Journal of Science, Technology and Environmental Studies. Vol. 1 | No. 1

PROTECTIVE OUTCOMES OF SPICES AGAINST CASSAVA MEAL CONTAINING VACUUM GAS OIL INDUCED TOXICITY IN WISTAR RATS

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ABSTRACT: Spices with medicinal properties are gift from nature to humanity; considerably improving general well-being and consumption of vacuum gas oil (VGO) may have toxic effect. This study aimed to determine the protective outcomes of spices (*Monodora myristica* and *Glycyrrhiza glabra)* in rats given cassava flour meal comprising vacuum gas oil (CFM-VGO). A total of fifty four (54) male Wistar rats weighing 200–250 g were randomly divided into nine (9) groups. Group 1: normal control. Group 2 were fed CFM-VGO only. Groups 3, 4, and 5, were given normal diet and then treated with *M. myristica*, *G. glabra*, and *M. myristica* + *G. glabra* extracts. Group 6, 7, 8 and 9 were given CFM-VGO and then treated with *M. myristica*, *G. glabra*, *M. myristica* + *G. glabra* extracts and 2-methyl cellulose respectively. The rats were euthanized using carbon dioxide after experimental period of 28 days. Significant ($p < 0.05$) increases were observed in alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), malondialdehyde (MDA) levels and decrease in albumin, total protein (TP) and antioxidant enzymes (superoxide dismutase (SOD) and catalase) in the serum and liver of rats fed with CFM-VGO when compared with normal control. However, treatment with *M. myristica* and *G. glabra* extracts significantly restored altered ALT, AST, ALP, MDA and antioxidant enzymes activities. In conclusion, the data obtained confirm the protective effect of spices against vacuum gas oil tainted cassava meal induced toxicity in rats. Therefore, sustainable medicinal spices production could be necessary in securing future sourcing and improvement of good health which may boost economic development.

Keywords: Cassava meal; Liver function; Medicinal spices; Toxicity; Vacuum gas oil

Introduction

Heavy oil leftover from petroleum distillation is referred to as vacuum gas oil. This oil can be refined even further in a cracking machine. Refineries can produce more diesel and gasoline with this intermediate feedstock (TradeFord, 2021). The technique of hydrocracking petroleum heavy vacuum gas oil is widely employed in the manufacturing of premium liquid fuels for automobiles and aircraft (TradeFord, 2021). All things considered, VGO contributes significantly to the refining process and the creation of petrochemical goods and necessary fuels (Vela et al., 2024). Vacuum gas oil is the primary component used to make lubricant oil. Additionally, food and consumer goods may become contaminated with petroleum-derived materials (vacuum gas oil) as a result of technological procedures and environmental exposure (such as coming into contact with lubricants used in food machinery) (Market Research Reports, 2024). VGO-contaminated cassava may be hazardous to the liver, kidneys, and brain when consumed through machinery lubricants (Okpoghono et al., 2024a).

Research has indicated that due to their high phenolic component concentration, spices and herbs are great providers of antioxidants (Ejueyitsi et al., 2022; Ibobo et al., 2024; Okpoghono at al., 2023a, 2023b; Otuaga et al., 2020a, b). *Monodora myristica* (Gaertn, Dunal.) is mostly called African nutmeg (family Anonaceae) and is primarily used to make pepper soup in southern part of Nigeria (Ejueyitsi et al., 2023). The spice is a good natural source of antioxidants (Ejueyitsi et al., 2024; Omoike et al., 2022; Okpoghono et al., 2021). The seed of *M. myristica* has been reported to be helpful in treating a variety of conditions, including headaches, rheumatism, pain, coughing, and neuralgia (Sultan et al., 2023). The flowering plant *Glycyrrhiza glabra* L. is a member of the Fabaceae family of beans. The primary bioactive ingredient (4–10%) in *G. glabra* roots is thought to be glycyrrhizin, a triterpenoid saponin glycoside (Noreen et al., 2021). *G. glabra* has the potential to be utilized biologically in the production of medications, dietary supplements, cosmetics, food additives, and flavors (Okpoghono et al., 2024b). The antiviral, anti-inflammatory, anticancer, and hepatoprotective properties of glycyrrhizin are well-known (Noreen et al., 2021). However, recent research has proven the importance of vacuum gas-oil-induced reactive oxygen species (ROS), pro-inflammatory responses, and altered neurotransmitters in the brain (Okpogono et al., 2024a). Therefore, antioxidants such as natural polyphenols with known anti-inflammatory potentials may exert inhibitory effects against VGO toxicity in the brain (Okpoghono et al., 2024b).

The liver is a sizable organ in the abdomen that is responsible for a number of vital processes, such as blood filtration (Meunier & Larrey, 2019). Detoxification, drug elimination, digestion, and metabolism are all greatly aided by the liver (Meunier & Larrey, 2019). Aspartate and alanine transaminases (ALT and AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), serum bilirubin, total protein, and albumin are commonly measured as part of liver function tests (Meunier & Larrey, 2019). Hepatocellular disease is indicated by elevated ALT and AST that are not proportionate to elevated alkaline phosphatase levels (Algefare et al., 2024). The ability of the liver to manufacture albumin and vitamin K-dependent clotting factors can be used to grade the liver's true function (Algefare et al., 2024).

Oxidative stress happens when reactive oxygen species (ROS) surpass the antioxidant defense system of the cell, which can happen due to a rise in ROS levels or a fall in the antioxidant capacity of the cell (Cordiano et al., 2023). It is discovered that antioxidant enzymes and inflammation are closely related. Superoxide dismutases (SOD), catalase (CAT), and glutathione peroxidase (GPx) are important antioxidant enzymes (Janicka et al., 2024). Accordingly, hepatic protection is favorably connected with

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increase in antioxidant activity and suppression of ROS (Janicka et al., 2024;Onakurhefe et al., 2020). Alongside the consequences of VGO toxicity, the present study aims to investigate the protective outcomes of spices (*Monodora myristica* and *Glycyrrhiza glabra*) in rats given cassava flour meal comprising vacuum gas oil (CFM-VGO).

Materials and methods

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. Pictures of *M. myristica* and *G. glabra* are shown in Figure 1.

***Monodora myristica* seeds** ***Glycyrrhiza glabra* (banga soup sticks)**

Figure 1. *M. myristica* and *G. glabra*

Preparation of the Spice Extract

The extracts of *M. myristica and G. glabra* were obtained using the extraction technique as previously described by Okpoghono et al. (2018). 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.

Preparation of cassava flour for diet formulation

The tubers were peeled using a sharp knife, then washed. The washed tubers manually chopped into smaller pieces. The chips were oven dried (45°C). The chips were milled to yield oven-dried cassava flour. The food items, including the cassava flour were mixed together and manually made into pellets to feed albino rats.

Table 1 Percentage of food items in diet formulation

Ingredient	CFM-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 meal containing vacuum gas oil (CFM-VGO), Cassava flour meal not containing vacuum gas oil (CFM-NVGO).

Experimental Procedures

A total of fifty four male Wistar rats weighing 200–250 g were allowed to adjust to the conditions of the laboratory for 1 week.

The rats were divided into nine groups, each group having six rats;

group 1: normal control (rats were fed CFM-NVGO only),

group 2: CFM-VGO only,

group 3: normal + *M. myristica* diethyl ether extract (DEE),

group 4: normal + *G. glabra* DEE,

group 5: normal + *M. myristica* and *G. glabra* DEE,

group 6: CFM-VGO + *M. myristica* DEE,

group 7: CFM-VGO + *G. glabra* DEE,

group 8: CFM-VGO + *M. myristica* and *G. glabra* DEE,

group 9: CFM-VGO plus 2-methyl cellulose.

The administration of the cassava diet and extracts at established dose of 400 mg/kg b.wt using oral cannula was carried out for a period of 28 days.

Preparation of serum and Homogenate

The rats were euthanized utilizing carbon dioxide asphyxiation at a rate of 50% volume per minute displacement for 2 minutes on the 29 day after overnight fast. Blood sample were collected by cardiac puncture using syringe and needle into plain test tubes and allowed to clot. The liver was harvested and 1 g of the liver tissue was homogenized in 9 mL of normal saline. The clotted blood and homogenized liver tissue were centrifuged at 2, 500g for 15 mins to obtain the serum and supernatant which was stored in the refrigerator, for further biochemical analysis.

Determination of biochemical parameters

The activities of ALT, AST, ALP, albumin and total protein were determined using Randox laboratory kits. The activities of SOD, CAT and MDA level were analyzed using standard methods descried by Misra & Fridovich (1972), Aebi (1984), Buege & Aust (1978) respectively.

Statistical analysis

The data obtained were expressed as mean ±SD and analyzed using analysis of variance (ANOVA) and the group means were compared by least significant difference (LSD). The SPSS-PC programme package (version 21.0) was used for statistical analysis.

Results

Changes in AST, ALT, ALP, total protein and albumin level in the serum of rats administered CFM-VGO treated with *Myristica* and *G. glabra* extract are shown in Table 2 and 3. Significant ($p < 0.05$) increases were observed in AST, ALT, ALP and decrease in total protein and albumin levels in the serum of rats fed with CFM-VGO only and CFM-VGO plus 2-methyl cellulose when compared to the control. However, rats fed with CFM-NVGO and extracts of *M. Myristica* and *G. glabra* (groups 3, 4, and 5) showed a significant increase ($p<0.05$) in TP and Alb levels and a significant decrease ($p<0.05$) in AST, ALT, and ALP activities when compared to the normal control (group 1). Significant decrease ($p<0.05$) in AST, ALT, and ALP activities and increase TP and Alb levels were observed in the serum of rats fed with CFM-VGO plus extracts of *M. Myristica* and *G. glabra* (group 6, 7 & 8) in comparison with CFM-VGO only.

Table 4, shows the changes in antioxidants (SOD and CAT) and lipidpeoxidation (MDA) in the liver of rats administered CFM-VGO, *M. myristica* and *G. glabra* extract. A significant decrease ($p<0.05$) was observed in antioxidants and increased MDA level in the liver of rats fed with CFM-VGO only (group 2) when compared with the control group. Rats administered CFM-NVGO with extracts from *G. glabra* and *M. myristica* (group 5) demonstrated a significant increase ($p<0.05$) in antioxidants activities in the liver in comparison to rats fed CFM-NVGO alone. Significant increases ($p<0.05$) were observed in antioxidants in the liver of rats fed with CFM-VGO plus *M. myristica* and *G. glabra* extracts (group 8) when compared with rats fed CFM-VGO only.

Table 2. Changes in AST, ALT and ALP activities in the serum of rats given formulated CFM-VGO treated with *M. myristica* and *G. glabra* extracts

Groups	Serum		
	AST (U/L)	ALT (U/L)	ALP (U/L)
group 1: Normal control (rats were fed CFM-NVGO only)	25.74 ±12.34[a]	38.50 ±13.30[a]	470.23±31.30[a]
group 2: CFM-VGO only	58.90 ±13.35[b]	69.65 ±12.55[b]	665.15±39.20[b]
group 3: Normal plus *M. myristica* extract	20.60 ± 8.45[a,f]	35.74 ± 6.40[a,f]	430.25±31.23[e]
group 4: Normal plus *G. glabra* extract	18.45 ± 9.00[f]	30.65 ± 7.10[f]	420.15±40.43[f]
group 5: Normal plus *M. myristica* and *G. glabra extract*	14.30 ± 7.05[f]	23.35 ± 5.15[g]	407.30±55.40[g]
group 6: CFM-VGO plus *M. myristica* extract	50.65 ±10.35[c,d]	60.05 ±9.15[c]	616.38±30.30[c]
group 7: CFM-VGO plus *G. glabra* extract	45.85 ± 8.30[c]	50.00 ± 7.35[d]	564.45± 67.50[d]
group 8: CFM-VGO plus *M. myristica* and *G. glabra* extract	30.42 ± 8.20[a,c]	40.52 ± 11.25[a]	472.45± 50.19[a]
group 9: CFM-VGO plus 2-methyl cellulose	58.80 ±11.15[b]	68.50 ±15.05[b]	667.11±46.80[b]

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.

Table 3. Serum albumin and total protein levels of rats given formulated CFM-VGO treated with *M. myristica* and *G. glabra* extracts

Groups	Serum Albumin (g/dL)	Serum Total protein (g/dL)
group 1: Normal control (rats were fed CFM-NVGO only)	18.65± 5.24[a]	30.12±8.54[a]
group 2: CFM-VGO only	6.10 ± 0.57[b]	10.32±1.95[b]

group 3: Normal plus *M. myristica* extract	21.22 ± 2.09 [a,d]	32.31±9.34 [a,d]
group 4: Normal plus *G. glabra* extract	22.23 ± 2.55 [d]	35.45±10.13 [d]
group 5: Normal plus *M. myristica* and *G. glabra extract*	27.51 ± 5.04 [e]	44.42±12.08 [e]
group 6: CFM-VGO plus *M. myristica* extract	8.15 ± 1.32 [b,c]	13.43±5.56 [b,c]
group 7: CFM-VGO plus *G. glabra* extract	12.55 ±1.32 [c]	16.55±8.55 [c]
group 8: CFM-VGO plus *M. myristica* and *G. glabra* extract	19.08 ± 5.70 [a]	29.18±9.62 [a]
group 9: CFM-VGO plus 2-methyl cellulose	6.50 ± 1.42 [b]	9.98±0.93 [b]

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.

Table 4. Changes in SOD, CAT activities and MDA level in the liver of rats given formulated CFM-VGO treated with *M. myristica* and *G. glabra* extracts

Groups	Liver		
	CAT (units/g wet tissue)	SOD (units/g wet tissue)	MDA (units/g wet tissue)
group 1: Normal control (rats were fed CFM-NVGO only)	36.65 ± 8.13 [a]	42.57 ± 7.52 [a]	2.58 ± 0.15 [a]
group 2: CFM-VGO only	15.20 ± 6.35 [b]	19.30 ± 3.54 [b]	10.10 ± 1.65 [b]
group 3: Normal plus *M. myristica* extract	41.50 ± 10.01 [a,d]	46.82 ± 1.04 [a,d]	2.00 ± 0.10 [a]
group 4: Normal plus *G. glabra* extract	45.20 ± 8.25 [d]	49.05 ± 8.10 [d]	1.58 ± 0.22 [a]
group 5: Normal plus *M. myristica* and *G. glabra* extract	57.40 ± 9.54 [e]	54.45 ± 10.04 [e]	0.23 ± 0.02 [c]
group 6: CFM-VGO plus *M. myristica* extract	17.25 ± 4.92 [b,c]	22.47 ± 8.51 [b,c]	6.34 ± 1.02 [d]

group 7: CFM-VGO plus *G. glabra* extract	21.54 ±4.50^{c}	27.93 ± 5.54^{c}	5.22 ± 1.04^{d}
group 8: CFM-VGO plus *M. myristica* and *G. glabra* extract	34.07 ± 9.70^{a}	39.15 ± 5.22^{a}	3.30 ± 0.15^{a}
group 9: CFM-VGO plus 2-methyl cellulose	16.56 ± 6.32^{b}	19± 5.18^{ab}	9.90 ± 1.15^{b}

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

Discussion

The most widely used biochemical indicators of liver damage are serum levels of ALT, AST, and ALP (Ibioku et al., 2024). The activities of ALT, AST, and ALP in the serum significantly increased in the current investigation caused by repeated CFM-VGO consumption (Table 2). The extracts of *G. glabra* and *M. myristica* were able to significantly lessen the liver damage brought on by CFM-VGO by lowering the rise in serum enzyme activities. It has long been recognized that serum aminotransferase activity are sensitive markers of liver damage (Ogbonnaya et al., 2024). Damage to the hepatocytes changes the permeability of their membranes and their transport function, which allows the cells' enzymes to escape (Dibua et al., 2024; Hussain et al., 2020). Consequently, significant hepatic tissue membrane damage after CFM-VGO consumption is indicated by the considerable release of AST and ALT into the bloodstream. As proven by lowered AST, ALT, and ALP activities, the present study's results showed that treatment with *M. Myristica* and *G. glabra* extract effectively protected the rat against CFM-VGO -induced hepatotoxicity.

An additional sign of liver impairment linked to CFM-VGO treatment in rats were the decreased in serum total proteins and albumins. The main protein produced by the liver and found in blood is serum albumin. According to Starnes et al. (2024) and Okpoghono et al. (2023), it is a clinically meaningful indicator of hepatic synthetic function. The ability of *M. myristica* and *G. glabra* to protect hepatocellular integrity in this present study (Table 3) may be due to hepatocyte membrane stabilization by active phytochemicals like phenols, alkaloids, flavonoids and water soluble glycosides which are reported constituents of *M. myristica* and *G. glabra* (Okpoghono et al., 2024b; Okpoghono et al., 2018).

The hepatic cells have several enzymatic defense mechanisms against free radicals that are reactive. Protecting against reactive oxygen species is known to be possible for two families of enzymes: CAT and SOD. According to the current investigation, the administration of *M myristica* and *G. glabra* extract increased the activities of SOD and CAT (Table 4), suggesting that these two substances' hepatoprotective effects also involved hepatic antioxidant enzyme modulation. Onuoha & Chukwuma (2023) have discovered that consuming hazardous substances might cause changes in gene expression as well as a reduction in the liver SOD and CAT activity. Following the treatment of CFM-VGO, MDA a product of lipid peroxidation was more strongly prevented by the administration of *M myristica* and *G. glabra* extract. This result is in agreement with the earlier report of Okpoghono et al. (2024c) who used other type of treatments in VGO toxicity. One of the main causes of liver damage is lipid peroxidation, which is mediated by the free radical derivatives of CFM-VGO consumption (Ferenczi et al., 2020; Longobardi et al., 2024). To protect the liver from damage caused by CFM-VGO, antioxidant activity and/or the suppression of free radical production are crucial.

Antioxidant enzymes such CAT, SOD, and GST typically exhibit reduced activity in CFM-VGO toxicity due to their facile inactivation by lipid peroxides or reactive oxygen species. This is in line with earlier investigation of Okopogono et al. (2024c) who stated that decrease in antioxidant enzymes may be the result of excess free radical production, which is a sign of oxidative stress brought on by tissue damage from consumption of diet containing VGO. Free radicals in CFM-VGO intoxication are a major pathway of non-enzymatically induced lipid peroxidation, which subsequently affect various enzyme activities and therefore may also be linked to enzymatically induced lipid peroxidation (Longobardi et al., 2024). As a result, this suggest that *M myristica* and *G. glabra* extract may protects against significantly decreased CAT and SOD activities and increase MDA in rats given CFM-VGO compared with control rats.

Conclusion

Results obtained from this study indicated that, CFM-VGO intake reliably induced hepatotoxicity and oxidative stress in rats. The group administered CFM-VGO displayed elevated activities of AST, ALT, ALP and MDA level and attenuated SOD and CAT levels. Interestingly, administration of the extracts efficiently protected the experimental rats from oxidative stress-induced changes. However, it would be interesting to explore the hepatoprotective properties of the isolated phytochemicals from *M myristica and G. glabra extracts* in future studies.

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