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Monodora myristica seed extracts ameliorate crude oil contaminated diet-induced cardiotoxicity in rats

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ABSTRACT

Aim: This study investigated the ameliorative effects of *Monodora myristica* seed extracts in serum and heart of rats fed crude petroleum oil-polluted diet (CPOPD).). **Materials and Methods:** Thirty adult Wistar rats (180 ± 5 g) were used for the study. They were divided into six groups and treated as follows: Group A (normal control), Group B (CPOPD control), Group C (CPOPD plus 1 ml/kg tween 80), Group D (CPOPD plus 200 mg/kg *M. myristica* aqueous extract), Group E (CPOPD plus *M. myristica* ethanol extract, 200 mg/kg), and Group F (CPOPD plus *M. myristic*a diethyl ether extract, 200 mg/kg). The CPOPD and extracts were prepared following standard methods and administered for a period of 28 days, after which the animals were sacrificed and tissues processed for biochemical and histological examinations. **Results:** Exposure of rats to CPOPD significantly increased ($P < 0.05$) the activities of lactate dehydrogenase and glucose-6-phosphate dehydrogenase and the level of lipid peroxidation, but significantly decreased the activities of glutathione peroxidase, glutathione reductase, superoxide dismutase, and catalase compared to rats in the control group. In addition, histological analysis of the heart showed considerable necrosis and degeneration of cardiac cells in CPOPD-exposed rats compared to control. However, the treatment of CPOPD exposed rats with various extracts *M. myristica* significantly reversed these trends compared to rats maintained on CPOPD alone with the diethyl ether extract showing the greatest ameliorative potentials. **Conclusion:** This shows that *M. myristica* extracts have antioxidant capacity against crude oil toxicity.

Keywords: Crude petroleum, heart, *Monodora myristica*, oxidative stress, rat

INTRODUCTION

Each year, large numbers of oil spills are recorded, leading to significant contamination of the marine environment.[1,2] Upon entry into living cells, petroleum hydrocarbons are metabolized into free radicals and other activated cellular metabolites, which have harmful effects on cell membranes and other tissues and organs.[3,4] Petroleum hydrocarbons can get into tissues of animals directly or indirectly through the food web.[5] In this light, Patrick-Iwuanyanwu *et al*.[6] noted that human and animal exposure to petroleum hydrocarbons is on the increase given the rise in crude oil exploration and usage.

Several studies have shown the adverse effects of crude oil in rats fed diets contaminated with crude oil. Onwurah and Eze[7] reported that the consumption of petroleum hydrocarbon-contaminated diets caused enlargement of the liver, suppression of growth, and adverse histological changes, while Sunmonu and Oloyede[5] reported that there was impairment of liver function in rats following ingestion of diet containing catfish exposed to crude oil. In the same vein, Oguwike *et al*.[8] noted that kerosene and petrol contaminated fish had toxic effects on rat liver, while Achuba *et al*.[9] and Okpoghono *et al*.[10] showed that the feeding of the rats with crude petroleum oil contaminated diet resulted in adverse effects in rat's liver, kidney, and brain. However, studies on the effect of crude oil contaminated diet in the heart, which is also very susceptible to toxic effects, are limited. The heart pumps blood needed to give enough perfusion to body cells, tissues, and organs through the circulatory system. This cannot be achieved if the heart or blood vessels are impaired.[11]

The increase in crude oil pollution has led to studies aimed at ameliorating crude oil toxic effects in plants, animals,

and the ecosystem in general. Antioxidants have been shown to possess potent ameliorative effects against the toxic effect of free radicals.[12] Thus, the use of herbs, spices, and other plant products and extracts has been advocated as a remedy against oxidative stress induced by crude oil. One spice that has shown great antioxidant potentials is *Monodora myristica*, a perennial member of the Annonaceae family.[13] Studies by Erukainure *et al*.,[14] Eze-Steven *et al*.[15] Akinwunmi and Oyedapo,[16] Moukette *et al*.,[17] and George and Okpoghono[18] showed that various extracts of *M. myristica* seed possess significant antioxidant properties.

This study is, therefore, designed to investigate the ameliorative effect *M. myristica* seed extract on crude oil contaminated diet-induced toxicity in the serum and heart of rats.

MATERIALS AND METHODS

Ethical Consideration

The Faculty of Science, Delta State University Abraka ethical committee gave approval for this research with approval number ETH/17/18/PG225016. The animals were handled in accordance with the principles of laboratory animal care as contained in the NIH Guide for laboratory animal care.

Chemicals and Reagents

The chemicals and reagents used for this study were all of the analytical grade. Reduced glutathione, Gallic acid, and hydrogen peroxide (H_2O_2) were purchased from Sigma-Aldrich, Germany, while all other chemicals were procured from BDH Chemical Laboratory, England, United Kingdom.

Preparation of the Spice Extract

Extracts of *M. myristica* seeds obtained from local dealers in Delta State and identified by a specialist in the Department of Botany, Faculty of Science, Delta State University, Abraka were obtained following the extraction procedures as previously described by George and Osioma.[19] First, the seeds were dried to constant weight and then grounded into a fine powder. Thereafter, 100 g of the powdered spice was extracted with 500 ml of the hot water (60°C), ethanol, and diethyl ether. The extracts were left to stand for 48 h and were then evaporated to dryness using a rotary evaporator connected to a vacuum pump. Residues obtained were kept in the refrigerator until used.

Simulation of Crude Oil Pollution

The crude oil used for this study was obtained from the Nigerian National Petroleum Cooperation, refinery, Warri, Delta State. Fifty healthy catfish (*Clarias gariepinus*) (weighing 250–300 g) used for diet formulation were obtained from a commercial farm in Delta State, acclimatized for 1 week and then randomly divided into two groups in 30 L plastic aquaria. Fish in Group one (control) was cultured in borehole water, while those in Group 2 was cultured in water exposed to 823.3 µl/l of crude oil in line with the method described by Ikeogu *et al.*[20] for 4 weeks. The catfish were fed *ad libitum* with fish meal and the water changed every 24 h.

Experimental Diet Formulation

This was done following the method described by Sunmonu and Oloyede.[5] The catfish were dried at 40°C in an oven and used as a source of protein for the experimental rats. The diet was made by mixing known quantity source of each food classes: Corn starch (52%), oil (4%), maize cob (4%), granulated refined sugar (10%), a mixture of vitamins-minerals (5%), and the catfish as a source of protein (25%). These were thoroughly mixed together with tween 80 and then manually processed into pellets to feed the rats.

Experimental Rats and Experimental Design

Thirty adult male Wistar rats (180 ± 5 g) obtained from the animal house of the Faculty of Basic Medical Sciences, Delta State University, Abraka were divided into six groups and were treated as follows: Group A (normal control), Group B (crude petroleum oil-polluted diet [CPOPD] control), Group C (CPOPD plus 1 ml/kg of tween 80), Group D (CPOPD plus 200 mg/kg of *M. myristica* aqueous extract), Group E (CPOPD plus *M. myristica* ethanol extract 200 mg/kg), and Group F (CPOPD plus *M. myristic*a diethyl ether extract, 200 mg/kg). The CPOPD and extracts were administered orally for a period of 28 days. The rats were housed in standard wooden cages in a room (25 ± 2°C) with adequate light and handled in line with standard principles of laboratory animal care. They were acclimatized for 1 week before the commencement of experiments and allowed free access to clean tap water and standard growers mash diet all through the experimental period.

Sample Preparation

At the end of the exposure period, the rats were sacrificed after 24 h fast. Blood was obtained through puncturing of the heart with a syringe and needle and then put into an anticoagulant free test tube. Serum was thereafter obtained by centrifugation at 2500 g for 15 min. From each rat, the heart was also harvested. 0.5 g of the heart tissue was homogenized in 4.5 ml of normal saline then and centrifuged at 2500 g for 15 min. The supernatants obtained were stored in the refrigerator until used for biochemical assays. Heart tissue for histological analysis was processed according to the method described by Zhang *et al.*[21]

Biochemical Analysis

Glucose -6-phosphate dehydrogenase (G6PDH) activity was measured by monitoring G6PDH catalyzed oxidation of glucose -6-phsphtate to 6-phosphogluconate and reduction of NADP to nicotinamide adenine dinucleotide phosphate (NADPH), the rate of formation of NADPH being proportional to the G6PD activity and measured spectrophotometrically as in the increase in absorbance at 340 nm as described by Beutler *et al.*[22] The activity of glutathione peroxidase (GPx) was measured by the method described by Rotruck *et al*.[23] based on the reduction of H_2O_2 to water in the presence of reduced glutathione while the activities of lactate dehydrogenase (LDH) and glutathione reductase (GR) were monitored as described by Stentz[24] and Carlberg and

Mannervik,[25] respectively. The GR assay was based on the GR-catalyzed reduction of glutathione disulfide (GSSG) to glutathione (GSH), which depends on NADPH as monitored by a decrease in absorbance at 340 nm.

The activity of superoxide dismutase (SOD) was measured using the method of Misra and Fridovic[26] based on the autoxidation of epinephrine by superoxide radical (0_2).

0.2 ml of the supernatant was added to 2.5 ml of 0.05 M carbonate buffer and 0.3 ml freshly prepared epinephrine was added as a substrate to start the reaction and mixed by inversion. The increase in absorbance at 480 nm due to the adrenochrome formed was monitored every 30 s for 120 s and SOD activity was expressed as unit/g wet tissue (1 unit being the amount of the enzyme necessary to cause 50% inhibition of the oxidation of epinephrine to adrenochrome during 60 s). Catalase (CAT) activity was determined by the method of Kaplan *et al*.,[27] which is based on the conversion of H_2O_2 directly into water and oxygen by CAT and measured spectrophotometrically. H_2O_2 (2 ml) was added to 1 ml of sample in the reaction cuvette and thereafter absorbance was read at 360 nm for 70 s. Using the molar extinction coefficient, $\varepsilon = 39.4\ M^{-1}cm^{-1}$, the disappearance of H_2O_2 was calculated. The level of lipid peroxidation as determined following the method described by Varshney and Kale[28] based on the understanding that under acidic condition, malondialdehyde (MDA) produced from the peroxidation of membrane fatty acid react with a chromogenic reagent to yield a pink colored complex, which has a maximum absorbance at 532 nm. An aliquot of 0.4 ml of the sample was mixed with 1.6 ml of Tris-KCL buffer, to which 0.5 ml of 30% trichloroacetic acid was added. Then, 0.5 ml of 0.75% thiobarbituric acid was added and placed in a water bath for 45 min at 80°C. This was then cooled in ice and centrifuged at 3000 g. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532 nm.

Histological Procedure

A known portion of the heart was harvested and fixed in 10% formalin solution. The fixed tissues were processed, embedded in paraffin, and sectioned (5–6 μ). The sections were stained with hematoxylin and eosin and observed under a light microscope. The heart sections were examined for fibrotic strands (FS) in cardiac wall (CW) and ballooning degeneration (BD) of cardiac muscle (CM) cell, which are indices of pathology as against the normal heart histology features normal cardiac tissue, thick CW, CM, and cardiac vein (CV).

Statistical Analysis

Results are expressed as mean ± standard deviation and analyzed using analysis of variance. A comparison between group means was done by the least significant difference test using the SSPC-PC 20.0 software.

RESULTS

Biochemical Parameters

Exposure of rats to CPOPD significantly increased ($P < 0.05$) LDH activity in the serum and heart compared to rats in the control group [Table 1]. There was no significant difference in the LHD activity of rats feed CPOPD alone (Group B) and those which received tween 80 in addition to CPOPD (Group C). The treatment of CPOPD exposed rats with *M. myristica* aqueous, ethanol, and diethyl ether extracts (Groups D-F) significantly reduced LDH activity in both the serum and heart compared to rats maintained on CPOPD alone with the diethyl ether extract showing the greatest ameliorative effect.

The ameliorative effect of *M. myristica* seed extracts on G6PD activity in the serum and heart of rats fed CPOPD is shown in Table 2. G6PDH activity was significantly increased ($P < 0.05$) in rats exposed to CPOPD compared to the control, but the activity of the enzyme was significantly higher in rats exposed to CPOPD alone (Group B) compared to CPOPD-exposed rats treated with various extracts of *M. myristica* seed. As observed with LDH activity, the diethyl ether extract showed the highest ameliorative effect.

GPx activity was significantly decreased in rats exposed to CPOPD alone (Group B) compared to the control [Table 3]. No significant difference was observed in the activity of this enzyme in rats exposed to CPOPD alone (Group B) and those given tween 80 in addition to CPOPD (Group C). Conversely,

Table 1: Ameliorative effect of *M. myristica* seed extracts on LDH activity in the serum and heart of rats fed CPOPD

Groups	LDH (mg/dl)	
	Serum	Heart
A	250.39±8.15[a]	300.47±3.64[a]
B	310.56±3.85[b]	350.42±3.62[b]
C	311.48±3.87[b]	351.37±3.71[b]
D	299.17±2.25[c]	341.14±4.05[a]
E	279.00±3.80[d]	326.46±3.86[a]
F	271.75±9.98[d]	314.54±3.00[a]

Values are presented as mean±SD and as units/mg protein $n=5$. Values on the same column with different superscripts differ significantly ($P<0.05$). Groups: A (normal control), B (Crude petroleum oil-polluted diet [CPOPD] control); C (CPOPD plus tween 80); D (CPOPD plus *M. myristica* aqueous extract); E (CPOPD plus *M. myristica* ethanol extract); F (CPOPD plus *M. myristica* diethyl ether extract). *M. myristica: Monodora myristica*, LDH: Lactate dehydrogenase, SD: Standard deviation

Table 2: Ameliorative effect of *M. myristica* seed extracts on G6PD activity in the serum and heart of rats fed CPOPD

Groups	G6PD (mg/dl)	
	Serum	Heart
A	41.41±3.67[a]	52.32±3.55[a]
B	70.48±4.62[b]	77.40±4.99[b]
C	71.42±2.22[c]	76.48±3.85[b]
D	65.54±3.73[c]	68.57±3.79[c]
E	55.53±4.36[d]	61.61±3.99[c]
F	46.45±4.53[e]	53.45±2.62[d]

Values are presented as mean±SD and as units/mg protein $n=5$. Values on the same column with different superscripts differ significantly ($P<0.05$). Groups: A (normal control), B (Crude petroleum oil-polluted diet [CPOPD] control); C (CPOPD plus tween 80); D (CPOPD plus *M. myristica* aqueous extract); E (CPOPD plus *M. myristica* ethanol extract); F (CPOPD plus *M. myristica* diethyl ether extract). *M. myristica*: *Monodora myristica*. SD: Standard deviation

CPOPD-exposed rats treated with aqueous (Group D), ethanol (Group E), and diethyl ether (Group F) extracts had significantly higher GPx activities compared to rats maintained on CPOPD alone. Thus, the treatment of CPOPD-exposed rats with the extracts greatly reversed the CPOPD-induced reduction in GPx activity and restored the activity of the enzyme to a level higher than the activity recorded in the control rats.

The ameliorative effect of *M. myristica* seed extracts on GR activity in the serum and heart of rats feed CPOPD is presented in Table 4. The administration of CPOPD to rats (Group B) significantly reduced the activity of GR compared to rats in control (Group A). GR activity was not significantly affected when CPOPD-exposed rats received tween 80 compared to rats fed with CPOPD alone. However, there was a significant increase in GR activity when CPOPD-exposed rats received various extracts of *M. myristica* seed compared to rats maintained on CPOPD alone. The greatest increase was observed in CPOPD-exposed rats treated with diethyl ether extract of *M. myristica* seed. This was observed in the serum as well as in the heart tissue.

There was increased lipid peroxidation in rats upon exposure to CPOPD compared to rats in control [Table 5]. However, treated of CPOPD-exposed rats with various extracts of *M. myristica* seed significantly reduced the level of lipid peroxidation compared to rats exposed to CPOPD alone. Conversely, the activities of SOD and CAT were significantly reduced in rats exposed to CPOPD compared to control, whereas treatment of CPOPD-exposed rats with the various extracts of *M. myristica* seed significantly increased the activities of the enzymes compared to rats maintained on CPOPD alone. Again, the diethyl ether extract of *M. myristica* seed had the greatest ameliorative properties.

Table 3: Ameliorative effect of *M. myristica* seed extracts on GPx activity in the serum and heart of rats fed CPOPD

Groups	GPx (unit/g)	
	Serum	Heart
A	10.68±1.51[a]	25.44±7.99[a]
B	5.63±1.97[b]	14.20±5.06[b]
C	5.30±3.58[b]	13.20±4.06[b]
D	7.54±3.95[c]	17.53±3.75[c]
E	27.45±3.26[c]	19.61±7.12[d]
F	34.30±3.25[d]	24.46±3.23[e]

Values are presented as mean±SD and as units/mg protein $n=5$. Values on the same column with different superscripts differ significantly ($P<0.05$). Groups: A (normal control), B (Crude petroleum oil-polluted diet [CPOPD] control); C (CPOPD plus tween 80); D (CPOPD plus *M. myristica* aqueous extract); E (CPOPD plus *M. myristica* ethanol extract); F (CPOPD plus *M. myristica* diethyl ether extract). *M. myristica: Monodora myristica,* GPx: Glutathione peroxidase. SD: Standard deviation

Table 4: Ameliorative effect of *M. myristica* seed extracts on GR activity in the serum and heart of rats fed CPOPD

Groups	GR (unit/ml)	
	Serum	Heart
A	35.38±3.84[a]	42.61±3.08[a]
B	10.66±1.59[b]	22.31±4.09[b]
C	10.30±2.35[b]	22.36±3.27[b]
D	18.41±3.91[c]	34.53±5.17[c]
E	27.45±3.26[c]	30.49±7.99[d]
F	34.30±3.25[d]	40.55±7.20[e]

Values are presented as mean±SD and as units/mg protein $n=5$. Values on the same column with different superscripts differ significantly ($P<0.05$). Groups: A (normal control), B (Crude petroleum oil-polluted diet [CPOPD] control); C (CPOPD plus tween 80); D (CPOPD plus *M. myristica* aqueous extract); E (CPOPD plus *M. myristica* ethanol extract); F (CPOPD plus *M. myristica* diethyl ether extract). *M. myristica*: *Monodora myristica*, GR: Glutathione reductase. SD: Standard deviation

Histology of the Heart

Figures 1-6 show the histology of the heart tissue of rats in the various experimental groups. Rats in the control group had normal heart histology features [Figure 1]: Normal cardiac tissue, thick CW, CM, and CV. However, exposure to CPOPD altered the normal histology of the heart, as shown in Figure 2, in which FS in CW and BD of CM cell was observed. A similar feature was observed in CPOPD-exposed rats given tween 80 [Figure 3]. Administration of various extracts of *M. myristica* seed to CPOPD-exposed rats caused significant improvement in the CPOPD-induced histological alterations, as shown in Figures 4-6. Figure 6 shows reduced FS and BD of CM, indicating a slight improvement in cardiac tissue architecture, while Figures 5 and 6 show more improvement in cardiac tissue architecture with a minimal area of FS and improved CM cell.

DISCUSSION

Given the many toxic effects of crude petroleum oil and the reported antioxidant potentials of *M. myristica* seed, this study was designed to show the ameliorative effects of various extracts of *M. myristica* seed in rats exposed to CPOPD.

Exposure of rats to CPOPD significantly increased ($P < 0.05$) the activity of LDH [Table 1] and G6PDH [Table 2], but significantly reduced GPx [Table 3] and GR [Table 4] activities compared to rats in the control group. These results are manifestations of toxic effects of crude petroleum which is in consonance with earlier studies.[29] Achuba *et al*.[30] reported that crude oil present in food or diet has the ability to elicit free

Table 5: Ameliorative effect of *M. myristica* seed extracts on activities of SOD, CAT, and level of MDA in the heart of rats fed CPOPD

Groups	Parameters		
	SOD (unit/g)	CAT (unit/g)	MDA (unit/g)
A	88.74±6.14[a]	74.57±3.20[a]	0.52±0.20[a]
B	57.66±2.58[b]	42.37±4.42[b]	4.15±1.46[b]
C	57.40±5.29[b]	42.31±7.09[b]	4.28±2.63[b]
D	62.81±3.34[b]	53.31±2.90[c]	2.20±0.82[c]
E	71.20±2.50[a]	62.31±1.72[d]	1.51±0.21[d]
F	80.37±7.60[a]	60.27±29.41[d]	0.60±0.15[d]

Values are presented as mean±SD and as units/mg protein $n=5$. Values on the same column with different superscripts differ significantly ($P<0.05$). Groups: A (normal control), B (Crude petroleum oil-polluted diet [CPOPD] control); C (CPOPD plus tween 80); D (CPOPD plus *M. myristica* aqueous extract); E (CPOPD plus *M. myristica* ethanol extract); F (CPOPD plus *M. myristica* diethyl ether extract) Histology of the heart. *M. myristica*: *Monodora myristica,* SOD: Superoxide dismutase, CAT: Catalase, MDA: Malondialdehyde. SD: Standard deviation

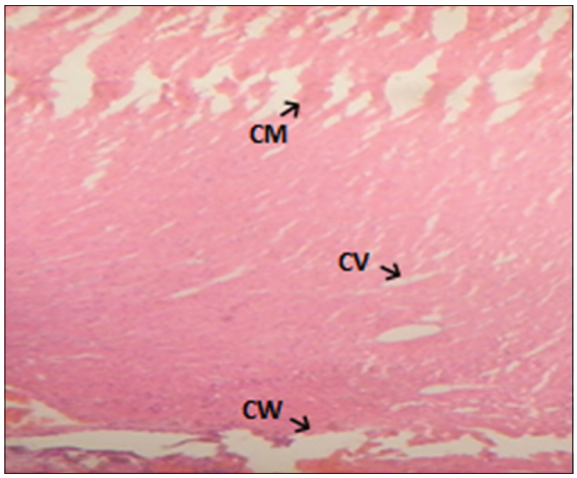

Figure 1: Group A (hematoxylin and eosin stain ×200)

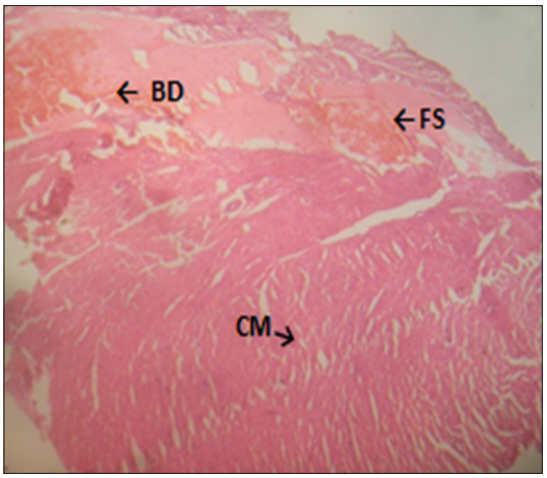

Figure 4: Group D (hematoxylin and eosin stain ×200)

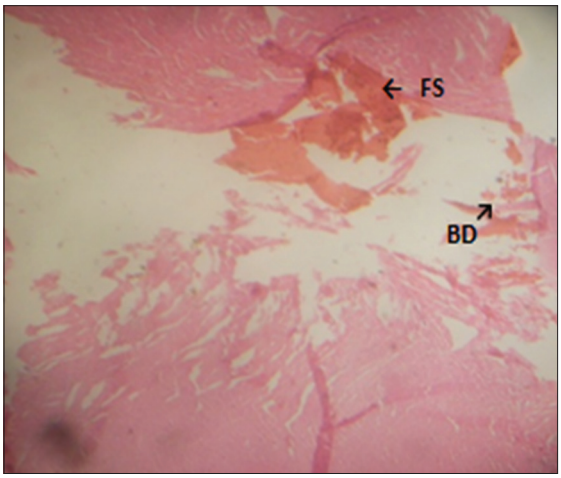

Figure 2: Group B (hematoxylin and eosin stain ×200)

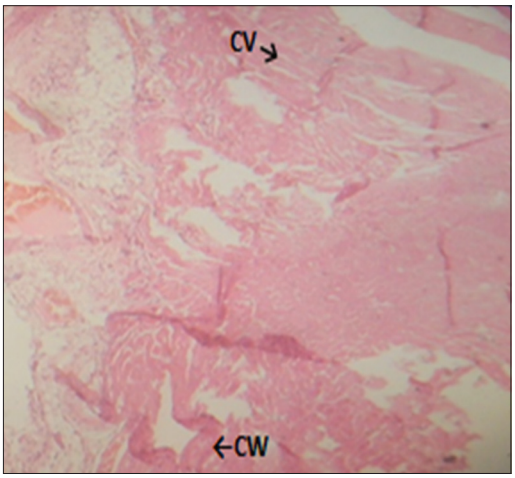

Figure 5: Group E (hematoxylin and eosin stain ×200)

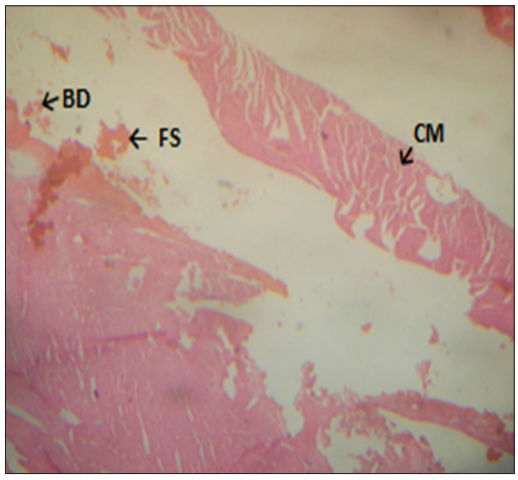

Figure 3: Group C (hematoxylin and eosin stain ×200)

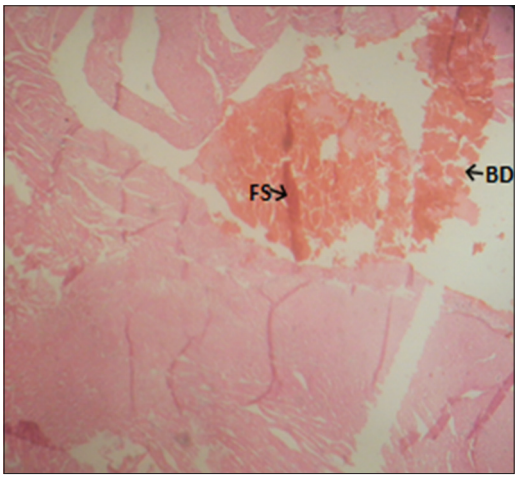

Figure 6: Group F (hematoxylin and eosin stain ×200)

radical production in the serum and heart of rats resulting in an increase in oxidative stress in cellular tissues which in turn leads disease states such as cancer and cardiac dysfunction.

LDH, a marker of cardiac function, catalyzes the reversible formation of lactate and pyruvate in tissues and its activity is elevated under anaerobic conditions to compensate for energy loss when oxygen is not available to facilitate the breakdown of pyruvate through the citric acid pathway.[31] Thus, the ability of various extracts of *M. myristica* seed to reverse the CPOPD-induced elevation in LDH activity in this

study is indicative of the antioxidant potentials of the extracts in combating CPOPD-induced oxidative stress probably by mopping up reactive oxygen species (ROS). The seeds *M. myristica* contain powerful antioxidants and pharmacological compounds such as flavonoids, Vitamin A, saponin, alkaloids, and phenols that are capable of quenching the oxidative stress induced by the consumption of crude oil contaminated diet.[32] Plants of the Annocacea family have also been shown to be rich in flavonoids, which are highly active antioxidants in living systems.[33]

G6PD controls the flow of substrate into the pentose phosphate pathway being the major source of reduced NADPH that is used by the glutathione pathway to combat ROS.[34-37] Thus, CPOPD-induced elevation in the activity of G6PD observed in this study could be a compensatory mechanism necessary to oppose the CPOPD-increased generation of ROS in heart tissue. It has been observed that the reduction of G6PD activity in cardiac cells reduces their antioxidant capacity, thereby increasing their susceptibility to ROS-induced cell death.[38] Conversely, the abundant molecules in the extracts of *M. myristica* seed reduced oxidative stress, which explains the reduction in G6PD activity observed in CPOPD-exposed rats treated with the extracts of *M. myristica* seed. The most important role of NADPH in cells is the regeneration of reduced glutathione catalyzed by GR. GR also plays an important role in the detoxification of xenobiotics. The inhibition of GR may lead to a low concentration of GSH and a corresponding high level of reactive species in the tissues resulting in oxidative stress.[39] This is in consonance with the result of this study, where CPOPD induced reduction in GR activity.

The activities of SOD and CAT, endogenous antioxidants, were also studied and it was observed that exposure of rats to CPOPD caused a significant decrease in tissue SOD and CAT activities and a contaminant increase MDA levels [Table 5]. An increase in MDA is an indication of oxidative stress, which has been shown to result from the ingestion of crude petroleum oil contaminated diets.[4,10,40] According to Patra *et al*.[41] CAT, SOD, and GR are the prime endogenous antioxidant enzymes that sequester ROS and protect cells from oxidative damage. Thus, the CPOPD-induced reduction in the activity of these three enzymes witnessed in this study confirms the toxic effects of CPOPD. Conversely, the ability of the various extracts of *M. myristica* seed to cause an elevation in the activities of SOD, CAT, and GPx may be due to the antioxidant molecules and properties of the extracts. This is in consonance with the works of Erukainure *et al*.[14] and Hamza and Al-Harbi[42] which showed that *M. myristica* has protective potentials against free radicals *in vitro*. Adefegha and Oboh;[43] Akinwunmi and Oyedapo;[16] and Moukette *et al*.[17] also showed that extracts of *M. myristica* inhibit lipid peroxidation. Thus, this study agrees with George and Osioma[19] that *M. myristica* has antioxidant properties that can be used by food and pharmaceutical industries as sources of natural antioxidants.

There was no significant difference in almost all the parameters examined between rats maintained on CPOPD alone (Group B) and those which received tween 80 in addition (Group C). Similar observations have previously been made by Rowe *et al*.[44] and by Okpoghono *et al.*[10] and it points to the fact that tween 80 used as a solvent stabilizer in these experiments is non-toxic. In all the parameters examined, the diethyl ether extract of *M. myristica* seed had higher antioxidant and ameliorative effects compared to the aqueous and ethanol extracts. This is in agreement with the report of George and Okpoghono,[18] who examined the antioxidant activity of water, ethanol, and diethyl ether extracts of *M. myristica in vitro*. This is probably because it is a better solvent for the extraction of phytochemical content in *M. myristica* and, as such contained, more bioactive compounds compared with the other two extracts.

Histological examination of heart tissue revealed FS in CW and BD of CM in CPOPD-exposed rats compared with the control, which are indications of the toxic effects of CPOPD already seen in the various biochemical analysis. This result is in consonance with the report of Anakwue and Otamiri[45] where exposure to petroleum products caused marked negative effects on cardiac tissues. The ability of *M. myristica* seed extracts to ameliorate the CPOPD-induced changes in heart histology may be attributed to the antioxidant powers of the extracts, which have also been attested to by George and Okpoghono.[18]

CONCLUSION

This study showed that selected oxidative stress markers in the serum and heart (SOD, CAT, MDA, LDH, G6PDH, GPx, and GR) were significantly ($P < 0.05$) altered due to ingestion of CPOPD and is attributable to the oxidative stress induced by CPOPD. Histological analysis of the heart showed considerable damage in heart tissues leading to necrosis and degeneration of cardiac cells. However, this study has shown that the induced toxicity in serum and hearts of rats fed CPOPD was markedly reduced in rats treated with *M. myristica* extracts due to their antioxidant properties which effectively quenched the CPOPD-induced oxidative stress.

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