats in this group had a histopathological score of three (3) characterized by peri-septal hepatitis and portal inflammation and Focal lytic necrosis, apoptosis, and focal inflammation based on the histopathological scoring system described in a previous study[36]. Sections of the liver presented in this group showed mild multifocal aggregations of mononuclear inflammatory leukocytes around the portal triads (arrow). Portal triad (P) (Figure 6).

Rats in this group had a histopathological score of one (1) characterized by portal inflammation based on the histopathological scoring system described in a previous study[36]. Sections of the liver presented in this group showed mild multifocal aggregations of mononuclear inflammatory leukocytes around the portal triads with areas of piece-meal necrosis (red arrow). Central vein (V); Portal triad (P) (Figure 7).

Rats in this group had a histopathological score of two (2) characterized by peri-septal hepatitis and portal inflammation based on the histopathological scoring system as described in a previous study[36]. Sections of the liver presented in this group showed mild multifocal aggregations of mononuclear inflammatory leukocytes around the portal triads (arrow). Central vein (V); Portal triads (P) (Figure 8).

Rats in this group had a histopathological score of one (1) characterized by portal inflammation based on the histopathological scoring system described by[36].

Sections of the liver presented in this group showed mild centrilobular necrosis of the hepatocytes (black arrow) with mild portal-to-portal fibrosis (red arrow). Central vein (V); Portal triad (P) (Figure 9).

Rats in this group had a histopathological score of one (1) characterized by confluent necrosis based on the histopathological scoring system as described in a previous study[36].

At the conclusion of the experiment, the results of week 8 showed that the wide range of weekly swings of fasting blood glucose concentration had normalized and there was no difference in fasting blood glucose concentration in comparison to the basal control diet group, in each of the treatment groups. The lowest value of the fasting glucose concentration was seen at the end of week 3 correlating with the substantial weight loss observed across all the groups during the period of acclimatization.

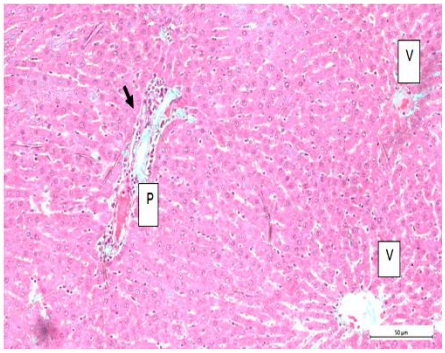

Figure 4: Photomicrograph of liver sections in animals fed basal diets only.

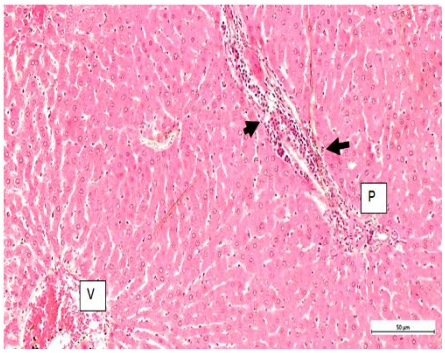

Figure 5: Photomicrograph of liver sections in animals fed high-fat diets only.

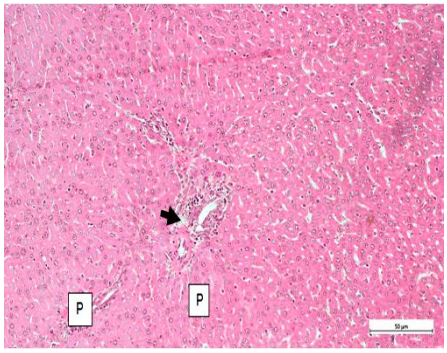

Figure 6: Photomicrograph of liver section of animals fed with Monosodium Glutamate only.

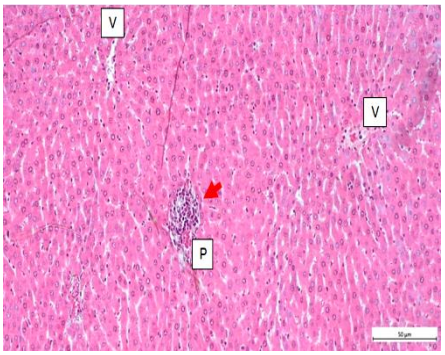

Figure 7: Photomicrograph of liver sections of rats fed MSG + HFD for 8 weeks and 5% *V. amygdalina* incorporated HFD for 4 weeks.

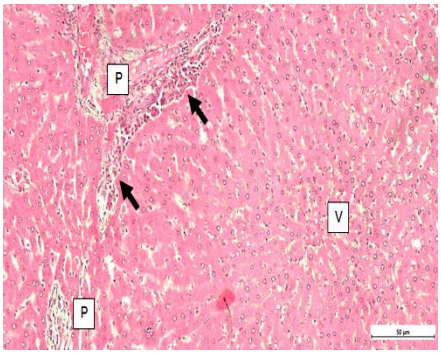

Figure 8: Photomicrograph of liver sections of rats fed MSG + HFD for 8 weeks and 10% *V. amygdalina* incorporated HFD for 4 weeks.

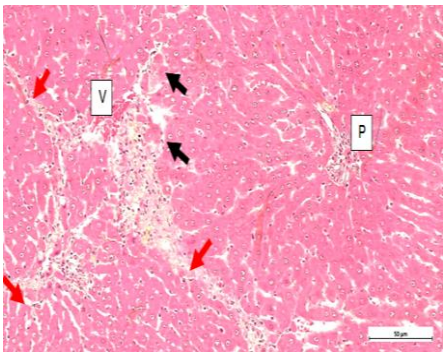

Figure 9: Photomicrograph of liver sections of rats fed MSG + HFD for 8 weeks and Orlistat 10 mg/kg plus HFD for 4 weeks.

The highest value of glucose concentration was recorded by MSG only group at the end of week 2 while the HFD only group peaked with fasting blood glucose level at the conclusion of the sixth week. Peak fasting glucose concentration at the conclusion of week twelve was recorded for the MSG-only group. Reports by various authors have pointed to the role of MSG in the induction of insulin resistance[37]. These findings confirm the reports.

The combination of MSG+HFD did not significantly alter fasting glucose concentration between week 0-week 8 which confirms the absence of any additive effect of chronic MSG+HFD administration on fasting blood glucose concentration. At the end of week 12, 10% *V. amygdalina* incorporated HFD was superior compared to the standard drug Orlistat 10 mg/kg in reducing the fasting blood glucose concentration.

The study's findings confirm the low-glycemic impact of dietary incorporation of *V. amygdalina* in managing insulin resistance. These findings are in line with reports from earlier works by Egedigwe *et al.*,[38] on the benefits of dietary incorporation of *V. amygdalina* in insulin resistance. Possible mechanisms of action of *V. amygdalina* in controlling fasting blood glucose levels include delaying intestinal absorption of dietary fats and carbohydrates via inhibition of digestive enzymes[38]. Taken together, the results suggest no additive effects or interaction of chronic high dose MSG +HFD in fasting blood glucose concentration and confirm the hypoglycaemic properties of diet incorporated *V. amygdalina* and its efficacy in managing insulin resistance which is a component of metabolic syndrome. Weight measurements in experimental animals are useful in obesity experimentation in determining food substances that have the

tendency of increasing or decreasing weights as they could find application in the management of obesity. Figure 2 shows the body weight gain of animals fed with a basal control diet and HFD for eight weeks. At the end of week two, there was a substantial decrease in terms of body mass which may be credited with the period of acclimatization and quality of protein source utilized in the feed formulation. Immediately after the feed protein was changed from crayfish dust (*Palaemon hastatus*) to Atlantic yellow croaker (*Micropogonias undulatus*), there was an upward trend in weight gain across all the groups.

Table 6: Histopathology score of the liver.

Group	Periportal or Peri septal Hepatitis (Piece-meal necrosis)	Confluent Necrosis	Focal lytic necrosis, apoptosis, and focal inflammation	Portal Inflammation
A	1	0	0	1
B	1	0	1	1
C	0	0	0	1
D	1	0	0	1
E	0	0	0	1
F	0	1	0	0

At the end of eight weeks, the MSG-only group recorded maximum weight increase in contrast to the basal (control) diet, HFD-only, and HFD +MSG diets. While rats fed with HFD recorded a substantial weight gain compared to the basal control diet.

Table 7: Liver histopathology score for each treatment group.

Group	Score (Out of 18)
A	2
B	3
C	1
D	2
E	1
F	1

The result of the MSG-only group could be attributed to the neurotoxic effect of chronic administration of high-dose MSG, various authors including He *et al*.,[39] reported that the intake of MSG was conclusively, among seemingly healthy chinesse people, longi-tiudinally linked to the development of overweight, possible mechanisms included modification of the hypothalamic signalling pathway involving leptin action. Egbuonu *et al*.,[40] also reported on the effect of low-dose MSG administration in adult male albino rats over 28 days and observed a significantly increased body weight.

The combination of MSG +HFD for eight weeks did not show any additive effect on weight gain in the experimental animals. For the first time, the study reveals that chronic co-administration of MSG + HFD did not significantly alter the weight in experimental animals in contrast to the MSG-only group and the HFD-only group. At the end of week 12, the orlistat group had the highest reduction in weight gain from week 9 to the end of week 12 followed by the 5% incorporated *V. amygdalina* HFD and the 10% incorporated *V. amygdalina* HFD while the group with the highest weight gain at the conclusion of week twelve was the MSG only group. The results show the efficacy of 5% and 10% incorporated *V. amygdalina* HFD in weight reduction similar to the activity of the standard drug, Orlistat 10 mg/kg. Possible mechanisms of action of *V. amygdalina* in weight reduction include delaying intestinal absorption of dietary fats and carbohydrates via inhibition of digestive enzymes[28].

Taken together, the results corroborate previous reports from studies done by Egedigwe *et al*.,[38] on the anti-obesity potential of diet-incorporated *V. amygdalina*. It also shows that the current obesity epidemic might be due to interactions of various dietary factors excluding the combination of MSG and HFD. The Lee's obesity index is an objective criterion used in confirming the attainment of obesity in experimental animals and is widely used in obesity studies. The results from the induction of obesity through the measurement of the adiposity index revealed at the end of week 8, none of the groups receiving HFD or MSG alone or MSG +HFD achieved Lee's obesity index. This may be attributed to the two weeks of acclimatization at the beginning of the study period where there was a negative weight gain. The highest adiposity index at the end of week 8 was seen in the HFD-only group showing a trend similar to earlier reports by Egedigwe *et al*.,[38] on HFD-induced obesity in experimental rats which could be attributed to the high-fat composition of the formulated diet. At the end of the study period, there was no increase in the adiposity index recorded for the 5%, 10%, and Orlistat 10 mg/kg group but they were all higher than all other treatment groups but not enough to confirm the presence of obesity. The 5%, 10% *V. amygdalina* incorporated HFD and Orlistat 10 mg/kg were able to prevent the attainment of Lee's obesity index at the end of the study period as there was no increase in the adiposity index between the ends of week 8 to the end of the study period. The results however could not be verified as there was also a reduction of the adiposity index for the HFD-only group between the end of week 8 and the end of the study period. When considered collectively, the findings indicate that the composition of the high-fat diet was sufficient to cause a considerable increase in the weights of the animals although the presence of obesity was not confirmed likely due to the loss of weight experienced by the animals at the beginning of the study period due to the quality of protein source in the formulated diet while the dietary incorporation of *V. amygdalina* was sufficient in preventing the attainment of obesity with results similar to those

obtained for the standard drug Orlistat 10 mg/kg. Serum protein and albumin concentrations are assayed to detect chronic long-term inflammation of the liver. Levels of albumin and globulin are determined by the total protein test with high levels indicating the presence of inflammation or infection and low levels showing secretory problems of the liver[41]. From the results from Table 5, there was no difference between the serum total protein of the basal control diet group compared to all the other treatment groups. The highest total protein concentration value was seen in the 5% *V. amygdalina* incorporated HFD group. There was evidence of an additive effect of MSG +HFD for eight weeks on total protein as the value of the 5% *V. amygdalina* incorporated HFD was higher than what was obtained for the MSG-only group and the HFD-only group.

The 10% *V. amygdalina* incorporated HFD was effective in mitigating the effect of chronic MSG intoxication as it produced a reduction in albumin concentration restoring it to similar levels obtained in the basal control diet group. Orlistat 10 mg/kg however produced a superior reduction in albumin levels. *V. amygdalina* was effective in ameliorating the effect of chronic MSG+HFD intoxication.

Table 8: Description of liver histopathology scoring parameters.

Periportal or Peri septal Hepatitis (Piece-meal necrosis)	Score	Confluent Necrosis	Score	Focal lytic necrosis, apoptosis, and focal inflammation	Score	Portal Inflammation	Score
Absent	0	Absent	0	Absent	0	None	0
Mild (Focal, Few portal areas)	1	Focal confluent necrosis	1	One focus or less per 10x objective	1	Mild, some or all portal areas	1
Mild/moderate (Focal, Most portal areas)	2	Zone 3 necrosis in some areas	2	Two to four foci per 10x objective	2	Moderate, some or all portal areas	2
Moderate (Continuous, around <50% of tracts or septa)	3	Zone 3 necrosis in most areas	3	Five to ten foci per 10x objective	3	Moderate/marked, all portal areas	3
Severe (continuous, >50% of tracts or septa	4	Zone 3 necrosis + occasional portal-central (P-C) bridging	4	More than ten foci per 10x objective	4	Marked, all portal areas	4
		Zone 3 necrosis + multiple P-C bridging	5				
		Peri-acinar or multiacinar necrosis	6				
Total Hepatitis Activity Index score (HAI) = 18.							

Albumin concentrations were not affected by HFD in comparison to the basal control diet group, and albumin values obtained in the 5%, 10% *V. amygdalina* incorporated HFD groups and the Orlistat treatment groups showed no additive effect of chronic MSG + HFD administration for eight weeks. The identification of structural changes in sensitive organs after exposure to various chemical substances is achieved through histological studies. Microscopic analysis of the liver sections of experimental rats fed basal control diet showed mild periportal septal hepatitis with mild portal inflammation with a histopathological score of two (2) based on Ishak *et al*.,[36] scoring system. The high histopathological score could be related to age-related decline in the liver's endogenous antioxidant system after twelve weeks, Tung *et al*.,[42] reported an increased liver oxidative injury, particularly to the glutathione-dependent system associated with aging.

Rats fed on the HFD only showed the presence of periportal septal hepatitis, the presence of focal lytic necrosis, apoptosis, and focal inflammation, and a mild portal inflammation with the highest histopathological score of 3 of all the treatment groups. This high score may be attributed to the composition of the diet with the high fat content being able to affect the liver negatively[42]. Various studies have reported the tendency of HFD to induce fatty liver disease[43] and these results confirm such reports.

Rats treated with MSG only had a low histopathological score of 1 with the presence of portal inflammation; these results suggest the relative safety of high-dose chronic administration of MSG or the ability of the liver's endogenous antioxidants to detoxify the MSG toxicant. There was no additive or increased toxicity on the liver cells by the combination of MSG + HFD for eight weeks as seen with the histopathological scores as the results obtained after the study period for all treatment groups with 5% and 10% *V. amygdalina* incorporated HFD and Orlistat 10 mg/kg group being lower than the histopathological score of HFD only group upon the conclusion of the study term. The 10% *V. amygdalina* incorporated HFD group provided superior protection to the liver compared to the 5% *V. amygdalina* incorporated HFD group after MSG + HFD for eight weeks with a histopathological score of 1:2 but was similar to the standard drug with a histopathological score of 1.

Taken together, the results show the benefits of short-term dietary incorporation of *V. amygdalina* in reversing fatty liver disease. Possible mechanisms include upregulation of the liver's endogenous antioxidant systems and could be advantageous in the treatment of fatty liver disease in humans.

Limitations of study

The limitation of this study is the lack of data on various liver function tests. In future publications, appropriate data will be provided to further support the hepatoprotective claims of *V. amygdalina* in High fat diet and monosodium glutamate co-intoxicated male wistar rats.

CONCLUSIONS

Dietary incorporated *V. amygdalina* in HFD was effective in the management of hyperglycemia after co-intoxication of experimental animals with MSG + HFD for eight weeks, with the 10% incorporated *V. amygdalina* HFD surpassing the standard drug Orlistat 10 mg/kg in hypoglycemic effect. It also significantly protected the liver from MSG + HFD-induced toxicity while efficiently reversing weight gain following HFD and MSG intoxication. There was no significant additive toxic effect from MSG and HFD co-intoxication. Dietary *V. amygdalina* may be effective for the alternate management of insulin resistance, obesity, and fatty liver disease in humans due to its availability, safety, and efficacy.

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AUTHOR'S CONTRIBUTIONS

Ubah EE: experiment work, writing original draft. **Ijeh II:** designed the study, supervised the experiment, and read the literature review. **Egbuonu ACC:** assisted with the study design, literature review survey. All authors revised the article and approved the final version.

DATA AVAILABILITY

Data will be made available to anyone on request with the corresponding author.

CONFLICT OF INTEREST

None to declare.

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