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Short Communication

Protective effect of *Monodora myristica* extracts on crude petroleum oil-contaminated catfish (*Clarias gariepinus*) diet in rats

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ABSTRACT

Humans and animals are constantly exposed to crude petroleum contaminated diets in petroleum producing areas of the world. As a result, researches are on-going to find simple ameliorative agent against crude petroleum contaminated diet toxicity. The aim of this study was to evaluate the protective effect of *Monodora myristica* on some biochemical parameters of rats fed with crude petroleum oil contaminated catfish diet (CPO-CCD). Thirty male albino rats were separated into six groups of five rats as follows: group 1: control, group 2: rats were fed CPO-CCD only, group 3: CPO-CCD plus 1 ml/kg of 1 % tween 80, group 4: CPO-CCD plus *M. myristica* water extract (MWE), group 5: CPO-CCD plus *M. myristica* ethanol extract (MEE) and group 6: CPO-CCD plus *M. myristica* diethyl ether extract (MDEE). The feeding of the rats with CPO-CCD and administration of extracts orally lasted for 28 days. The results showed significant ($P < 0.05$) increase in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) in the serum and tissues (liver, kidney and brain) and decrease in total protein, albumin and globulin in the serum and liver of group 2 and 3 when compared with group 1. Significant ($P < 0.05$) decrease in AST, ALT, ALP activities and increase in total protein, albumin and globulin levels were observed after treatment with *M. myristica* extracts (group 4, 5, and 6) when compared with group 1. However, it could be concluded that MDEE revealed a strong effect when compared with the MEE and MWE.

1. Introduction

Crude petroleum oil (CPO) contains high amount of toxic chemicals, which can cause a wide range of health effects in people and wildlife, depending on the level of exposure and susceptibility [1]. The constituents of crude petroleum oil are very complex. It contains aliphatic, alicyclic, polycyclic aromatic hydrocarbons, oxygen, sulphur and nitrogen containing substances [2]. The polycyclic aromatic hydrocarbon content of CPO consists of fused aromatic benzene [3].

Exposure of human and animals to these chemicals depends on different stage of CPO usage and environmental level [4]. Crude petroleum oil has been reported as a mediator in oxidative stress [5], which may lead to various dreadful diseases like cancer [6], dementia, atherosclerosis, multiple sclerosis, cardiac dysfunction, blood disorders, hepatic morphological abnormalities [6], nephrotoxic effects [7], etc.

The use of antioxidant in ameliorating the deleterious effect of free radicals has been the subject of previous investigations [8–11]. Hence, use of spice with antioxidants property is necessary to suppress oxidative stress in a healthier way [12]. Spice and herbs have been studied for their antioxidant activities. Although there are some studies on the antioxidant activity of *M. myristica*, its effect on CPO-CCD induced toxicity is scarce in literature. The study was carried out to examine the protective effect of *M. myristica* extracts on crude petroleum oil contaminated catfish (*Clarias gariepinus*) diet stimulated toxicity in rats. The findings of the study could be helpful to the inhabitants of the Niger Delta region of Nigeria and other parts of the world where CPO is produced to be enlightened on the benefit of including such antioxidant spice in their diet.

2. Materials and methods

2.1. Chemicals 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. ALT, AST, ALP, albumin and total protein kits were obtained from Randox laboratories limited (Antrim, England). All other reagents used were of

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analytical grade.

2.2. Spice

The spice *M. myristica* was purchased from the local market in Obiaruku, Delta State, Nigeria. The spice was identified at the Department of Botany, Delta State University, Abraka, Delta State, Nigeria.

2.3. Preparation of spice extracts

The spice (*M. myristica*) was sun-dried to constant weight for two weeks and then crushed into fine particles using electric blender. Extraction was carried out using one hundred grams of the powdered spice with 500 mL of the respective solvent (hot water (60 °C), ethanol (95% v/v), and diethyl ether, 95% v/v). The mixture was allowed to stand for 48 h and then filtered using a clean muslin cloth. The filtrate was evaporated to dryness using rotary evaporator attached to a vacuum pump. From the crude extracts (dry samples) 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.

2.4. Stimulation of crude petroleum oil pollution

The CPO was obtained from the Nigerian National Petroleum Cooperation (NNPC), refinery, and reported to have been extracted from Warri (Warri Excarvos light crude oil) in Delta State, Nigeria. The fish used for this study was collected from artificial pond in Obiaruku, Nigeria. They were allowed to acclimatize for 7 days and then divided into two groups.

Group 1: control: the catfish was cultured in plastic aquaria with 30 L borehole water for four weeks. Group 2: the catfish was cultured in plastic aquaria with 30 L borehole water and then polluted with crude petroleum oil (LD 50 toxicity in catfish, 823.3 μL/L) as described by Ikeogu et al. [13] for four weeks. The water was changed and re-polluted every 24 h.

2.5. Formulation of diet

The formulation of diet was carried out by the method described by Sunmonu and Oloyede [14]. The catfish were used as a source of protein (25%) to formulate diet for the rats, after it has been oven dried at 40 °C. The diet for each group was formulated using corn starch (52%), oil (4%), maize cob (4%), granulated refined sugar (10%) and vitamin/mineral mixture (5%).

2.6. Experimental procedure

Male albino rats were used for the study to avoid oestrous cycle complication. They were allowed to acclimatize for two weeks and had free access to water. The rats were supplied with standard growers mash diet gotten from Lagos State (Bio-Ingredients Limited Ikeja, Lagos). They were maintained in accordance with the National Institutes of Health (NIH) guidelines and then placed in six groups (5 rats each) as follows:.

Group 1: Control
Group 2: Crude petroleum oil contaminated catfish diet (CPO-CCD) control
Group 3: CPO-CCD plus tween 80
Group 4: CPO-CCD plus 200 mg/kg b. wt. of MWE
Group 5: CPO-CCD plus 200 mg/kg b. wt. of MEE
Group 6: CPO-CCD plus 200 mg/kg b. wt. of MDEE

Rats in groups 1–6 received tap water daily throughout the experiment and then sacrificed after 24 h fast on the last day of the study. The blood was collected into anticoagulant free test tubes. The organs were harvested immediately. One gram (1 g) of various tissues was homogenized in 10 mL of normal saline. After wards the clotted blood and the tissue homogenate were centrifuged at two thousand five hundred revolution per minutes (RPM) for fifteen minutes to separate the serum and tissues supernatants, which was stored in the refrigerator (−4 °C) for further biochemical analysis.

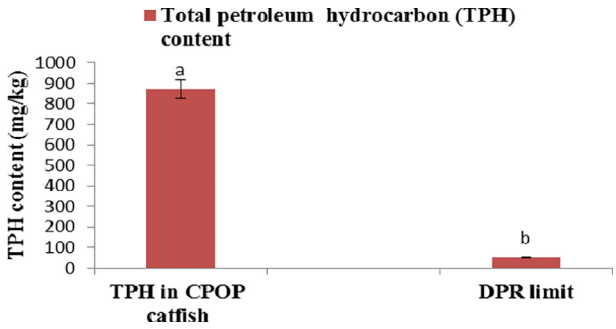

Fig. 1. Level of total petroleum hydrocarbon content in crude petroleum oil polluted (CPOP) catfish. Mean bars with different superscript letters (a, b) differ significantly ($P < 0.05$). DPR limit for crude oil polluted catfish = 50 mg/kg [21]

2.7. Biochemical analysis

2.7.1. Determination of total hydrocarbon content

The total hydrocarbon content in the catfish sample was determined using a modified method of Parageau et al. [15]. Two grams (2 g) of catfish sample were extracted with 20.0 mL of dichloromethane (CH_2Cl_2) then filtered using filter paper. The filtrate was collected in a clean dry pre weighed conical flask. The oil-solvent mixture was treated with anhydrous sodium sulphate, in order to remove the residual water from the extract. Fifty grams (50 g) of sodium sulphate was added and shaken vigorously to remove all traces of water that are present in the aqueous medium. The extract was reduced to a volume of 10 mL by evaporation. The total hydrocarbon content concentration in the sample was extrapolated from a standard curve obtained by the preparations of various concentrations of the crude oil (0.10, 1.0 and 10.0 mg/mL) with absorbance's (0.01, 0.03 and 0.3) at a wavelength of 430 nm.

The total hydrocarbon content in the catfish was calculated using to the following equation:

$$C = \frac{RxD}{X}$$

C = Total hydrocarbon content (THC) (mg/kg), R = Concentration from graph, X = Sample (kg), D = Total volume of solvent

2.7.2. ALP assay

The method of Bessey et al. [16] was used in the assay of ALP activity. Step 1, zero point five milliliters (0.5 mL) of alkaline phosphatase substrate was added in labeled test tubes and equilibrate to 37 °C for three minutes. Step 2, at time interval, 0.05 mL of each standard, control and sample was added to respective test tubes and mixed gently. Deionized water was used as sample blank and this was incubated for exactly ten minutes at 37 °C. Following the same sequence in step 2, 2.5 mL ALP colour developer were added at time interval and mixed well. Absorbance was read against reagent blank at a wavelength of 580 nm.

2.7.3. AST and ALT activities assay

The method of Reitman and Frankel [17] was adopted for the assay of AST and ALT activities. Reagents were prepared according to standard procedures using commercially available diagnostic kits supplied