



The assessment of the ameliorative effects of *Monodora myristica* extracts on kidney function indices in crude petroleum oil induced nephrotoxicity

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ABSTRACT

Consumption of diet contaminated with crude petroleum oil (CPO) generates reactive oxygen species which may cause kidney damage in humans and animals. *Monodora myristica* is a spice commonly consumed in the Niger Delta region of Nigeria. This study evaluated the ameliorative effects of *M. myristica* extracts on kidney function indices in CPO induced nephrotoxicity. Thirty adult male Wistar rats (180 ± 5 g) were randomly placed in six groups of 5 rats each. Group 1 served as the control; group 2 had crude petroleum oil contaminated catfish diet (CPO-CCD); group 3 received CPO-CCD plus tween 80; group 4 received CPO-CCD plus 200 mg/kg b. wt. of *M. myristica* aqueous extract (MAE); group 5 was given CPO-CCD plus 200 mg/kg b. wt. of *M. myristica* ethanol extract (MEE) and group 6 took CPO-CCD plus 200 mg/kg b. wt. of *M. myristica* diethylether extract (MDEE). The feeding of the rats with CPO-CCD and administration of extracts orally lasted for 28 days. Serum creatinine, urea, uric acid and malondialdehyde (MDA) increased significantly ($p < 0.05$) in rats fed CPO-CCD. Similarly, serum electrolyte levels increased significantly ($p < 0.05$) in this group compared with group 1. However, treatment with MAE, MEE and MDEE resulted in a decrease in kidney function parameters, MDA and electrolyte levels compared to group 2 given crude petroleum oil contaminated catfish diet. Also, MDEE had better renal restorative activity compared with MEE and MAE. Therefore, *M. myristica* extracts can suppress the toxicity effects of crude petroleum oil-induced renal dysfunction.

Keywords: Nephrotoxicity, Spice, *Monodora myristica*, electrolyte, petroleum oil

INTRODUCTION

Crude petroleum oil exploration in the Niger Delta region of Nigeria has brought huge financial advantage for human and infrastructural development in the country. However, this exploration has also endangered both land and marine lives; the aboriginal people who depend on the ecosystem for their survival have been confronted with starvation, poverty, displacement, and diseased conditions. Crude oil is a mixture of hydrocarbons; it exists in liquid phase in natural underground reservoirs and remains liquid at atmospheric pressure after passing through surface separation facilities (Azeez *et al.*, 2013). The crude oil and its products on consumption generate free radicals and promote a variety of chemical reactions, such as depletion of antioxidant parameters or inducing lipid peroxidation leading to oxidative stress (Achuba, 2018). Oxidative stress is an imbalance between oxidant and antioxidants in favour of the oxidant causing cell, tissue and organ damage (Hryciak and Bandiera, 2015).

Consumption of diet contaminated with crude petroleum oil generates reactive oxygen species which may cause neurotoxicity in humans and animals (Achuba, 2018; Azeez *et al.*, 2013; Khan *et al.*, 1986). The kidneys play an important role in the regulation of fluids and electrolytes and when they malfunction it often leads to electrolytes imbalance. Electrolytes are charged particles involved in the transmission of impulses in nerve and muscles. When

imbalances occur, this can lead to serious complications and even death (Enwereuzoh *et al.*, 2015).

Monodora myristica is popularly known as nutmeg, it is a tropical spice that originated from the West African continent. *M. myristica* is a perennial plant that belongs to the family of Annonaceae (Omobuwajo *et al.*, 2003) and its seeds are an aromatic spice that act as a local nutmeg substitute. *M. myristica* has its common names as African nutmeg, calabash nutmeg, and in Nigeria, it is called “ere” in Urhobo, “Ehuru” in Igbo, “Abolakoshe” in Yoruba and “Ebenoyoba” in Benin (Chinelo *et al.*, 2018). *M. myristica* is a spice commonly consumed in the Niger Delta part of Nigeria especially by the Itsekiri, Urhobo and Ukwuani people (Okpoghono *et al.*, 2018). The aromatic fragrance of nutmeg makes it suitable for spicing assorted food types such as vegetables, confections, sausages, sauces, meats and puddings etc (Esiekpe *et al.*, 2020). The roasted powdered seed relief headache and prevent skin diseases (Chinelo *et al.*, 2018); a preparation of the seed serves as stimulant to aid digestion and control passive uterine hemorrhage in women immediately after child birth (Chinelo *et al.*, 2018). The essential oil from the seed of *M. myristica* have been reported to have reducing effect on oxidative level of other oil when they are mixed (Esiekpe *et al.*, 2020).

Presently, there are limited literature reports on the health protective role of *M. myristica* seed on exposure to crude

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oil pollution. This study is therefore centered on the ameliorative effect of *M. myristica* extracts on kidney

MATERIALS AND METHODS

Chemical and reagents

Dichloromethane, ethanol and diethyl ether were all purchased from BDH chemical laboratory England. Anhydrous sodium sulphate was purchased from Sigma chemical company, London, England. All other chemicals used in this investigation were of analytical grade.

Collection of *M. myristica*

The plant spice, *M. myristica* was bought from Agbrha-Otor market in Ughelli North Local Government area of Delta State, Nigeria. The seed was authenticated at the Department of Plant Botany, Delta State University, Abraka, Delta State, Nigeria. The plant scientific name was checked with <http://www.theplantlist.org> on 9th October, 2021.

Preparation of *M. myristica* extracts

The seeds of *M. myristica* were air-dried to constant weight for 14 days. The spice was pulverized using Binatone blender (BLG-595). The extraction of pulverized *M. myristica* seeds was done by macerating 100 g in 500 mL of the respective solvents: hot water, 60 °C, ethanol (99.5% v/v) and diethylether (95 % v/v) for 48 h. The infusions from the seeds were then filtered and concentrated to dryness with a rotary evaporator (RE 300, Bibby Scientific, UK). one gram of each extract was dissolved in 9 mL aqueous tween 80 (1% tween 80 in 90 mL of distilled water (v/v)). The samples were kept in the refrigerator at - 4 °C.

Simulation of crude petroleum oil pollution

The crude petroleum oil sample (Warri Excavos light crude oil) was collected from the Nigerian National Petroleum Cooperation Refinery, Warri, Delta State, Nigeria. Fifty healthy catfish (*Clarias gariepinus*) weighing 250–300 g were collected from an artificial pond at Agbrha-Otor in Ughelli North Local Government area of Delta State, Nigeria. They were allowed to acclimatize for 7 days and then randomly divided into two groups in 30 L plastic aquaria. Group 1 served as the control because the water was not polluted while group 2 was cultured in water exposed to 823.3 µl/ of crude oil (Okpoghono *et al.*, 2018) for 4 weeks. The catfish were fed ad libitum with fish meal and the water changed every 24 h. At the end of the experimental 4 weeks period, the catfish were harvested and then used to formulate diet for the rats.

Experimental diet formulation

The components of the formulated diet in percentage were: Corn starch (52%), oil (4%), maize cob (4%), granulated refined sugar (10%), a mixture of vitamins-minerals (5%), and the catfish oven dried at 40 °C (25%) (Okpoghono *et al.*, 2018). These were formed into pellet after mixing with tween 80.

function indices namely serum creatinine, urea, uric acid and electrolytes levels.

Experimental procedure

Thirty adult male Wistar rats (180 ± 5 g) were obtained from the animal house of the Faculty of Basic Medical Sciences, Delta State University, Abraka. They were maintained in accordance with the National Institutes of Health (NIH, 1985) guidelines. They had free access to clean tap water and standard growers mash diet. The rats were housed in standard wooden cages in a room (25 ± 2 °C) with adequate light. These rats were allowed to acclimatize for one week and randomly placed in six groups of 5 rats each. Group 1: Control; Group 2: Crude petroleum oil contaminated cat fish diet (CPO-CCD); Group 3: CPO-CCD plus tween 80; Group 4: CPO-CCD plus 200 mg/kg b. wt. of *M. myristica* aqueous extract (MAE) Group 5: CPO-CCD plus 200 mg/kg b. wt. of *M. myristica* ethanol extract (MEE); Group 6: CPO-CCD plus 200 mg/kg b. wt. of *M. myristica* diethylether extract (MDEE). These rats were fed with CPO-CCD and orally administered the extracts for four weeks and then sacrificed after 24 h fast on the last day of the study. The blood was collected into anticoagulant free test tubes. After wards the clotted blood was centrifuged at two thousand five hundred revolution per minutes (RPM) for fifteen minutes to separate the serum, which was stored in the refrigerator (- 4 °C) for further biochemical analysis.

Biochemical Assays

The assessment of kidney function profiles was done with commercial Tecco diagnostic kits for potassium ion, sodium ion, calcium ion, chloride ion, bicarbonate ion and creatinine (Cr). Randox diagnostic kit was used for the determination of serum urea level. Lipid peroxidation (LPO) was determined based on the reaction of malondialdehyde (MDA) with thiobabituric acid (TBA) to form a MDA – TBA adduct that absorbs light strongly at 532 nm (Zeb and Ullah, 2016).

Statistical Analysis

The statistical analysis was performed using the statistical package for social science (SPSS) for windows, version 16.0 (SPSS Inc., Chicago, IL, USA). The single Factor analysis of Variance (ANOVA) test was used to determine significance differences between the groups. Post hoc analysis (comparisons across Groups) was done using Bonferroni at P < 0.05 level of significance.

RESULTS

Effect of *M. myristica* seed extracts on renal function indices in the serum of rat fed crude petroleum oil contaminated catfish diet (CPO-CCD).

The changes in serum creatinine, urea and uric acid in rats fed CPO-CCD and administered *M. myristica* seed extracts are shown in Table 1. Serum creatinine, urea and uric acid increased significantly ($p < 0.05$) in rats fed only CPO-CCD when compared with the control. However, treatment with MAE, MEE and MDEE resulted in decrease in serum creatinine, urea and uric acid when compared with CPO-CCD only (Table 1). The decrease in these renal markers in the group treated with MDEE was significant ($p < 0.05$)

when compared with CPO-CCD only. Administration of extracts to CPO-CCD fed rats resulted in a decrease in lipid peroxidation (LPH). Serum malondialdehyde (MDA) was

significantly lower ($p < 0.05$) in the extract treated groups compared with the group fed only CPO-CCD.

Table 1: Serum creatinine, urea, uric acid levels and MDA concentration of rats fed CPO-CCD and administered *M. myristica* seed extracts

Groups	Creatinine (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)	MDA (units/mL)
Control	5.54 ± 0.83 ^a	8.51 ± 1.80 ^a	16.38 ± 2.46 ^a	0.33 ± 0.06 ^a
CPO-CCD only	12.60 ± 1.66 ^b	20.39 ± 0.65 ^b	30.43 ± 4.80 ^c	4.96 ± 1.08 ^d
CPO-CCD + Tween 80	12.30 ± 2.39 ^b	20.53 ± 1.84 ^b	31.50 ± 0.96 ^c	3.93 ± 0.98 ^{cd}
CPO-CCD + MAE	10.14 ± 2.48 ^{ab}	18.24 ± 3.34 ^b	26.52 ± 2.84 ^{bc}	2.50 ± 0.35 ^{bc}
CPO-CCD + MEE	7.30 ± 1.13 ^{ab}	17.38 ± 2.78 ^b	20.34 ± 1.34 ^{ab}	0.81 ± 0.22 ^{ab}
CPO-CCD + MDEE	5.16 ± 1.13 ^a	8.50 ± 1.40 ^a	16.78 ± 2.93 ^a	0.40 ± 0.12 ^a

Values are expressed as mean ± SEM, n = 5/ group. Mean values with different alphabet superscript in the same column are significantly different ($P < 0.05$). Where: MAE, *M. myristica* aqueous extract; MEE, *M. myristica* ethanol extract and MDEE = *M. myristica* diethyl ether extract

Effect of *M. myristica* seed extracts on electrolyte levels of rat fed crude petroleum oil contaminated catfish diet (CPO-CCD).

According to Table 2, there was a significant ($p < 0.05$) increase in serum electrolyte level of the group fed on CPO-CCD only when compared with the control (Table 3). Treatment of CPO-CCD fed rats with the extracts (MAE, MEE and MDEE) resulted in a decrease in serum electrolytes when compared with the group fed only CPO-

CCD. The decrease in sodium, calcium, chloride and bicarbonate ion was only significant ($p < 0.05$) in the group administered MEE and MDEE. Treatment of CPO-CCD rats with MDEE resulted in electrolyte level that were not significantly ($p > 0.05$) different from the control.

Table 2: Serum electrolyte levels of rats fed CPO-CCD and administered *M. myristica* seed extracts

	Na ⁺ (mEq/L)	K ⁺ (mEq/L)	Ca ²⁺ (mEq/L)	Cl ⁻ (mEq/L)	HCO ₃ ⁻ (mEq/L)
Control	80.41 ± 1.59 ^a	9.68 ± 0.46 ^a	12.78 ± 0.62 ^a	14.43 ± 1.45 ^a	34.24 ± 1.19 ^a
CPO-CCD only	170.36 ± 3.53 ^c	18.36 ± 2.65 ^c	20.59 ± 1.05 ^b	35.38 ± 1.55 ^d	56.36 ± 4.72 ^{bc}
CPO-CCD + Tween 80	170.51 ± 3.50 ^c	18.41 ± 1.47 ^c	21.07 ± 1.78 ^b	35.59 ± 1.64 ^d	60.44 ± 1.99 ^c
CPO-CCD + MAE	150.27 ± 17.02 ^c	16.27 ± 2.70 ^{bc}	16.50 ± 2.29 ^{ab}	29.50 ± 1.31 ^c	52.41 ± 2.17 ^b
CPO-CCD + ME	120.41 ± 6.96 ^b	14.28 ± 1.33 ^{abc}	14.45 ± 2.21 ^a	20.47 ± 1.72 ^b	40.41 ± 1.62 ^a
CPO-CCD + MDEE	100.42 ± 6.40 ^{ab}	10.36 ± 1.87 ^{ab}	12.61 ± 0.72 ^a	15.39 ± 1.83 ^a	33.74 ± 1.13 ^a

Values are expressed as mean ± SEM, n = 5/ group. Mean values with different alphabet superscript in the same column are significantly different ($P < 0.05$). Where: MAE, *M. myristica* aqueous extract; MEE, *M. myristica* ethanol extract and MDEE = *M. myristica* diethyl ether extract.

DISCUSSION

In this investigation, assessment was done on changes in renal function indices, electrolyte balance and oxidative stress parameter of rat fed with catfish diet contaminated with crude petroleum oil and treated concurrently with *M. myristica* seed extracts. The study of the toxicant suppressive effect of *M. myristica* in laboratory animals is of great relevance because crude oil spilled into natural water body in the Niger-delta region of Nigeria does accumulate in aquatic animals like fish; humans get expose to these harmful hydrocarbons when fish is consumed as a source of protein. These toxicants perturb homeostatic balance, increase oxidants levels and damage internal organs (Okpoghono *et al.*, 2018).

The significant increase in creatinine, urea and uric acid levels in the serum of rats fed with crude petroleum oil contaminated catfish diet (CPO-CCD) concurred with earlier literature reports (Azeez *et al.*, 2013; Momoh and Oshin, 2015). The rise in serum urea levels could suggest reduced reabsorption at the epithelial of the kidney while elevated level in creatinine shows kidney dysfunction cause by CPO-CCD. The coadministration of MAE, MEE and MDEE to rats placed on CPO-CCD countered the toxicity of the crude oil and resulted in decreased creatinine, urea and uric acid levels in the serum. The animals treated with MDEE were the most protected because the kidney function parameters were almost restored to basal levels. The protective effects of plant materials against crude oil

induced organ toxicity was previously reported (Okpoghono *et al.*, 2018; Esiekpe *et al.*, 2020). The literature data show induction in monoamine oxidase, xanthine oxidase, aldehyde oxidase and sulphite oxidase on administration of *Vernonia amygdalina* to rat fed with hydrocarbon polluted diet. These oxidases help to breakdown the harmful constituent of hydrocarbon toxicants, namely sulfoxides, N-oxides, and aromatic nitro compounds and 1,2-benzisoxazole derivatives (Achuba, 2018). *M. myristica* may have similar mode of action with *Vernonia amygdalina* in aiding rapid clearance of hydrocarbon to abate petroleum induced nephrotoxicity and enhance the ability of the kidney to excrete creatinine, urea, and uric acid with subsequent reduction in serum levels in their values.

The rise in serum and tissue MDA in CPO-CCD fed rats in this investigation agrees with previous data on crude oil toxicity in which there was an increase in free radical production (Lee *et al.*, 1985; Khan *et al.*, 1986) and lipid peroxidation (Achuba, 2018). There are many possible reasons for oxidant increase associated with hydrocarbon toxicity. First, constituents of crude oil can induce cytochromes P450 (CYPs) enzymes. CYPs are a superfamily of enzymes containing heme as a cofactor that functions as part of NADPH/O₂-dependent microsomal electron transport system (Hrycay and Bandiera, 2015).

The monooxygenase activity (the incorporation of atomic oxygen from O₂ into organic compounds) and peroxygenase activity (the use of atomic oxygen from H₂O₂ to oxygenate substrates) of CYPs enzyme ensure hydrocarbon catabolism. However, the process of oxygen transfer is not perfect because substrate target can be missed such that there will be uncoupling, i.e., the atomic oxygen is erroneously used to produce superoxide radicals (O₂⁻) and hydroxyl radicals (•OH); these ROS can initiate the production of other oxidants as well as react with the lipid component of the cell membrane to produce MDA as a byproduct. Second, the constituents of crude oil can inhibit the mitochondrial β-hydroxybutyrate and succinate-supported electron transfer activity as well as impair the mitochondria membrane electron transport system; these events do result in electron leakage with concomitant generation of tissue damaging ROS (Khan *et al.*, 1986).

The reduction in MDA on administration of MAE, MEE and MDEE is suggestive of the free radical scavenging activity of *M. myristica* in CPO-CCD induced oxidative stress. The seed of *M. myristica* is rich in bioactive compounds like saponins, alkaloids, phenols etc (Iwu, 2014; Chinelo *et al.*, 2018). These phytochemicals have electron transfer and hydrogen atom contributing activity to neutralize harmful ROS (Irabor *et al.*, 2021). These pharmacologically active substances could also boost endogenous antioxidants like SOD, catalase, and glutathione peroxidase through induction in their gene expression and RNA transcription. SOD inhibits free radical by dismutating superoxide radical into hydrogen peroxide while catalase catalyzes hydrogen peroxide into water and molecular oxygen; glutathione peroxidase uses reduced glutathione to peroxidize hydrogen peroxide into water and oxidized glutathione (Irabor *et al.*, 2021).

Though data obtained in this present study show significant increase in major serum electrolytes (potassium, sodium, calcium, chloride, and bicarbonate ions) in rats administered only petroleum tainted diet (CPO-CCD). However, previous study indicated significant elevation on selected serum electrolytes (potassium and calcium ions) (Achuba, 2018). There is an agreement between these two independent research since an alteration in potassium level is the most convincing electrolyte marker for renal dysfunction (Chen *et al.*, 2013; Ratliff *et al.*, 2016).

Furthermore, the increase in serum potassium is probably as a result of decreased filtration and decreased secretion of potassium in distal tubule during renal perturbation. This electrolyte imbalance may have been caused by the nephrotoxic effect of hydrocarbon through enhance ROS (Ratliff *et al.*, 2016). The increase in free radical has been implicated in the development of proximal tubular disease which can lead to renal cell damage and death. The downstream effect of this event is a reduced Na⁺/K⁺ ATPase activity and weak tubular transport of electrolyte. Co-administration of MAE, MEE and MDEE with hydrocarbon tainted diet was able to gradually restore electrolyte levels to normal in respective animals.

The bioactive constituents in these plant extracts may have mop up free radical associated with hydrocarbon catabolism. MDEE was the most active in restoring electrolyte concentration to basal levels; the pharmacologically active component of nutmeg could be less soluble in MAE and MEE and more soluble in MDEE. Therefore, the restorative potency of MDEE may be related to the presence of high contents of flavonoids, phenolics (George and Okpoghono, 2017), saponins, alkaloids, tannins, proanthocyanidins and other phytochemicals. Generally, flavonoids are known to inhibit lipid peroxidation, however, the availability of other phytoconstituents in high concentration in MDEE (George and Okpoghono, 2017) may have resulted in synergistic phytochemical action to maintain the integrity of the kidneys through the scavenging of free radicals and the enhancement of renal functions.

CONCLUSION

The results of this investigation show that consumption of crude petroleum oil contaminated catfish diet was hazardous to the kidney with the consequence of debilitation in renal function. But the administration of *M. myristica* seed extracts suppressed the induced negative effects of crude petroleum oil and restored renal function indices close to normal. Furthermore, this assessment revealed that treatment with MDEE was better in mitigating crude petroleum oil induced renal toxicity than MEE and MAE. Therefore, the use of *M. myristica* spice to supplement diet may be a wise nutrition option to protect the health of people living in areas where there is crude oil exploration.

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