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Effect of Sub-chronic Dietary *Vernonia amygdalina* in Monosodium Glutamate and High-fat Diet Fed Male Wistar Rats on Antioxidant Status and other Biochemical Parameters

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ABSTRACT

Monosodium glutamate (MSG) has been shown to have a flavour-enhancing effect in foods, however, its safety and systemic side effects have not been fully clarified. *Vernonia amygdalina* (VA) is a medicinal plant used in traditional medicines. This study evaluated the effects of MSG intoxication on antioxidant status, renal histopathology, and other biochemical markers in Wistar rats given a high-fat diet (HFD), as well as the effects of VA. Male Wistar rats (36) were randomly allocated into six groups of six rats each, with average weights ranging from 150 to 220 grams.5%, 10% dietary incorporated VA, and Orlistat 10 mg/kg were given to the animals for 4 weeks after treatment with MSG 8000 mg/kg orally for the first 8 weeks while on HFD. The control group and HFD group received basal (control) diet and HFD for 12 weeks respectively while the MSG group received MSG 8000 mg/kg for 8 weeks and basal (control) diets for 12 weeks. MSG increased urea and creatine levels while a 10% VA diet reduced it significantly, MSG also reduced liver catalase (CAT) enzyme while Orlistat 10 mg/kg was > than 5% and 10% VA in restoring it. HFD increased liver Malondialdehyde (MDA) while 10% VA diet was > than 5% VA and Orlistat 10 mg/kg in antioxidant effect. MSG increased brain superoxide (SOD) similar to a 10% VA diet. Nephrotoxicity was prominent in the MSG group while dietary incorporation of VA provided some benefit.VA ameliorated the deleterious effects observed with MSG +HFD co-intoxication and could provide benefits in MSG and HFD-induced antioxidant depletion.

Keywords: *Vernonia amygdalina*, High fat diet, Monosodium glutamate, Toxicity, Antioxidants.

Introduction

Due to more African communities embracing the Western diet, dietary imbalance in Nigeria and throughout Africa has increased.[1]The urgency of the issue and the requirement for health authorities to take action have been highlighted by more recent reports on the emergence of a dietary transition in Sub-Saharan Africa from diets characterized by the risk of hunger to those marked by the risk of diet-related non-communicable diseases (DR-NCD), such as diabetes and obesity.[2]

The safety and potential systemic adverse effects of monosodium glutamate (MSG), a popular dietary additive, are currently unknown.[3] Glutamic acid, a non-essential amino acid found in nature and many foods, contains a sodium salt known as MSG, which has been shown to improve flavor.

El Tabbal, [4] investigated the effects of MSG on type 2 diabetes to shed light on the contradicting findings regarding MSG and type 2 diabetes. He discovered a negative pattern of effects for MSG at oral dosages greater than or equivalent to 2000 mg/kg of body weight, as well as lower doses by gavage or injection, but smaller oral doses did not provide conclusive evidence.

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Egbuonu *et al*.,[5] reported on the role of MSG in inducing oxidative stress in the heart of experimental animals leading to heart dysfunctions and inflammations after oral administration of MSG (8000 mg/kg) for 14 days while the calcium channel blocker amlodipine dose-dependently reversed the MSG induced oxidative damage.

One of the most distinguishing features of a Western diet is its high fat content. A high-fat diet is one in which fats comprise at least 35% of the total calories consumed.[6] The impact of fat consumption on the gut microbial community has been determined. HFD in the stomach alters the microbiota makeup, which can contribute to chronic inflammation and metabolic problems. Short-term investigations have indicated that HFD-induced dysbiosis occurs before the onset of obesity.[7] The total and widespread adoption of Western cuisine in Africa has resulted in a recent increase in the incidence of Western lifestyle disorders such as cancer, cardiovascular, and neurological diseases.

Vernonia amygdalina Del. (Asteraceae), also known as bitter leaf in Africa and Asia, is a medicinal plant well known for its use in various traditional medicines in various diseases including wound healing, malaria, hypertension, and diabetes mellitus.[8,9] Other authors have reported hypolipidemic activities of the leaves of the plant in various high-fat diet (HFD) obese animal models.

Djeujo *et al*.,[10] investigated the efficacy of aqueous decoctions of *Vernonia amygdalina* leaves and roots in treating diabetes. The scientists concluded that *Vernonia amygdalina* roots contained more vernodalol than leaves, while leaves contained more luteolin. The root extracts were shown to have vernodalol-dependent anti-proliferative activity, whilst the leaf extracts had luteolin-dependent antioxidant and anti-diabetic properties.

This study will aim to elucidate the possible mechanisms of protection of dietary *Vernonia amygdalina* in MSG intoxicated HFD fed male wistar rats. A double assault from HFD and MSG intoxication could equally be responsible for the current upsurge in various diseases linked to chronic inflammation and endogenous antioxidant depletion like

cancer, cardiovascular, and neurodegenerative diseases in Nigeria but few studies have considered this problem to the best of our knowledge. Hajhasani *et al*., [11] suggested the use of various natural products as safeguards against MSG-induced toxicity with no information on the benefits of *Vernonia amygdalina* leaves.

Materials and Methods

Collection of Plant Materials

Fresh *Vernonia 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.

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 Tables 1 and 2, respectively. All feeding supplies were obtained locally from Jocan Agro Feeds Ltd, Umuahia, Abia State, Nigeria.

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 5ml syringe as described by Ijeh *et al.*,[12]. After pelleting, it was dried in an oven at a temperature of 35^0C. 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 % *Vernonia 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 were fed *ad libitum w*ith 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 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 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% *Vernonia amygdalina* incorporated HFD respectively as required, the animals were sacrificed with anesthesia, Blood samples were collected by retro-orbital bleeding from the eyes.

Calculation of relative organ weights

The kidney 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.

Relative organ weights were calculated thus:

w/W were:

w = the weight of the organ

W =The final body weight of the animal before it was sacrificed.[13,14]

Determination of anti-nutrients

Hydrocyanide content, Tannin content, Alkaloids, and Total Phenol content were determined according to standard procedures described by Sofowora[15] and Evans.[16]

Determination of Antioxidant parameters

Catalase activity as described by Goth,[17] Lipid Peroxidation as described by Draper and Hadley,[18]

Reduced glutathione (GSH) as described by Ellman,[19] as recently described by Alam *et al.*,[20] Superoxide dismutase as described by Sun and Zigman.[21]

Determination of Urea and Creatinine: Tiez *et al*., [22] as described in Randox commercial kit.

Table 1: High-Fat Diet Composition

Ingredients	(g/1000kg)	(g/100g)
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 2: Basal (Control) Diet Composition

Ingredients	(g/1000kg)	(g/100g)
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	1000.00	100.00

Histological studies of the kidney

Tissue preparation and staining

Sections of the kidney from each group were collected in a sterile universal container containing 10% formal saline solution based on the method described by Batra. [23]

Statistical analyses

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 relative organ weight measurement is a useful parameter in assessing the toxicity profile of various chemicals in biological systems. The results of the relative organ weights are shown in Table 7. The heart was altered by MSG and MSG+HFD co-administration while the Orlistat 10 mg/kg was able to ameliorate the toxic effect on the heart, The VA supplementation failed to reverse the observed toxicity in the heart tissue. A similar trend of results was also observed in the lungs. This could be explained by oxidative stress, a sub-chronic course of Orlistat 10 mg/kg, the production of many pro-inflammatory cytokines, and the activation of caspase pro-apoptotic proteins.[24]

The relative organ weight of the kidney was unaffected by chronic MSG only, HFD only and MSG +HFD diet administration alluding to their safety in these organs after long-term exposure.

The anti-nutrient compositions of various prepared diets are significant in determining the presence of pharmacologically active components that could either positively or negatively impact the nutritional value of the diet.

The results of the anti-nutrient content of the various formulated diets are shown in Table 5. It revealed that the tannin content of the HFD-only group was significantly higher than the basal (control) diet while 10% and 5% *Vernonia amygdalina* incorporated HFDs significantly reduced the amount of tannin present in the HFDs. The alkaloid content was highest in the 5% incorporated *Vernonia amygdalina* HFD compared to all the other formulated diets. Dietary incorporation of *Vernonia amygdalina* could provide a readily available source of alkaloids to man. Alkaloids have been reported to possess potent anti-inflammatory, anticancer, antimicrobial, and analgesic properties.[25]

Total phenol content was highest in the 10% *Vernonia amygdalina* incorporated HFD while the lowest amount was seen in the HFD group only. Incorporation of *Vernonia amygdalina* in HFD increased the total phenol content for both the 5% and 10% *Vernonia amygdalina* incorporated HFD. Dietary incorporation of phytochemicals is now an area of active research interest. Functional foods which can be natural or processed foodstuffs for basic consumption in the diet provide an essential nutritional level and also share potentially positive effects on the host's health by optimizing the immune system and reducing the incidence of diseases. [26] Gasparre and Rossel, [27] reported on increased efforts by the food industry to incorporate nutritional enrichment strategies with plant-based ingredients (phytochemicals) into foods such as bakery products, pasta-like products, snacks, and beverages to increase the public's vegetable intake, thereby increasing the availability of their bioactive components, which play critical roles in biological systems.

Cyanide content was higher in the basal (control) diet compared to the HFD groups. This may be from the composition of the formulation with the high amount of corn starch. Dietary incorporation of 5% and 10% *Vernonia amygdalina* into HFD increased the amount of cyanide when compared to the HFD-only formulation. Food items containing cyanogenic glucosides might cause unfavorable health consequences, such as nausea, vomiting, diarrhea, dizziness, and weakness when high quantities of hydrogen cyanide are subsequently consumed. [28] According to Nzwalo and Cliff, [29] neurological disorders such as tropical ataxic neuropathy and konzo can result from prolonged exposure to elevated hydrogen cyanide concentrations.

The urea level (Table 6) of the MSG-only group was significantly higher than the HFD-only group and the basal (control) group. High serum urea is indicative of a kidney pathology.[30]The 10% incorporation of *Vernonia amygdalina* in HFD significantly reduced the high urea content in the MSG+HFD treatment group and its effect was higher than the standard drug,10 mg/kg Orlistat group. The observed elevated urea recorded in the MSG-only group corresponds with previous reports by Onwubiko *et al.*,[31] on the nephrotoxicity of chronic administration of high-dose MSG in rats while there was no additive effect of chronic MSG+HFD on nephrotoxicity as seen in urea concentration values in the 5% and 10% incorporated *Vernonia amygdalina* HFD and 10 mg/kg Orlistat groups that were less than the MSG only group. The results confirm the nephrotoxic risk of chronic consumption of MSG on kidney function.

Health authorities should carefully consider the risk of consumption of MSG chronically and the possible benefits of dietary incorporation of *Vernonia amygdalina* in mitigating the effects on the kidneys.

Creatinine levels (Table 6) were also significantly higher in the MSG-only group. High creatine concentrations are seen in various conditions affecting the kidneys including acute kidney injury.[32]

Table 3: 5% *Vernonia amygdalina* Incorporated High Fat Diet Composition

Ingredients	(g/1000kg)	(g/100g)
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 4: 10 % *Vernonia amygdalina* Incorporated High Fat Diet Composition

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

From the results obtained, the effect of the 10 % incorporated *Vernonia amygdalina* HFD group was comparable in efficacy to the standard drug Orlistat 10mg/kg in significantly reducing the creatinine concentration in treated rats.

Creatinine levels were considerably higher in the MSG-only group compared to the Basal (control). The 10% *Vernonia amygdalina* HFD produced a substantial reduction in creatinine levels compared to the MSG-only group.

These results reveal the absence of any additive effect in the effect of MSG + HFD on nephrotoxicity as creatinine levels for 5% and 10% incorporated *Vernonia amygdalina* HFD and 10mg/kg Orlistat

treatment after MSG+HFD eight weeks induction were lower than the creatine levels of MSG only group.

Taken together, these results reveal a clear trend of nephrotoxicity attributable to the chronic high-dose administration of MSG on the kidneys and the possible health challenges when chronically administered to humans while dietary incorporation of *Vernonia amygdalina* was effective in mitigating the deleterious effects of the toxicants. Possible mechanisms employed include the upregulation of endogenous antioxidants by phytochemicals present in the leaves of *Vernonia amygdalina*. Future studies would be needed to isolate the fractions of the plant responsible for kidney protection which could lead to the development of newer drug candidates in the management of kidney problems.

A significantly reduced amount of the catalase enzyme (Figure 1) in the MSG-only group when compared to the basal (control) diet group at the end of week 12 was observed. The result is in line with earlier results by Hamza and Diab., [33] on the reduction of catalase after chronic high-dose MSG exposure. The possible mechanism involved includes the depletion of antioxidant enzymes due to the overproduction of reactive oxygen and nitrogen species.

A high-fat diet only also produced a reduction in catalase activity at the end of week 12 but the result was not significant when compared to the basal (control) diet group. From the results, there was no additive effect on catalase enzyme activity after long-term MSG+HFD administration as seen with 5%,10% *Vernonia amygdalina* incorporated HFD and Orlistat 10 mg/kg treatment groups with catalase activity higher than the MSG-only group and the HFD only group value for the same enzyme.

Dietary incorporation of *Vernonia amygdalina* increased the catalase concentration at the end of week 12 but the effect was not significant when compared to the basal (control) group. The Orlistat 10 mg/kg group was superior to 5%,10% *Vernonia amygdalina* incorporated HFD in restoring catalase activity at the end of week 12 as it restored catalase concentration to a level that was not significantly different when compared to the basal (control) group and the HFD only group.

The results for the HFD-only group on catalase activity show a non-significant reduction in concentration at the end of 12 weeks.

While there was no additive effect of chronic MSG+HFD administration on catalase activity, long-term exposure to either MSG or HFD could deplete endogenous catalase concentration of the liver while dietary incorporation of *Vernonia amygdalina* could help preserve the catalase concentration.

Various reports have noted the effect of phytochemicals in preserving endogenous antioxidants. Akpoveso *et al.*,[34] reviewed the role of antioxidant phytochemicals as a potential therapy in various diabetic complications, concluding that amongst other mechanism, they acted by upregulating endogenous antioxidants. The results of this study also revealed for the first time the effect of chronic MSG + HFD administration on brain catalase activity in Wistar rats. At the end of the study period, there was no significant difference in the catalase concentration of all treatment groups when compared to the basal (control) diet group. The highest catalase concentration was observed in the 10% *Vernonia amygdalina* incorporated HFD group which was higher than the basal (control) group while the HFD-only group had the lowest catalase concentration. One explanation for the observation might be the presence of the Blood Brain Barrier (BBB) preventing the exposure of neurons to the various toxicants administered chronically while *Vernonia amygdalina* incorporated HFD could be a potent enzyme inductor leading to de-novo synthesis of the catalase enzyme. This trend is also seen in the 5% *Vernonia amygdalina* incorporated HFD group both of which were non-significantly higher than the Orlistat 10 mg/kg treatment group.

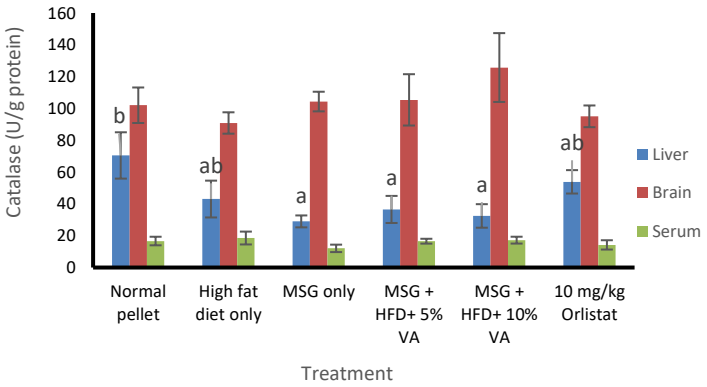


Figure 1: The effects of HFD, MSG, HFD+MSG and *Vernonia amygdalina* incorporated HFD on Catalase antioxidant of the Liver, Brain and Serum.
Values are mean±SD, n = 6. The different superscripts ([abc]) are statistically significant ($p<0.05$)

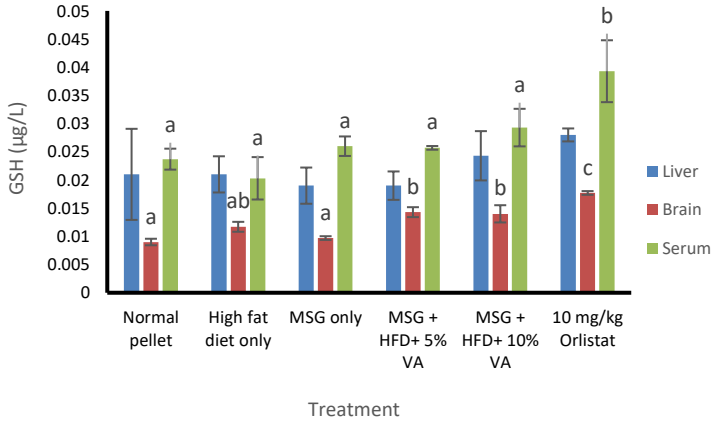


Figure 2: The effects of HFD, MSG, HFD+MSG and *Vernonia amygdalina* incorporated HFD on Glutathione antioxidant of the Liver, Brain and Serum.
Values are mean±SD, n = 6. The different superscripts ([abc]) are statistically significant ($p<0.05$)

Table 5: The Antinutrient Composition Of The Various Formulated Feeds

Parameter	HFD	NP	10% VA	5% VA
Tannins (%)	4.07 ± 0.00[d]	1.29 ± 0.03[b]	2.32 ± 0.03[b]	2.69 ± 0.05[a]
Alkaloid (%)	2.04 ± 0.04[b]	0.62 ± 0.01[a]	1.88 ± 0.03[a]	2.69 ± 0.05[a]
Total phenol (mg/100g)	3.01 ± 0.01[c]	6.62 ± 0.01[d]	10.94 ± 0.02[d]	5.80 ± 0.01[c]
Cyanide (mg/100g)	0.87 ± 0.00[a]	3.98 ± 0.00[c]	4.12 ± 0.00[c]	3.30 ± 0.00[b]

Values are Mean ± Standard Deviation of five determinations .The different superscripts ([abc]) are significant ($p<0.05$) across the row (horizontally)

Taken together, the results suggest a minimal impact of chronic MSG+HFD administration on brain and serum catalase activity while the liver concentration was affected. One explanation for the observed result could be that since the liver is the site of bio-transformation and detoxification of various chemicals, it makes first contact with the toxicants and tries to neutralize them via endogenous catalase activity leading to the more pronounced depletion of the liver catalase compared to that of the brain and the serum while dietary incorporation of *Vernonia amygdalina* was able to restore catalase concentration in the

liver. A more pronounced effect was observed in the 5%,10% *Vernonia amygdalina* incorporated HFD group with catalase concentration higher than the basal (control) diet group. Further studies will be needed to assess if dietary incorporation of *Vernonia amygdalina* could be beneficial in inducing the production of brain catalase enzyme as this could find application in the management of various neurodegenerative diseases like Alzheimer's where endogenous antioxidant depletion is implicated.

The results from the reduced glutathione assay (Figure 2) reveal the activity of *Vernonia amygdalina* in preserving endogenous GSH activity necessary for the prevention of oxidative stress and could be beneficial in the management of the oxidative stress component of metabolic syndrome. The liver concentrations of MDA (Figure 3) were lowest for the MSG-only group which was significant when compared to the HFD-only group and the basal (control) diet.

The HFD only group had a significantly higher MDA value compared to all the other treatment groups. This result is in line with previous reports by Othman *et al*.,[35] on the effect of chronic HFD administration on MDA.

The low value of MDA in the MSG only group could be due to adaptive mechanisms including enzyme induction from MSG-induced oxidative stress thereby reducing lipid oxidation through endogenous antioxidant upregulation. Similar reports have been published by Abolaji *et al*., [36] There was no additive effect of long-term MSG + HFD on lipid oxidation as a result of antioxidant depletion as 5%,10% *Vernonia amygdalina* incorporated HFD group and Orlistat 10 mg/kg group had MDA concentration that was lower than the HFD only group after 12 weeks with 5 % *Vernonia amygdalina* incorporated HFD group providing a better protection compared to 10% *Vernonia amygdalina incorporated* HFD group and Orlistat 10mg/kg all of which was better than the basal (control) diet group.

An uncharacteristic high level of SOD (Figure 4) was seen for the MSG-only group similar to the findings made for MDA probably due to an adaptive response.[37] Further studies will be needed to clarify the mechanism involved. The HFD-only group produced a reduction in SOD concentration when compared to the basal (control) group, but the level was not significant. The 5 % and 10% *Vernonia amygdalina* incorporated HFD group also showed SOD concentration that was significantly higher when compared to the Orlistat 10mg/kg group which was also higher than the basal (control) group although not significant. A possible explanation for the increased amount of SOD in the brain could be via enzyme induction by *Vernonia amygdalina* while other mechanisms still need to be investigated to understand the high levels of the enzyme observed. In a nutshell, the antioxidant assays reveal the benefit of dietary incorporation of *Vernonia amygdalina* against exogenous toxicants and age-related antioxidant depletion.

We also report for the first time the effect of MSG+HFD on the brain's endogenous antioxidants. No additive effects were observed with the chronic usage of MSG +HFD on the brain CAT and reduced glutathione while an uncharacteristic effect on brain SOD and MDA levels in the MSG-only group was observed. Further studies are recommended to better understand this finding.

The histopathological study of the kidney reveals the harmful effects of long-term administration of high-dose MSG in experimental rats and the benefits of short-term *Vernonia amygdalina* incorporated diets in protecting the kidney. MSG only group (Plate 3) at the end of the study showed a high histopathological score of 5 (Table 8) characterized by infiltration of renal interstitial with inflammatory mononuclear leukocytes, dilation of renal tubules, presence of eosinophilic tubular casts in the lumen of some renal tubules. The high histopathological score for MSG only group correlates with the high values of urea and creatinine obtained in the kidney function assay and confirms the deleterious impact of high dose chronic consumption of MSG on kidney function while dietary incorporation of *Vernonia amygdalina* provided some benefit in protecting the exposed kidneys.

Table 6: The effects of HFD, MSG, HFD+MSG and *Vernonia amygdalina* incorporated HFD on Serum Kidney Function Marker

Group	Urea (mg/dL)	Creatinine (mg/dL)
Normal pellet	38.00 ± 1.00^{ab}	0.42 ± 0.05^{ab}
High fat diet only	33.20 ± 1.36^{a}	0.30 ± 0.04^{a}
MSG only	52.75 ± 2.50^{c}	0.58 ± 0.05^{c}
MSG + HFD+ 5% VA	45.00 ± 1.90^{bc}	0.46 ± 0.05^{bc}
MSG + HFD+ 10% VA	40.20 ± 2.48^{ab}	0.40 ± 0.03^{ab}
10 mg/kg Orlistat	52.00 ± 6.44^{c}	0.36 ± 0.04^{ab}

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

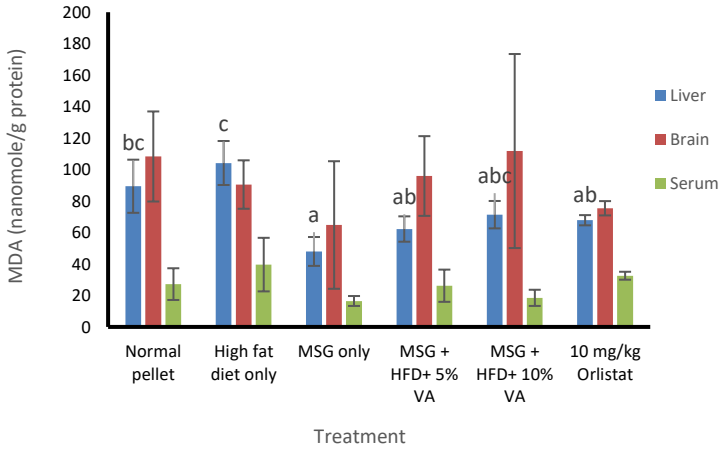


Figure 3: The effects of HFD, MSG, HFD+MSG and *Vernonia amygdalina* incorporated HFD on Malondialdehyde (MDA) of the Liver, Brain and Serum

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

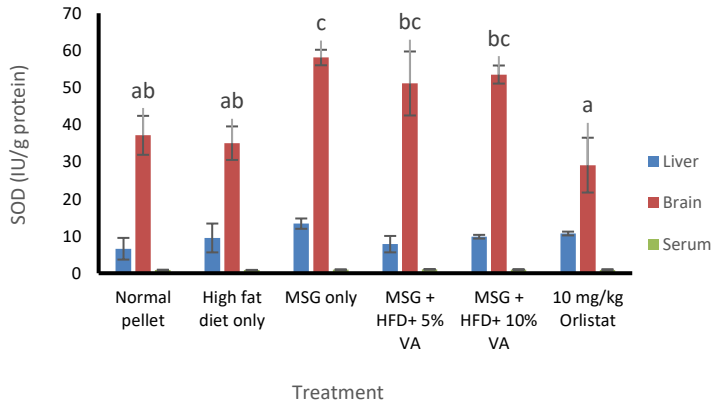


Figure 4: The effects of HFD, MSG, HFD+MSG and *Vernonia amygdalina* incorporated HFD on Superoxide dismutase (SOD) of the Liver, Brain and Serum.

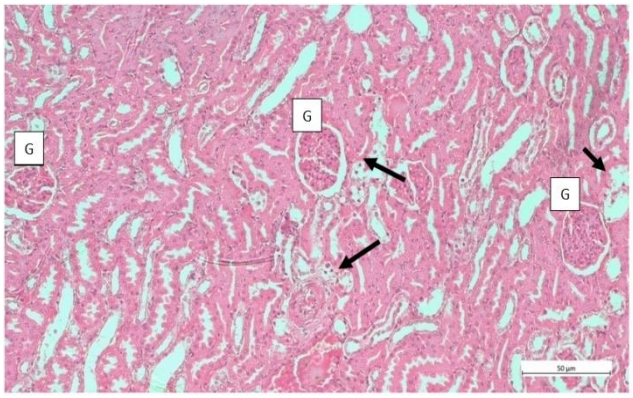
Values are mean±SD, n = 6.The different superscripts (^{abc}) are statistically significant ($p<0.05$)

Table 7: The effects of HFD, MSG, HFD+MSG and *Vernonia amygdalina* incorporated HFD on Relative Organ weights

Group	Liver (%)	Kidney (%)	Spleen (%)	Heart (%)	Lungs (%)	Testicle (%)

Normal pellet	3.20 ± 0.18^{ab}	0.68 ± 0.05^{a}	0.21 ± 0.01^{ab}	0.38 ± 0.03^{b}	0.88 ± 0.13^{b}	1.20 ± 0.13^{abc}
High fat diet only	3.09 ± 0.17^{ab}	0.62 ± 0.01^{a}	0.21 ± 0.03^{ab}	0.33 ± 0.02^{ab}	0.59 ± 0.07^{a}	0.99 ± 0.10^{a}
MSG only	2.86 ± 0.05^{a}	0.62 ± 0.02^{a}	0.17 ± 0.01^{a}	0.30 ± 0.01^{a}	0.58 ± 0.04^{a}	1.09 ± 0.08^{abc}
MSG + HFD+ 5% VA	3.24 ± 0.17^{ab}	0.66 ± 0.03^{a}	0.16 ± 0.01^{a}	0.30 ± 0.02^{a}	0.62 ± 0.09^{a}	1.36 ± 0.07^{c}
MSG + HFD+ 10% VA	3.40 ± 0.10^{b}	0.59 ± 0.02^{a}	0.20 ± 0.01^{ab}	0.30 ± 0.01^{a}	0.55 ± 0.02^{a}	1.33 ± 0.08^{bc}
10 mg/kg Orlistat	3.47 ± 0.15^{b}	0.90 ± 0.16^{b}	0.24 ± 0.02^{b}	0.33 ± 0.01^{ab}	1.01 ± 0.06^{b}	1.06 ± 0.09^{ab}

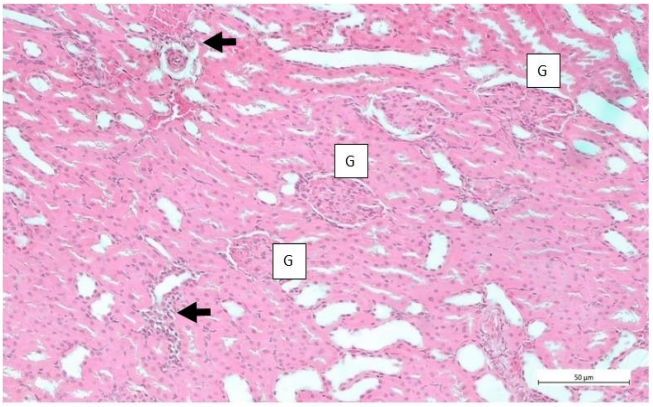
Values are mean±SD, n = 6. The different superscripts (^{abc}) are significant ($p<0.05$) across the Column (vertically)



H & E X200

Plate 1: Photomicrograph of kidney sections in animals fed basal diets only

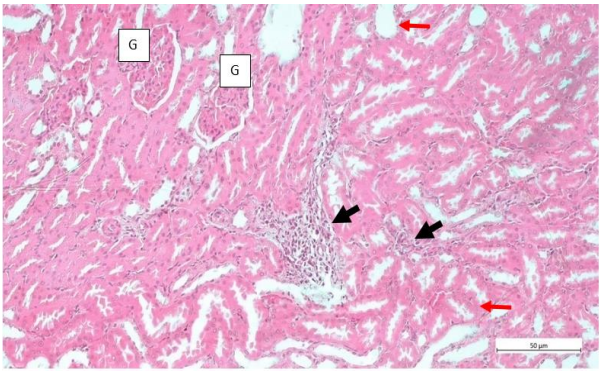
Section of the kidney presented in this group showed mild multifocal widespread vacuolar degeneration of epithelial lining of the renal tubules in the cortex (arrow). Glomeruli (G) with a histopathological score of one characterized by loss of brush border in less than 25% of tubular cells, integrity of basal membrane was intact.



H & E X200

Plate 2: Photomicrograph of kidney sections in animals fed high fat diets only

Section of the kidney presented in this group showed multifocal areas of infiltration of the renal interstitial with inflammatory mononuclear leukocytes (arrow). Some of the Glomeruli (G) in the cortex showed sclerosis of the tuft with a histopathological score of two characterized by thickening of bowman's capsule and tubulo-interstitial inflammation and haemorrhage in less than 25% of tissue.



H & E X200

Plate 3: Photomicrograph of kidney sections in animals fed MSG only

Section of the kidney presented in this group showed focal areas of infiltration of the renal interstitial with inflammatory mononuclear leukocytes (black arrow), dilation of renal tubules (red arrow) in the outer medulla as well as the presence of eosinophilic tubular casts in the lumen of some of the renal tubules. Some of the Glomeruli (G) in the cortex showed sclerosis of the tufts with a histopathological score of five characterized by loss of brush border in more than 25% of tubular cells, thickened basal membrane, retraction of glomerular tuft/sclerosis with tubulo-interstitial inflammation and haemorrhage in less than 25% of tissue.

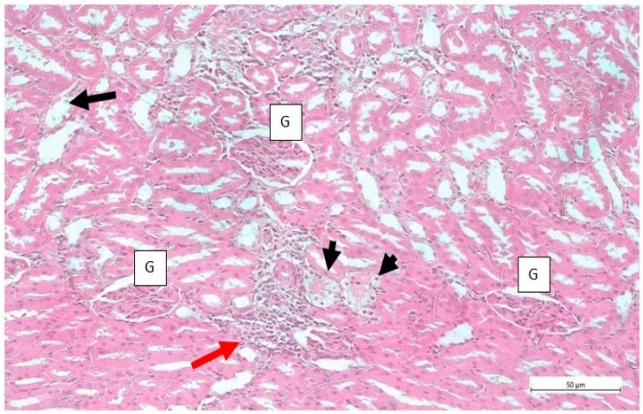
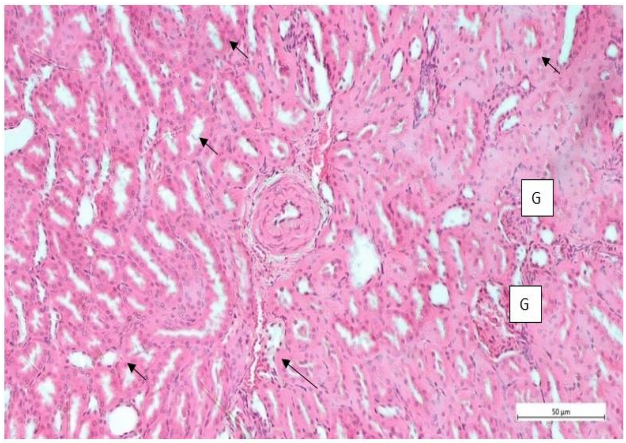


Plate 4: Photomicrograph of kidney sections in animals fed HFD +MSG and 5% *Vernonia amygdalina*

Section of the kidney presented in this group showed a moderate multifocal vacuolar degeneration of the epithelial lining of the renal tubules (black arrow), deposition of eosinophilic casts in the renal tubular lumen, and mild multifocal areas of infiltration of the renal interstitial by inflammatory mononuclear leukocytes (red arrow) Glomeruli (G) . Histopathological score of two characterized by loss of brush border in less than 25% of tubular cells with the integrity of basal membrane intact and inflammation and haemorrhage in less than 25% of tissue.

Table 7: Histopathology Score of the Kidney

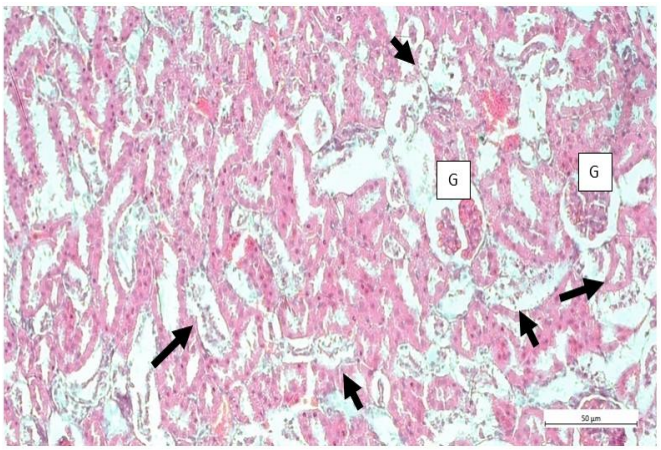
Group	Tubular Lesions	Endothelial Lesions	Glomerular Lesions	Tubular/Interstitial Lesions
A	1	0	0	0
B	0	0	1	1
C	2	0	2	1
D	1	0	0	1
E	2	0	1	3
F	0	0	0	0



H & E X200

Plate 5: Photomicrograph of kidney sections in animals fed HFD +MSG and 10% *Vernonia amygdalina*

Section of the kidney presented with severe multifocal vacuolar degeneration of the epithelial lining of the renal tubules (arrow) with mild multifocal areas of infiltration of the renal interstitial by inflammatory mononuclear leukocytes. Some the Glomeruli (G) in the cortex showed sclerosis of the tufts with a histopathological score of six showing Loss of brush border in more than 25% of tubular cells, thickened basal membrane, thickening of bowmans capsule and up to 60% tubular- interstitial necrosis.



H & E X200

Plate 6: Photomicrograph of kidney sections in animals fed HFD +MSG and Orlistat 10mg/kg

Section of the kidney presented in this group showed the normal renal histo-architecture. Normal Glomeruli (G), in their respective Bowman's capsules were observed, surrounded by a sea of normal renal tubules (arrow) with a histopathological score of zero.

Table 8: Kidney Histopathology Score for Each Treatment Group

Group	Score
A	1
B	2
C	5
D	2
E	6
F	0

Kidney Scoring Technique: EGTI (Endothelial, Glomerula, Tubular, Insterstitial) histology scoring system

Conclusion

The antinutrient components of the formulated feeds (5% and 10% *Vernonia amygdalina* incorporated HFD) revealed the presence of saponins, alkaloids, and phenols which makes it possible for the leaves of the plant to be used in the design of functional foods with the availability of various phytochemicals. The serum kidney function markers revealed a strong affinity of MSG for kidney damage while short-term dietary incorporation of *Vernonia amygdalina* was useful in reversing the observed toxicities. The antioxidant profiles of the brain, liver, and serum after chronic MSG, HFD, and MSG and HFD concomitant administration revealed depletion of endogenous antioxidants while short-term dietary incorporation of *Vernonia amygdalina* in HFD restored the depleted endogenous antioxidants via various mechanisms including enzyme induction. These results could provide new information for the dietary incorporation of *Vernonia amygdalina* in the management of various neurodegenerative disorders with characteristic endogenous antioxidant depletion while there was no observed additive effect on toxicity from chronic high-dose MSG and HFD co-administration.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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Table 9: Description of Kidney Histopathology Scoring Parameters

Tissue type	Damage	Score
Tubular	No damage	0
	Loss of brush border in less than 25% of tubular cells, Integrity of basal membrane intact.	1
	Loss of brush border in more than 25% of tubular cells, thickened basal membrane.	2
	Additional Inflammation, cast formation, Necrosis in up to 60% of tubular cells	3
	Additional Necrosis in more than 60% of tubular cells	4
Endothelial	No damage	0
	Endothelial swelling	1
	Endothelial disruption	2
	Endothelial loss	3
Glomerular	No Damage	0
	Thickening of Bowmans capsule	1
	Retraction of Glomerular tuft/sclerosis	2
	Glomerular fibrosis	3
Tubulo-Interstitial	No damage	0
	Inflammation/Haemorrhage in less than 25% of tissue	1
	Additional necrosis in less than 25% of tissue	2
	Necrosis up to 60%	3
	Necrosis more than 60%	4

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