Toxicology Reports 12 (2024) 244–252

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Neuroprotective aftermath of *Monodora myristica* and *Glycyrrhiza glabra* against cassava diet containing vacuum gas oil induced brain injury in Wistar rats

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ARTICLE INFO

Handling Editor: Prof. L.H. Lash

Keywords:
Cassava diet
VGO
Anti-neurotoxic
Monodora myristica
Glycyrrhiza glabra

ABSTRACT

Vacuum gas oil (VGO) is a hydrocarbon combination formed during crude oil extraction, and its consumption may have neurological repercussions. This study investigated the neuroprotective properties of *Monodora myristica* and *Glycyrrhiza glabra* in rats given cassava flour diet containing vacuum gas oil (CFD-VGO). Thirty rats were separated into six groups and treated as follows: Group 1 severed as normal control. Group 2 were fed CFD-VGO only. After a normal diet was given to groups 3, 4, and 5, *M. myristica*, *G. glabra*, and *M. myristica* plus *G. glabra* extracts were administered. Group 6, 7, 8 and 9 were given CFD-VGO and then treated with *M. myristica* extract, *G. glabra* extract, *M. myristica* plus *G. glabra* extracts and 2-methyl cellulose respectively. The rats were euthanized using carbon dioxide after experimental period of 28 days. The brain was excised for biochemical assays. The results showed that the concentration of the assessed 16 PAHs in CFD-VGO using GC-MS was 53.38 ppm. Significant ($p < 0.05$) increase were observed in malondialdehyde (MDA), total cholesterol (T.Chl), triacylglycerol (TAG), low density lipoprotein-cholesterol (LDL-C), and decrease in high density lipoprotein-cholesterol (HDL-C), acetylcholinesterase (AChE) and ATPases in the brain rats fed with CFD-VGO. On the other hand, administration of *M. myristica* and *G. glabra* extract effectively restored altered antioxidants, ATPases, and lipids in brain of rats fed with cassava diet containing VGO.

1. Introduction

Vacuum gas oil (VGO) is a mixture of hydrocarbons created during the extraction of crude oil. Beyond the obvious global economic benefits of crude oil, a wealth of information indicates that it poses significant ecological risks [1]. Natural gas and oil reserves abound in Niger Delta region of Nigeria. This region not only produces the majority of Nigeria crude oil, but it also has the highest levels of pollution caused by crude oil. Cassava grown in this area is negatively impacted [2]. The livelihoods of the indigenous people are negatively affected because they are reliant on cassava meal for survival [2]. According to Adeyemi and Adeyemi [2], crude oil spills that are either unintentional or intentional, leaking storage tanks and pipelines, and land disposal of petroleum waste are the main causes of pollution from crude oil. The global VGO market is expected annual growth rate (AGR) is 5.8% in the forecast period of 2023–2028 [3]. The global VGO market is being driven by the rising demand for gasoline, which is primarily consumed as a fuel for transportation. There are about 1177 VGO buyers and importers in Nigeria [3]. The country failure to reach its full potential is a textbook illustration of what scholars refer to as the "resource curse" due to pollution disaster since corruption undermines the integrity of the people and government institutions managing the enormous income necessary to reduce pollution adversity [4].

According to Olasehinde and Olaniran [1], chronic or recurrent exposure to meals containing VGO products may result in harm to organs such as the liver, thymus and blood. A naturally occurring class of compounds found in VGO and gasoline are called polycyclic aromatic hydrocarbons (PAHs). Coal, oil, gas, wood, trash, and tobacco are the causes of them [4]. Airborne particulates can attach to or produce PAHs. When meat and other meals are cooked at high temperatures, PAHs are produced [1]. Studies have demonstrated that exposure to PAHs may result to neurotoxic effect such as memory loss, cognitive impairment,

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https://doi.org/10.1016/j.toxrep.2024.02.001
Received 25 December 2023; Received in revised form 31 January 2024; Accepted 4 February 2024
Available online 7 February 2024

and behavioral problems [1]. The cassava plant, *Manihot esculenta* Crantz, belongs to the *Euphorbiaceae* family and is one of Africa's three principal staple crops and the third-biggest basis of carbohydrates in the tropics for human consumption, behind maize and rice [5]. Nowadays, cassava flour—made from cassava roots—is utilized in a variety of industries and has the potential to displace wheat flour [5]. Injury to the brain resulting from exposure to hazardous substances, whether naturally occurring or man-made, is referred to as neurotoxicity [6]. Neurotoxicants can directly impact several aspects of neurotransmission, such as the enzyme(s) responsible for synthesising a neurotransmitter, the processes involved in its release and absorption, the enzyme(s) that metabolize it, the receptors, and the post-synaptic events linked to receptor activation [7]. Certain neurotoxicants can interact with ATPases, sodium channels, energy metabolism, or other biological processes to indirectly disrupt neurotransmission [7]. A component of cholinergic neurotransmission is the enzyme acetylcholinesterase. According to Bondžic et al. [8], the enzyme degrades acetylcholine, which stops neurotransmission. Low intracellular Na^+ concentration and a gradient of high K^+ are necessary for optimal neuronal processes. ATPases (Na^+/K^+-ATPase and Ca^{2+}/Mg^{2+}-ATPase) are recognized to play significant roles in neuronal transmission [8]. Na^+/K^+-ATPase is a membrane-bound enzyme involved in maintaining the Na^+ and K^+ gradient across the cell membrane. It helps to maintain normal neuronal activity and is extensively expressed in neurons. This action facilitates membrane potential regulation. Abnormalities in Na^+/K^+-ATPase have been connected to a number of neurological conditions, including Alzheimer's disease, spongiform encephalopathy, and bipolar disorder [7].

The forebrain is a part of the growing vertebrate brain that consists of the cerebral hemispheres' telencephalon and the thalamus, hypothalamus, epithalamus, and subthalamus that lie beneath it. The processing of information pertaining to intricate cognitive functions, emotion management, and behavior regulation is primarily controlled by the forebrain. The prefrontal cortex, hippocampus (forebrain), and cerebellum (hindbrain) are the primary brain regions involved in remembering. The prefrontal cortex, however, is also the part of the brain that is most vulnerable to the negative consequences of oxidative stress. According to studies, PAHs can cause behavioral issues, cognitive dysfunction, and memory loss [9,10]. The midbrain is the highest point on the brainstem, which serves as the main nerve connecting the brain to the spinal cord. The colliculi, tegmentum, and cerebral peduncles are the three primary components of the midbrain. One of the main groups of organic compounds in particulate matter (PM) is PAHs. Recent research has demonstrated a high correlation between PM2.5 and brain damage, primarily midbrain cerebrovascular injury (stroke) and neurological damage [11]. Furthermore, PM2.5 may harm mitochondria of brain cells thereby producing ROS in brain cells [11].

The cerebellum, pons, and medulla, which make up the hindbrain, are crucial for enhancing movement and transmitting information to the spinal cord. Kim et al. [12] reveal that stress generated by PAHs has been linked to reduced volumes of the cerebellum and hippocampus. Several investigations have evaluated the structural deformities in brain regions exposed to PAHs, including the white matter [13], parietal, temporal, insular lobes, and caudate [14]. On the other hand, Cho et al. [9] reported that PAHs prompted stress exposure may perhaps cause a decrease in whole-brain cortical thickness. As a result, the current study used the entire brains of Wistar rats to determine the impact of vacuum gas oil.

Oxidative stress can lead to neurotoxicity [15]. It has been documented that crude oil contain abundant of toxic substances that causes oxidative stress [16]. Antioxidants can be utilized to lessen the involvement of oxidative stress [17,18]. Previous findings from studies have indicated that natural compounds can reduce oxidative stress [19, 20] and improve brain function [1]. *Monodora myristica,* often known as African nutmeg (family Anonaceae), is mostly grown in southern Nigeria [16]. The seeds are highly valuable both medicinally and economically, and the kernel that is extracted from the seed is a widely used condiment in Nigeria. *M. myristica* is an extremely useful natural source of antioxidants [16,17].

Glycyrrhiza glabra L. is a flowering plant belonging to the bean family Fabaceae. Glycyrrhizin, a triterpenoid saponin glycoside, is believed to be the main bioactive component (4–10%) in *G. glabra* roots [20]. Biologically, *G. glabra* could be used to produce drugs, dietary supplements, cosmetics, food additives and flavours [21]. The effect of naturally occurring spices in our environment to reduce the neuro-toxicological impact of cassava diet containing VGO has not been established. Thus, the present study focused on the characterization of PAHs in CFD-VGO and the neurodefensive properties of *M. myristica* and *G. glabra* in rats fed with CFD-VGO. This was done by monitoring some biochemical parameters in the brain tissues that are associated with oxidative stress, signal transduction and dyslipidemia, since diet intake could have significant influence on brain health.

2. Materials and methods

2.1. Spices and cassava materials

Cassava tubers harvested from loamy soil at 26–37 °C in Umuosele community Amai, Latitude: 5° North, Longitude: 6° East, Delta State, Nigeria, was used for the study. The spices *M. myristica* and *G. glabra* were purchased from a market in Amai and then identified at Forest Research Institute of Nigeria, Ibadan and Department of Plant Biotechnology, University of Benin with voucher number FHI 107259 and UBH_G 394 respectively deposited in their herbarium.

2.2. Experimental animals and VGO

Male albino rats (Wistar strain) of 7 – 9 weeks were used for the study. For two weeks, they were allowed to adjust to the conditions of the laboratory. The animals were maintained in accordance with the guidelines and protocol for care and standard use of laboratory animals by the Canadian Council and ethical committee of Novena University (Study number NUO/PG21/890). VGO was obtain from the Nigerian National Petroleum Cooperation (NNPC), refinery, Warri, Delta State, Nigeria.

2.3. Preparation of cassava flour for diet formulation

Freshly harvested matured cassava tubers were bought from local farmers within Amai village, Delta State. After being thoroughly cleaned with clean water to get rid of any dirt, the tubers were processed using the following techniques: the tubers were peeled using a sharp knife and then manually chopped into smaller pieces. It was then washed in clean water, spread thinly between sheets of clean polythene, and dried until it reached a consistent weight. This was achieved by turning at intervals in an oven (45°C) to enhance the drying process of the cassava chips. The chips were milled to yield oven-dried cassava flour. The food items,

Table 1
Gross composition (%) of diet formulation for rats.

Ingredient	CFD-VGO	CFD-NVGO
Cassava flour	54.64	54.64
Casein	11.26	11.26
Cellulose (corn cob)	5.00	5.00
Bone Meal	2.00	2.00
Oyster Shell	1.00	1.00
Vit-min(premix)	1.00	1.00
Glucose Monohydrate	5.00	5.00
Sucrose	-	10.00
Salt	0.20	0.20
VGO	10.00	-
Total	100	100

Cassava flour diet containing vacuum gas oil (CFD-VGO), Cassava flour diet not containing crude oil (CFD-NVGO).

including the cassava flour (Table 1) were mixed together and manually made into pellets to feed albino rats.

2.4. Extraction and analysis of PAHs in cassava tubers and cassava flour diet having VGO

The USEPA method 3550 C [22] was used to extract and analyse the samples for PAHs. A pre-conditioned Whatman Soxhlet thimble was filled with approximately 10.0 g of sample. Equal amounts of DCM and hexane were then mixed and extracted for 10 h. The extract volume was reduced to roughly 4 millilitres by using a rotary evaporator to remove the solvent combination. Following that, equal amounts of acidified silica gel, florisil, anhydrous Na_2SO_4, and copper powder were placed into a column to purify the concentrated extract. Eluting PAHs into a 50-ml conical flask involved adding 20 ml of DCM and hexane mixture each, then rotating the flask until around 2 ml was left behind. After the mixture was carefully placed into a tube, the content of the flask was emptied into the tube and the flask was twice rinsed with 2 ml hexane. This extract was once again nearly dried using a moderate stream of nitrogen gas, dissolved in 1.7 millilitres of ethyl acetate, and then put into an injection vial so that GC–MS analysis could begin. To measure the amount of PAHs in the sample extracts, an Agilent HP-5 − 60 to 325 °C GC column (30 m×320 μm×0.25 μm film thickness) was fitted with an Agilent mass selective detector (Agilent 5975B) (GC–MS). For the injection and detection temperatures, the GC was programmed to operate at 280 °C and 300 °C, respectively. In the initial stages, 50 °C was the column temperature for two minutes. It was then raised to 150 °C at 20 °C/min, 160 °C at 10 °C/min, and finally, 300 °C at 5 °C/min for fifteen minutes. The total runtime for the chromatographic separation was 32.25 min. The carrier gas was steady-state helium flowing at a rate of 0.8 ml/min. In splitless mode, 1 μL sample was added to the GC-MS. Agilent Chemstation software was utilized to acquire data while maintaining the chosen ion monitoring mode.

2.5. Preparation of the spice extract

Briefly the spices (*M. myristica* and *G. glabra*) were open air-dried to constant weight for two weeks. The spices were crushed into coarse particles using Warren blender for 3 min at high speed. One hundred grams (100 g) of the ground spices were extracted with 500 ml of diethyl ether and left to stand for 48 hrs. The mixtures were filtered using a clean muslin cloth. Thereafter the filtrates were evaporated to dryness using water bath. The extracts were stored in refrigerator until needed for further analysis.

2.6. Determination of dosage

Forty rats were used for the dose determination. The rats (n = 4) were divided into ten groups (*M. myristica* and *G. glabra* DEE extract having five group each). The *M. myristica,* and *G. glabra* DEE extract were administered separately to all the four rats in each group at starting single dose of 100 mg/kg b.wt (group 1). The rats were observed for the period of 1 h, occasionally for 3 h for severity of any toxic sign and mortality. If no mortality was observed at this dose, the same procedure was repeated for dose level of 200 g/kg b.wt (group 2), 400 mg/kg b.wt (group 3), 800 mg/kg b.wt (group 4), and 1600 mg/kg b.wt of extract (group 5). The LD_{50} was thus determined, which was selected for the neurotoprotective animals study. The animals were observed up to 7 days after the administration of extracts to find out for any delayed mortality. No mortality was observed in rats given *M. myristica* and *G. glabra* DEE extract from 100 - 1600 mg/kg body weight. The LD_{50} of the sample were greater than 1600 mg/kg b.wt and hence had high degree of safety. However, 400 mg/kg b.wt was chosen for the protective animal study due to the positive behavioural signs (such as new hair growth, increased locomotion, no respiratory abnormalities; cough and sneezing) of the rats given this dose.

2.7. Experimental procedures

Fifty four (54) male rats were divided into nine groups, each group having six rats; group 1: normal control (rats were fed CFD-NVGO only), group 2: CFD-VGO only, group 3: normal plus *M. myristica* diethyl ether extract (DEE), group 4: normal plus *G. glabra* DEE, group 5: normal plus *M. myristica* and *G. glabra* DEE, group 6: CFD-VGO plus *M. myristica* DEE, group 7: CFD-VGO plus *G. glabra* DEE, group 8: CFD-VGO plus *M. myristica* and *G. glabra* DEE, group 9: CFD-VGO plus 1 ml/kg of 2-methyl cellulose in normal saline (0.9% NaCl). The administration of the cassava diet and extracts at a dose of 400 mg/kg b.wt using cannula was carried out for a period of 28 days. The rats were euthanized utilizing carbon dioxide asphyxiation at a rate of 50% volume per minute displacement for 2 min on the 29 day after overnight fast. The brain was harvested and 1 g of the brain tissue was homogenized in 9 ml of normal saline then centrifuged at 2, 500 g for 15 mins to obtain the supernatant which was stored in the refrigerator, for further biochemical analysis.

2.8. Determination of antioxidant parameters in brain tissue

2.8.1. Estimation of reduced glutathione

The reduced glutathione concentration in the brain was estimated using the method of Ellman [23]. To 0.5 ml of tissue homogenate was added 2 ml 10% trichloroacetic acid and then centrifuged. One millilitre (1 ml) of supernatant was treated with 0.5 ml of Ellman's reagent and 3 ml of phosphate buffer. At 412 nm, the colour produced was read. Along with a blank that contained 3.5 millilitres of buffer, a series of standards were treated similarly.

2.8.2. Assay of superoxide dismutase activity

Misra and Fridovich [24] approach was utilized to measure the activity of SOD. The experiment was performed by mixing 2.5 ml of 0.05 M carbonate buffer, pH 10.2, with 0.2 ml of the supernatant. Freshly made 0.3 mM epinephrine (0.3 ml) was added as the substrate to the buffer supernatant mixture to initiate the reaction, which was rapidly mixed by inversion. The reference cuvette consists of 2.5 ml of buffer, 0.3 ml of substrate, and distilled water (0.2 ml). The absorbance at 480 nm was observed every 30 s for 120 s. The activity of the enzyme is expressed in units/g wet tissue. One unit is distinct as the sum of the enzyme that caused 50% inhibition of the autooxidation of epinephrine.

2.8.3. Assay of catalase activity

Aebi [25] method was adopted for the assay of catalase activity. Catalase breaks down H_2O_2 directly into water and oxygen. The decrease in H_2O_2 concentration may be monitored spectrophotometrically. About 2.4 ml phosphate buffer (50 mM, pH 7.0), 10 μL of 19 mM H_2O_2 and 50 μL sample were allowed to mix in a test tube. The absorbance was measured at 240 nm over a 3 min period at 25 °C against the blank on a spectrophotometer. Readings were taken at 0 s and 3 min

2.9. Estimation of parameters essential for signal transduction for normal brain function

2.9.1. Na^+/K^+-ATPase

The activity of Na^+/K^+-ATPase was assayed using the procedure of Hesket et al. [26]. The reaction mixture (0.1 ml of buffer, 0.2 ml of $MgSO_4$, 0.2 ml of NaCl, 0.2 ml of KCl, 0.2 ml of EDTA and 0.2 ml of ATP) was incubated for 15 min at 37 °C, and then the reaction was initiated by adding of 0.2 ml of sample. This was incubated at 37 °C for 15 min. About 1 ml of 10% TCA was added at the end of 15 min to arrest the reaction mixture. The supernatant Pi liberated was estimated.

2.9.2. Mg^{2+}-ATPase

The activity of Mg^{2+}-ATPase was assayed according to the method described by Ji, et al. [7]. Briefly, 0.1 ml of buffer, 0.1 ml of $MgCl_2$, 0.1 ml of ATP, 0.1 ml of water and 0.1 ml of sample were placed in test tube

and then incubated at 37 °C for 15 min. Thereafter, 0.5 ml of 10% TCA was added. The Pi free was estimated in the supernatant.

2.9.3. Ca^{2+}-ATPase

Ca^{2+}-ATPase activity was assayed as descried by Ji, et al. [7]. The reaction contained 0.1 ml of buffer, 0.1 ml of $CaCl_2$, 0.1 ml of ATP and 0.1 ml sample. The contents were incubated for 15 min at 37 °C. The reaction mixture was arrested by adding 0.5 ml of ice cold 10% TCA. The free Pi of supernatant was determined.

2.9.4. Total ATPases

Evans [27] approach was used to quantify the total activity of total ATPases. A final volume of 2 ml comprising 0.1 ml of 0.1 M Tris-HCl (~pH 7.4), 0.1 ml of 0.1 M NaCl, 0.1 ml of 01 M $MgCl_2$, 1.5 ml of 0.1 M KCl, 0.1 ml of 1 mM EDTA, and 0.1 ml of 0.01 M ATP was used to assess the ATPase activity in 0.1 ml of aliquot of the homogenates. Following a 20 min reaction period, 1 ml of 10% TCA was added. The combination was centrifuged at 3000 rpm for 10 min to estimate the amount of inorganic phosphate (Pi) released into the supernatant.

2.9.5. Inorganic phosphate (Pi) estimation

Randox diagnostic kit was used to determine the Pi released by total ATPases, Na^{+}/K^{+}-ATPase, Mg^{2+}-ATPase, and Ca^{2+}-ATPase using Henry's [28] approach. The enzyme activity in tissues were expressed as μg Pi release/mg protein.

2.9.6. Acetylcholinesterase assay

Acetylcholinesterase activity was measured using Ellman et al. [29] method. Briefly, 25 μL of sample was added to test tube having 2.925 μL of 0.1 M phosphate buffer (pH 8.0), 25 μL of 8 mM of DTNB (5, 5′-dithiobis-2-nitrobenzoate) and 25 μL of 45 mM acetylcholine iodide at room temperature (27 C). The contents were mixed, and the absorbance was read continuously at intervals of 30 s for 2 min at 412 nm. Standard curve was obtained using 5-thio-2-nitrobenzoic acid (TNB) (5- 30 nmol). Acetylcholinestrase activity was expressed as nmol/mg/min.

2.10. Estimation of brain lipid profile

2.10.1. Total cholesterol (T.Chol)

Total cholesterol was determined according to the method described by Allan et al. [30]. One millilitre (1 ml) of cholesterol regent was added to test tube labelled blank, standard and sample, then 10 μL of sample or standard was then transferred to the respective tubes and mixed. Tubes containing reagent and specimen were left at room temperature for five minutes. The solution turns pinkish red colour and absorbance were taken at 530 nm after blanking.

2.10.2. Triacylglycerol (TAG)

Triacylglycerol was determined according to the method of Fossati and Prencipe [31]. One millilitre (1 ml) of triacylglycerol regent was added to test tubes labelled blank, standard and sample. Thereafter 10 μL of sample, blank (distilled water) and standard reagent was transferred to the respective tubes and mixed. The tubes containing reagent and specimen were allowed to stand for 5 min at room temperature. The absorbance was determined at 540 nm after blanking.

2.10.3. High-density lipoprotein– cholesterol (HDL-C)

High-density lipoprotein – cholesterol was determined according to the method described by Badimon et al. [32]. Sample (0.20 ml) was added to 1 ml of precipitating reagent and let to stand for 10 min and then centrifuge for 15 min at 3000 rpm. Thereafter 25 μL of supernatant, blank (distilled water) and standard reagent was transferred to the respective tubes containing one millilitre (1 ml) of cholesterol regent. The content of each tubes were thoroughly mixed for five minutes at 25 ^{0}C at room temperature. Absorbance was recorded at 500 nm against reagent blank.

2.10.4. Low – density lipoprotein (LDL) cholesterol

LDL-C was calculated using Friedewald formula.

$$\text{LDL-C(mg/dl)} = (\text{Total cholesterol–HDL-C)–Triacylglycerol}/5$$

2.10.5. Malondialdehyde (MDA)

MDA was determined in brain using the method of Buege and Aust [33]. Sample (1 ml) was added to 2 ml of TCA-TBA-HCL reagent [0.37% Thioarbituric acid (TBA), 15% Tricarcoxylic acid (TCA) and 0.24 N Hydrochloric acid (HCl)] (1:1:1 ratio). The tube was stoppered loosely and immersed in boiling water for 15 min and swirled slightly at intervals. The mixture was cooled and centrifuged for 10 mins at 5000 g. The absorbance was read at 532 nm using the reagent blank. Lipid peroxidation in units/g of wet tissue was calculated with a molar extinction co-efficient of $1.56\times105\ M^{-1}$.

2.11. Statistical analysis

The data obtained were expressed as mean ± SD and analysed using analysis of variance (ANOVA). The group means were compared by least significant difference (LSD). The SPSS-PC programme package (version 210) was used for statistical analysis.

3. Results

3.1. PAHs content of VGO cassava flour diet

Table 2 illustrated the PAHs content of harvested cassava tubers and cassava flour diet containing VGO. Sixteen compounds such as naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, chrysene, benzo [b] fluoranthene, benzo [k] fluoranthene, fluoranthene, pyrene, benz [a] anthracene, benzo [a] pyrene, dibenz (a, h) anthracene, indeno (1,2,3-cd) pyrene and benzo [ghi] perylene) were targeted in cassava tubers and CFD-VGO. The chromatograms of the targeted compounds are shown in Figs. 1 and 2. Anthracene, fluoranthene, benz [a] anthracene, dibenz (a,h) anthracene, indeno (1,2,3-cd) pyrene, benzo [a] pyrene, benzo [k] fluoranthene, and benzo [ghi] perylene were not found in the harvested cassava tubers. The sum total concentrations of the targeted PAHs compounds in tubers and CFD-VGO

Table 2
PAHs content of cassava tubers and CFD-VGO.

	PAHs content (ppm)	
Target compounds	Harvested cassava tubers	Formulated CFD-VGO
1) Naphthalene	0.01	29.06
2) Acenaphthylene	0.02	1.25
3) Acenaphthene	0.01	1.88
4) Fluorene	0.02	0.87
5) Anthracene	0.00	0.10
6) Phenanthrene	0.04	18.55
7) Fluoranthene	0.00	0.35
8) Pyrene	0.05	0.19
9) Benz [a] anthracene	0.00	0.52
10) Chrysene	0.01	0.01
11) Benzo [b] fluoranthene	0.01	0.15
12) Benzo [k] fluoranthene	0.00	0.17
13) Benzo [a] pyrene	0.00	0.17
14) Dibenz (a,h) anthracene	0.00	0.03
15) Indeno (1,2,3-cd) pyrene	0.00	0.05
16) Benzo [ghi] perylene	0.00	0.03
Total (∑16 PAHs)	**0.17**	**53.38**

The PAHs of CFD-VGO was higher than the European commission (EU) limit of PAHs for food sample (0.2 mg/kg) [34]. Also, benzo(a)pyrene (BaP) in analyzed CFD-VGO gave values above the department of petroleum resources (DPR) intervention limit of 0.01 mg/kg of B(a)P in food sample [34]. However, the PAHs levels of the harvested tubers (0.17 ppm) used in formulation of the diet was lower than PAHs of cassava tuber (0.20 ppm) from Nsirimo, Umuahia - South Local Government Area in Abia State Nigeria, with no occurrence of crude oil or related activities as reported by Nwaichi et al. [35].

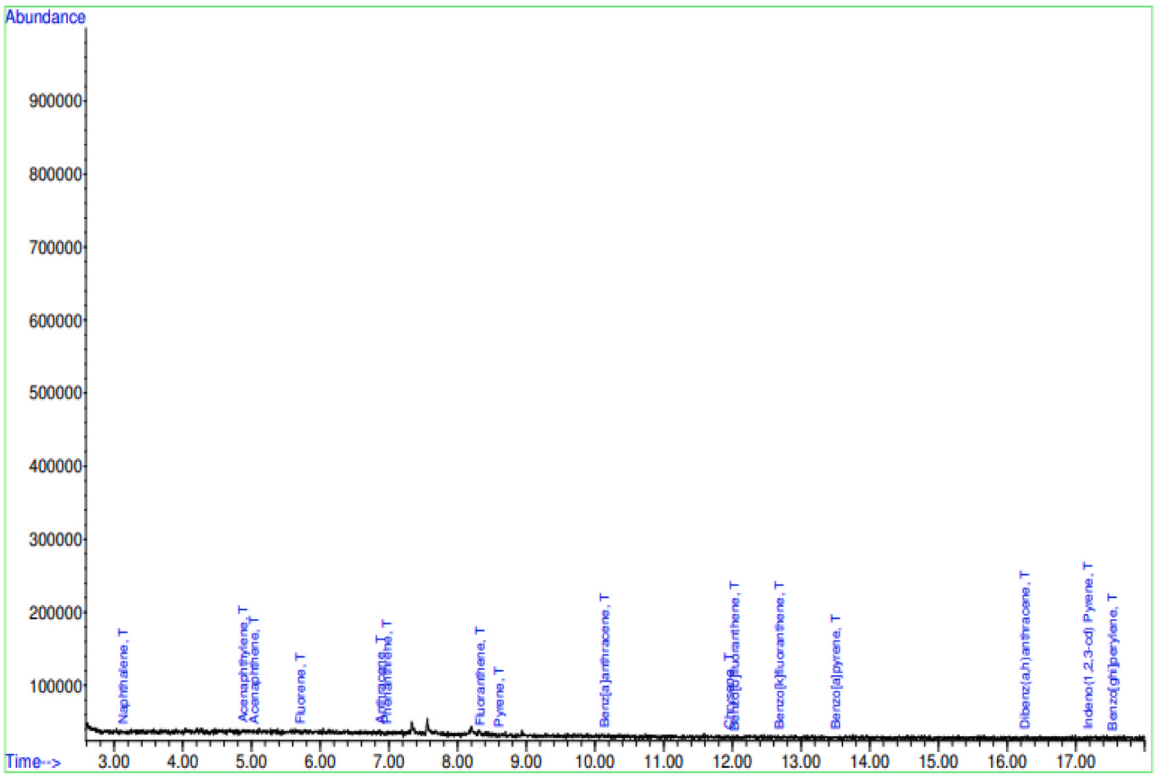

Fig. 1. Chromatogram of PAHs content of harvested cassava tubers.

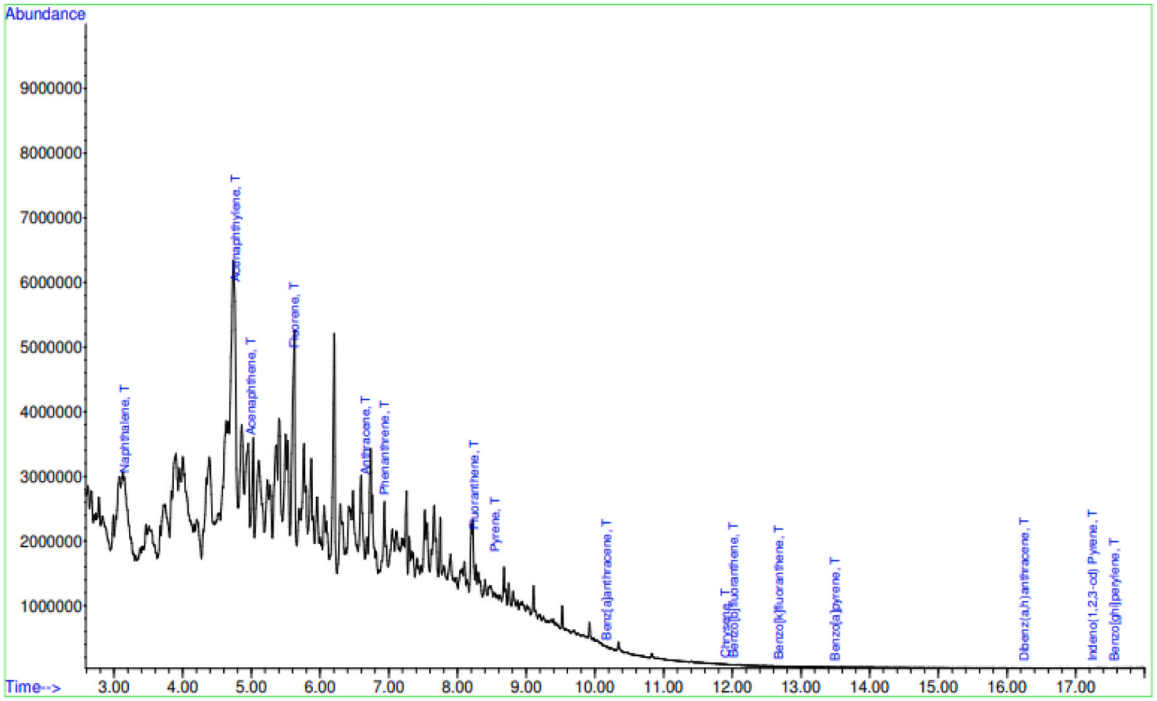

Fig. 2. Chromatogram of PAHs content of VGO cassava flour diet.

were 0.17 ppm and 53.38 ppm respectively,

3.2. Changes in antioxidants ATPases and acetylcholinesterase activities in the brain of rats

Tables 3, 4 and 5 shows the changes in antioxidants (GSH, SOD, CAT), ATPases (Total ATPases, Na^{+}/K^{+}ATPase, Mg^{2+}ATPase and Ca^{2+}ATPase) and acetylcholinesterase activity in the brain of rats administered CFD-VGO, *M. Myristica* and *G. glabra* extract. A significant decrease was observed in antioxidants, ATPases and acetylcholinesterase in the brain of rats fed with CFD-VGO only (group 2) when compared with the control group. Rats administered CFD-NVGO with

Table 3
Changes in GSH, SOD and catalase in the brain of rats given formulated CFD-VGO.

Groups	Brain		
	GSH (units/g wet tissue)	SOD (units/g wet tissue)	CAT (units/g wet tissue)
group 1: Normal control (rats were fed CFD-NVGO only)	27.65 ± 0.55[a]	32.52 ± 5.58[a]	26.58 ± 5.55[a]
group 2: CFD-VGO only	5.20 ± 0.55[b]	9.30 ± 0.55[b]	7.10 ± 1.65[b]
group 3: Normal plus *M. myristica* extract	31.50 ± 2.09[a,d]	36.82 ± 3.04[a,d]	32.00 ± 5.50[e]
group 4: Normal plus *G. glabra* extract	35.20 ± 2.55[d]	39.05 ± 5.10[d]	34.58 ± 8.25[e]
group 5: Normal plus *M. myristica* and *G. glabra extract*	41.40 ± 5.04[e]	44.40 ± 7.04[e]	40.23 ± 5.50[f]
group 6: CFD-VGO plus *M. myristica* extract	8.05 ± 0.92[b,c]	12.45 ± 0.50[b,c]	16.35 ± 2.13[c,d]
group 7: CFD-VGO plus *G. glabra* extract	12.55 ± 1.50[c]	17.93 ± 3.54[c]	19.25 ± 1.04[d]
group 8: CFD-VGO plus *M. myristica* and *G. glabra* extract	25.08 ± 3.70[a]	28.15 ± 5.62[c]	25.20 ± 2.05[a]
group 9: CFD-VGO plus 2-methyl cellulose	6.50 ± 0.30[b]	9.85 ± 0.28[a]	8.90 ± 1.15[a]

Values are represented in mean ± SD. n = 6. There is a significant difference ($p < 0.05$) between mean values in the same column with different superscripts. Cassava flour diet containing vacuum gas oil (CFD-VGO), Cassava flour diet not containing crude oil (CFD-NVGO).

Table 4
Changes in brain-ATPases in the brain of rats administered CFD-VGO treated with *M. myristica* and *G. Glabra* extract.

Groups	Brain			
	Total ATPases*	Na^+/K^+ATPase*	Mg^{2+}ATPase*	Ca^{2+}ATPase*
group 1: Normal control (rats were fed CFD-NVGO only)	7.22 ± 0.13[a]	3.25 ± 0.11[a]	2.45 ± 0.13[a]	2.35 ± 0.11[a]
group 2: CFD-VGO only	3.25 ± 0.15[b]	0.15 ± 0.04[b]	0.16 ± 0.02[b]	0.15 ± 0.05[b]
group 3: Normal plus *M. myristica* extract	8.45 ± 0.85[a,c]	4.15 ± 0.15[a,c]	3.32 ± 0.25[a,c]	3.42 ± 0.12[a]
group 4: Normal plus *G. glabra* extract	9.05 ± 1.23[a,c]	4.95 ± 1.04[a,c]	3.12 ± 0.13[a,c]	4.09 ± 0.53[a,c]
group 5: Normal plus *M. myristica* and *G. glabra extract*	11.23 ± 1.05[c]	6.53 ± 1.25[c]	5.31 ± 1.75[c]	6.24 ± 0.95[c]
group 6: CFD-VGO plus *M. myristica* extract	4.26 ± 0.35[a,b]	1.05 ± 0.05[a]	1.04 ± 0.09[a]	1.16 ± 0.04[a]
group 7: CFD-VGO plus *G. glabra* extract	4.98 ± 0.25[a,b]	1.72 ± 0.06[a]	1.17 ± 0.07[a]	1.25 ± 0.05[a]
group 8: CFD-VGO plus *M. myristica* and *G. glabra* extract	7.35 ± 0.11[a]	3.24 ± 0.15[a]	2.24 ± 0.04[a]	2.27 ± 0.15[a]
group 9: CFD-VGO plus 2-methyl cellulose	3.85 ± 0.52[b]	0.53 ± 0.16[b]	0.43 ± 0.16[b]	0.35 ± 0.14[b]

Values are given in mean ± SD. n = 6. Values with same superscript letter in the same column were not significant at $p < 0.05$. * µg pi liberated/min/mg protein

extracts from *G. glabra* and *M. myristica* (group 5) demonstrated a significant increase in ATPases, acetylcholinesterase, and antioxidants in the brain in comparison to rats fed CFD-NVGO alone. Significant increase were observed in antioxidants, ATPases and acetylcholinesterase in the brain of rats fed with CFD-VGO plus *M. myristica* and *G. glabra* extracts (group 8) when compared with rats fed CFD-VGO only.

Table 5
Impact of *M. myristica* and *G. glabra* extracts on AChE in rats given CFD-VGO.

Groups	Brain AChE activity (mol/min/g of tissue)
group 1: Normal control (rats were fed CFD-NVGO only)	4.52 ± 0.35[a]
group 2: CFD-VGO only	1.25 ± 0.45[b]
group 3: Normal plus *M. myristica* extract	4.98 ± 1.04[a]
group 4: Normal plus *G. glabra* extract	5.12 ± 0.81[a,c]
group 5: Normal plus *M. myristica* and *G. glabra extract*	8.89 ± 1.23[c]
group 6: CFD-VGO plus *M. myristica* extract	3.15 ± 0.50[a]
group 7: CFD-VGO plus *G. glabra* extract	3.80 ± 0.20[a]
group 8: CFD-VGO plus *M. myristica* and *G. glabra* extract	4.28 ± 0.10[a]
group 9: CFD-VGO plus 2-methyl cellulose	1.85 ± 0.15[b]

Values are indicated in mean ± SD. n = 6. Significant differences are observed at $p < 0.05$ for mean values in the same column that have different superscripts.

3.3. Lipid profile and MDA in the brain of rats

The lipid profile and MDA in the brain of rats administered CFD-VGO treated with *Myristica* and *G. glabra* extract are shown in Tables 6 and 7. A significant ($p < 0.05$) increase were observed in T.Chl, TAG, LDL-C, MDA and decrease in HDL-C in the brain of rats fed with CFD-VGO only and CFD-VGO plus 2-methyl cellulose when compared to the control. Rats fed with CFD-NVGO and extracts of *M. Myristica* and *G. glabra* (groups 3, 4, and 5) showed a significant increase in HDL-C and a significant decrease in T.Chl, TAG, LDL-C, and MDA in brain tissue when compared to the normal control (group 1). Significant decrease in T.Chl, TAG, LDL-C, MDA and increase HDL-C were observed in the brain of rats fed with CFD-VGO and treated with extracts of *M. Myristica* and *G. glabra* (group 6, 7 & 8) when compared with rats fed CFD-VGO only (group 2).

4. Discussion

The decrease in antioxidants activities in the brain in regard to CFD-VGO intake might indicate oxidative stress. Exposure to hydrocarbons causes DNA damage, sulfhydryl depletion, alterations in antioxidant

Table 6
Brain lipids of rats fed with CFD-VGO.

Group	TC (mg/dL)	TAG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
group 1: Normal control (rats were fed CFD-NVGO only)	477.23 ± 41.36[a]	346.45 ± 33.84[a]	289.20 ± 39.82[a]	118.74 ± 12.34[a]
group 2: CFD-VGO only	565.18 ± 9.23[b]	454.28 ± 27.62[b]	216.35 ± 28.12[b]	257.98 ± 21.34[b]
group 3: Normal plus *M. myristica* extract	430.25 ± 21.23[g]	300.25 ± 43.85[e]	319.23 ± 29.87[e]	50.97 ± 6.48[e,f]
group 4: Normal plus *G. glabra* extract	428.18 ± 31.43[g]	295.28 ± 43.85[e,f]	320.71 ± 40.33[e]	48.41 ± 5.56[f]
group 5: Normal plus *M. myristica* and *G. glabra extract*	417.33 ± 31.30[h]	276.40 ± 23.54[g]	328.70 ± 27.32[f]	33.35 ± 3.08[g]
group 6: CFD-VGO plus *M. myristica* extract	516.37 ± 27.30[c]	323.56 ± 30.66[c]	237.02 ± 27.11[c]	214.67 ± 17.34[c]
group 7: CFD-VGO plus *G. glabra* extract	464.44 ± 18.51[d]	315.09 ± 35.62[d]	299.54 ± 45.49[d]	101.89 ± 18.29[d]
group 8: CFD-VGO plus *M. myristica* and *G. glabra* extract	432.45 ± 10.19[e]	304.78 ± 27.12[e]	316.08 ± 15.17[e]	55.42 ± 12.27[e]
group 9: CFD-VGO plus 2-methyl cellulose	567.11 ± 46.82[b]	457.54 ± 41.51[b]	218.80 ± 1590[b]	256.81 ± 21.45[b]

Values are represented in mean ± SD. n = 6. Mean values with distinguished superscripts in the same column differ significantly ($p < 0.05$).

Table 7
Malondialdhyde (MDA) level in the brain of rats.

Group	Brain MDA (units/g wet tissue)
group 1: Normal control (rats were fed CFD-NVGO only)	0.50 ± 0.05^{a}
group 2: CFD-VGO only	9.56 ± 0.06^{b}
group 3: Normal plus *M. myristica* extract	0.25 ± 0.05^{a}
group 4: Normal plus *G. glabra* extract	0.12 ± 0.12^{a}
group 5: Normal plus *M. myristica* and *G. glabra extract*	0.09 ± 0.53^{d}
group 6: CFD-VGO plus *M. myristica* extract	3.16 ± 0.08^{c}
group 7: CFD-VGO plus *G. glabra* extract	3.22 ± 2.85^{c}
group 8: CFD-VGO plus *M. myristica* and *G. glabra* extract	1.02 ± 0.02^{a}
group 9: CFD-VGO plus 2-methyl cellulose	9.83 ± 0.25^{b}

Values presented in mean ± SD. n = 6. Means with different superscripts letter differ significantly at $p < 0.05$

cellular defences, and the production of reactive oxygen species (ROS) [1]. The decreased CAT, SOD and GSH levels (Table 3) may be as a result of the binding of the PAHs present in VGO with the GSH sulfhydryl group and the replacement of endogenous redox metals, which may alter the configurations of these enzymes leading to their inhibition [36]. The PAHs present in VGO (Fig. 2) may have some neurotoxic effects by encouraging degeneration of brain cells and oxidative damage. According to Olasehinde and Olaniran [1], being exposed to PAHs may be linked to higher levels of oxidative stress and neurotoxicity. Nevertheless, the results acclaimed that *M. Myristica and G. glabra* administration significantly alleviated the brain oxidative status induced by CFD-VGO administration. This indicates further that after consuming hydrocarbon-toxic diet, rats may be able to have their antioxidant activity restored by intake of *G. glabra* and *M. myristica*.

ATPases are enzymes responsible for the transfer of cations across the cell membranes at the expense of ATP by hydrolysis [8]. The outcomes in Table 4 indicated that animals fed CFD-VGO alone had significantly lower ATPase activity in the brain. Free radicals formed from the VGO polluted diet could have led to decrease in the activity of Total ATPase, Na^{+}/K^{+}ATPase, Mg^{2+}ATPase and Ca^{2+}ATPase. ATPase are sulfhydryl (-SH) containing enzymes and the decrease in ATPase activity by CFD-VGO might be as a result of the PAHs binding with –SH groups at the active sites of the enzymes. The usage of ATP is thus affected by the inhibition of ATPase activity [36,37]. Since the Na^{+}, K^{+} and Ca^{2+} ions are important in the development and conduction of action potential, the decrease in the activities of respective ATPases may alter the rate of influx and efflux of cations correlating with altered membrane permeability properties. Additionally, the reduction in ATPase activity reflects the lower ATP turnover, most likely as a result of oxidoreductase system suppression and oxidative phosphorylation uncoupling [36]. The reversal of ATPase activity in rats by treatment with the extracts might be due to their oxidative protective nature by preventing the oxidation of –SH groups of Na^{+}/K^{+}ATPase, Mg^{2+}ATPase and Ca^{2+}ATPase. In the current study, it appears that the bioactive components of the extracts may provide neuroprotection by altering ATPase activity either directly or indirectly, which may help cure seizure disorders.

Enhancement of cholinergic activity through the inhibition of AChE is one of the major therapeutic strategies for neurological disorder [38]. AChE is the primary enzyme that stops the action of acetylcholine at cholinergic synapses which hydrolyzes acetylcholine more quickly. AChE is also very effective at altering extracellular acetylcholine levels and controlling cholinergic neurotransmission. As a consequence of numerous toxins inducing the cholinesterase enzyme inactivation or inhibition, the central nervous system acetylcholine levels rise, thereby increasing muscarinic stimulation and finally resulting to memory impairments [8]. According to Olivares-Rubio and Espinosa-Aguirre [39], through behavioral analysis, neurotransmitter regulation, or transcriptional changes, PAHs may influence neuronal development and cause neurotoxicity. In this research, rats fed CFD-VGO had a significant decrease in brain AChE activity (Table 5). However, significant increase in AChE activity in the brain was observed upon the administration of extracts. The defensive role of these extracts may be attributed to their antioxidant properties and improvement of neuronal function and memory [40,41]. The increased AChE in the brain may be credited to the combined antioxidant and neuroprotective properties of extracts, suggesting a potential use of the combined extracts in the management of neurological pandemonium.

However, regarding to treatment effect of the *G. glabra* and *M. myristica* to normal rats, it can be noticed that the level of the GSH, SOD, CAT and ATPases were significantly increased even over the normal control. This may be happen due to two reasons: the *G. glabra* and *M. myristica* may act directly and scavenges the ROS derived by normal biochemical process/oxidation-reduction cycle with the cell or it may work in union with the existing antioxidants and may help to prevent their loss during the oxidative injury. In accordance to Lobe et al. [42], antioxidants scavenge free radicals, inhibit their synthesis, or encourage their breakdown to avoid tissue damage caused by free radicals.

Changes in lipid levels during toxicity are known to suggest a propensity for atherosclerosis, which is the underlying factor of dyslipidemia [43]. In the current investigation, rats fed CFD-VGO showed a significant ($p < 0.05$) increase in T.Chl, TAG, and LDL-C and a decrease in HDL-C in the brain (Table 6). This alteration in lipids is an indication that intake of CFD-VGO has an impact on lipid metabolism. High T.Chl, TAG and LDL-C levels have been stated to be an important risk factor as it influences lipid deposition clotting mechanism [44]. Similarly, recent report suggested that consuming crude oil components may affects lipid metabolism, impairing tissues capacity for lipid metabolism [45]. Additionally, the study shows that in the brains of rats fed CFD-VGO, the administration of extracts decreases T.Chl, TAG, LDL-C, and increases HDL-C. This suggests that the extracts might have the ability to reduce cholesterol levels in the brain. The ability of *G. glabra* and *M. myristica* to lower lipid levels may be the cause of this decrease. This may lessen inflammation, which could be a factor in elevated cholesterol [46]. One of the primary effects of injury mediated by free radicals is lipid peroxidation (LPO), which causes direct membrane damage and produces several downstream compounds with neurotoxic potential [4]. MDA generation is linked to the interactions between free radicals and unsaturated fatty acids in cellular membranes. It is thought to be a substantial sensitive marker of LPO and signals intracellular damage [47]. Exposure to VGO may initiates the metabolic mechanisms that lead to oxidative stress, which is directly responsible for many degenerative alterations in tissues by creating free radicals [4]. MDA levels in the brain of the rats administered CFD-VGO in this investigation were significantly ($p < 0.05$) elevated (Table 7). The elevation in the LPO marker could be from an overproduction of the superoxide anions which suppress the antioxidant enzymatic system as a result of injury to the cell membrane caused by free radical attack [48]. Nevertheless, administering the extracts to the rats fed CFD-VGO significantly reduces the high brain MDA level. The extract of *G. glabra* has a lot of polyphenolic elements that might serve as a source of anti-lipidemia thereby preventing microsomal LPO [49]. In addition, studies have demonstrated the anti-inflammatory and anti-hyperlipidemia effects of the flavonoids found in *M. myristica* seeds [45]. The rats given the combined extracts demonstrated lower levels of brain MDA, suggesting that the combined extracts (*G. glabra* and *M. myristica*) have a stronger shielding effect than the individual extract against brain cell membrane injury caused by CFD-VGO. This might be as a result of the synergetic effect of the combined extracts.

In comparison to the normal control, rats administered CFD-NVGO along with *M. myristica* and *G. glabra* demonstrated a significant increase in HDL-C and a decrease in T. Chl, TAG, LDL-C, and MDA in brain tissue (Tables 6 and 7). This may be due to the flavonoids and

polyphenols content of *M. myristica* and *G. glabra* extracts protective effect on brain lipids. Studies have shown that *G. glabra* and *M. myristica* are highly rich in polyphenols and flavonoids [50,51]. Dietary polyphenols and flavonoids have been reported to have a variety of impacts on the brain that are associated with their neuroprotective properties [52]. These effects include the capacity to reduce neuroinflammation, shield neurons from damage caused by neurotoxins, and enhance memory, learning, and cognitive function [52]. The significant decrease in lipidperoxidation as well as the decrease in T. Chl, TAG and LDL-C, which in essence increased HDL-C levels points to these plants as a potential hypolipidemic agents. The findings may also suggest that *M. myristica* and *G. glabra* are extremely important in the context of development of new anticholinestesterase antidotes against CFD-VGO.

5. Conclusion

The current study indicated that CFD-VGO may causes brain injury by inducing oxidative stress, lipid peroxidation and reduction of Na^+/K^+ - ATPase activity. The findings from this study showed that *M. myristica* and *G. glabra* extracts administration could suppress oxidative stress, and altered lipid levels. It is therefore recommended that dietary intake of antioxidant spices such as *M. myristica* and *G. glabra* may be safe in traditional therapies for regeneration of brain tissue in CFD-VGO induced oxidative stress.

Funding

There is no specific grant/fund available for this study.

CRediT authorship contribution statement

Onyesom Innocent: Writing – review & editing, Visualization, Validation, Supervision, Project administration, Funding acquisition. **Ukperegbulem Jonah Kanayo:** Software, Resources, Formal analysis, Data curation. **Okpoghono Joel:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

None.

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