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Influence of Monodora Myristica Extracts on Lipid Profile of Rats Fed with Crude Petroleum Oil **Contaminated Catfish Diet**

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Abstract

This study examined the influence of M. myristica extracts on lipid profile of rats fed crude petroleum oil contaminated catfish diet. Thirty albino wistar rats were divided into six groups of five rats each. Group 1: control, group 2: rats were fed crude petroleum oil contaminated catfish diet (CPO-CCD) only, group 3: **CPO-CCD** plus 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 of (MDEE). Treatment lasted for four weeks. Lipid profile was evaluated in serum and tissue homogenate and the results showed significant $(p<0.05)$ increase in total cholesterol (T-Chl). low density lipoprotein cholesterol (LDL-C), triacylglycerol (TAG) and decreased high density lipoprotein cholesterol (HDL-C) levels in the serum and tissues (liver, kidney and brain) of rats fed CPO-CCD only and CPO-CCD plus tween 80 when compared with controls. Treatments of rats with MWE, MEE and **MDEE** resulted in a significant $(p<0.05)$ decreased in T-Chl, LDL-C, TAG and increased HDL-C levels in the serum and tissues when compare with CPO-CCD only and CPO-CCD plus tween 80. No significant difference $(p$?0.05) was observed in the atherogenic index of all the experimental groups. It is therefore concluded that M. myristica extracts can be utilized in prevention of alterations in lipids in crude petroleum oil toxicity.

Key words: Lipid profile, petroleum, diet, atherogenic index

Introduction

Petroleum and natural gas are the components of crude oil. Petroleum occurs as a dark, sticky, toxic and viscous liquid (Jing et al., 2016). It is a mixture that comprises gaseous, liquid and solid alkanes, alkenes, cycloalkanes, aromatic hydrocarbon and others (Al-Sayegh et al., 2016; Ekperusi and Aigbodion, 2015; Rafik et al., 2013). According to Aluko (2004), fishing occupation which is a means of livelihood for the women in Niger Delta has been denied since their waters have been polluted and fishes died due to crude petroleum oil pollution. Abnormalities in lipid metabolism in crude petroleum oil induced oxidative stress have been reported (Ujowundu et al., 2012).

Lipids are diverse groups of biological substances which are made up of non-polar groups (Gurr and Harwood, 1991). Having the knowledge of lipid biochemistry is very important in nutrition and health (Gurr and Harwood, 1991). Hypercholesterolemia is a major health challenge. Atherosclerosis is connected to high levels of cholesterol in the blood, and to be specific high levels of LDLbound cholesterol (Murray et al., 2003). Low atherogenic indices are protective against coronary heart disease. Studies have shown that oxidative stress increases atherogenic index (Niroumand et al., 2015). Animal models give better understanding of the relationship between disorders in cholesterol metabolism and atherogenesis (Moghadasian et al., 2001).

Monodora myristica (family of flowering plants: *Annonecea*) is common spice use in the Niger

Delta part of Nigeria. The kernel of M. myristica is used to prepare pepper soup for women immediately after child birth to control passive uterine haemorrhage (Iwu, 2002). The aim of this study was to examine protective influence of M. *myristica* extracts on alterations of lipid profile in rats fed crude petroleum oil contaminated catfish prepared diet.

Materials and Methods Preparation of the Monodora myristica **Extracts**

M. myristica was obtained from Obiaruku, local market in Delta State and then identified at the Department of Botany, Delta State University, Abraka, Delta State. The method described by George et al. (2012) was adapted for the preparation of the *M. myristica* water, ethanol, and diethyl ether extracts.

Stimulation of Crude Petroleum Oil Pollution

The crude petroleum oil (CPO) was obtained from the Nigerian National Petroleum Cooperation (NNPC), refinery, Warri in Delta State, Nigeria.

Formulation of Diet

Formulation of diet was carried out following the method described by Sunmonu and Oloyede $(2007).$

Determination of Dosage

The rats were divided into five groups of four rats in each group. The M. *myristica* water extract was administered separately to 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 hour, occasionally for 3 h for severity of any toxic sign and mortality. If no mortality is observed at this dose, the same procedure was repeated for dose level of 200 mg/kg b. wt. (group 2), 500 mg/kg b. wt. (group 3), 1000 mg/kg b. wt. (group 4), and 2000 mg/kg b. wt. of extract (group 5). The LD_{50} was thus determined, which was selected to ensure that the the interest of the animals used for the study are protected. The animals were observed up to 7 days after M. myristica water extract administration to find out for any delayed mortality. The same procedure was repeated for M. myristica ethanol extract and diethyl ether extract.

Determination of LD

The LD_{50} value was calculated using the formula described by Lorke (1983)

 $LD_{50} = LD_{100} = ?(DdxMd)$ $\mathbf N$

- Ld_{50} = Dose that killed 50 $\%$ of a given population of rats
- Ld_{100} $\qquad \qquad =$ Dose that killed all the rats in the group.
- $?$ (Dd x Md) = Summation of all products of dose difference (Dd) and mean death (Md) of rats
- N $=$ Number of rats in each group.

Experimental Rats

Thirty male albino Wistar rats of $7 - 9$ weeks were used for the study. Acclimatization was allowed for a period of two weeks to suite the laboratory condition. During the period, the rats had free access to water and standard growers mash diet. The rats were used for the study following guide for the care and use of laboratory animals (NIH, 1985). The rats were divided into six groups of five rats. Rats in group 1 to 6 received tap water daily throughout the experiment. The administration of the crude petroleum oil contaminated catfish diet (CPO-CCD) and extracts orally using cannula was allowed for a period of four weeks.

- Group 1: Control
- Group 2: CPO-CCD control
- Group 3: CPO-CCD plus 1 ml/kg b. wt. of 1% tween 80.
- CPO-CCD plus 200 mg/kg b. wt. of Group 4: M. myristica water extract (MWE)
- CPO-CCD plus 200 mg/kg b. wt. of Group 5 : M. myristica ethanol extract (MEE)
- CPO-CCD plus 200 mg/kg b. wt. of Group 6 : M. myristica diethyl ether extract (MDEE)

Biochemical Analysis

Total cholesterol in the serum, liver, kidney and brain were determined according to the method described by Allan et al. (1979). Triacylglycerol in the serum, liver, kidney and brain was determined according to the method of Fossati and Prencipe (1982). High-density lipoprotein – cholesterol in the serum, liver, kidney and brain was determined according to the method described by Badimon et al. (1990). LDL-C was calculated using Friedewald formula (Martin et

al., 2013). LDL-C (mg/dl) = (Total cholesterol -HDL)-Triacylglycerol /5. Atherogenic index was calculated using the formula described by Dobiasova and Frohlich (2001). Atherogenic $index = log (Triacylycerol / High density)$ lipoprotein-Cholesterol)

Statistical Analysis

The data obtained in the experiment was expressed as means \pm standard deviation. The values were analyzed using analysis of variance and the group means were compare using last significant difference. A p-value of ≤ 0.05 was considered significant in all statistical analysis.

Results

The administration of M. myristica water, ethanol and diethyl ether extract orally to rats up to 2000 mg/kg b.wt (Tables 1, 2 and 3) after one week did not record any mortality. However, some clinical features such as growing of little new hairs were observed in the rats given 200 mg/kg b.wt. A mild clinical sign of roughness of hairs in the rats given the dosage of 500-2000 mg/kg b.wt were observed. Therefore, the LD_{so} may be in excess of 2000 mg/kg b. wt. Finally, the 200 mg/kgb. wt was chosen as a protective dose for the studies. Changes in T. Chl. TAG. HDL-C and LDL-C levels in the serum, liver, kidney and brain of rats fed CPO-CCD treated with extracts of M. myristica was demonstrated in Tables 4,5,6 and 7. The levels of T. Chl, TAG and LDL-C of rats fed CPO-CCD only and CPO- CCD + tween 80 in the serum, liver, kidney and brain were significantly $(p<0.05)$ increased while HDL-C was decreased when compared with the controls. On the other hand, among the rats fed CPO-CCD with MWE, MEE and MDEE, there were significant ($p<0.05$) decrease in T. Chl, TAG, LDL-C and increase HDL-C levels in serum, liver, kidney and brain when compared with rats fed CPO-CCD only and $CPO-CCD$ + tween 80. The level of atherogenic index was found not significant ($p<0.05$) in all the experimental groups (Table 8).

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Groups	N	Dose mg/kg	No. of death	$\frac{0}{0}$ mortality	Dose differences (Df)	Mean death (Md)	$\bf{D}d\bf{x}$ Md	Clinical features
	4	100	0	$\boldsymbol{0}$	100	$\boldsymbol{0}$	$\boldsymbol{0}$	۰.
	4	200	$\mathbf{0}$	$\boldsymbol{0}$	300	$\boldsymbol{0}$	$\boldsymbol{0}$	Growth of little new hairs
3	$\overline{4}$	500	θ	$\boldsymbol{0}$	500	$\boldsymbol{0}$	$\boldsymbol{0}$	Roughness of hairs
4	4	1000	$\overline{0}$	θ	1000	$\boldsymbol{0}$	$\boldsymbol{0}$	Roughness of hairs
	4	2000	θ	$\boldsymbol{0}$	\blacksquare	$\boldsymbol{0}$	$\boldsymbol{0}$	Roughness of hairs

Table 3: Mortality (LD50) effect of diethyl ether extract of M. myristica

Table 4: Total cholesterol (T. Chl) level in the serum, liver, kidney and brain of rats fed CPO-CCD treated with different extracts of M. myristica.

Values are given in mean \pm SD, n=5, Mean values with different superscript letter in the same column differ significantly at $p<0.05$.

CPO-CCD=crude petroleum oil contaminated catfish diet, MWE=M. myristica water extract. MEE=M. myristica ethanol extract. MDEE=M. myristica diethyl ether extract.

Table 5: Triacylglycerol (TAG) level in the serum, liver, kidney and brain of rats fed CPO-CCD treated with different extracts of M. myristica

	TAG (mg/dl)				
Groups		Serum	Liver	Kidney	Brain
1:	Control	200.34 ± 18.11 ^a	231.00 ± 31.39^{a} 221.43 ± 44.45^{a}		250.25 ± 19.82 ^a
2:	CPO-CCD only	$300.42 \pm 28.15^{\mathrm{b}}$	$331.41 \pm 12.42^{\mathrm{b}}$	$320.37 \pm 20.06^{\mathrm{b}}$	$350.34 \pm 9.96^{\mathrm{b}}$
3:	$CPO-CCD + Tween 80$	$305.13 \pm 38.04^{\mathrm{b}}$	$334.89 \pm 12.38^{\mathrm{b}}$	$320.41 \pm 26.40^{\mathrm{b}}$	$354.40 \pm 40.25^{\mathrm{b}}$
4:	$CPO-CCD + MWE$	$270.32 \pm 42.30^{\circ}$	301.90 ± 21.19 ^c	290.14 ± 23.07 °	336.86 ± 41.52 ^c
5:	$CPO-CCD + MEE$	250.77 ± 29.38 ^d	$280.95 \pm 37.50^{\text{ d}}$ $271.38 \pm 33.40^{\text{ d}}$		301.29 ± 10.56 ^d
6:	$CPO-CCD + MDEE$	220.48 ± 18.81 ^e	$250.88 \pm 13.40^{\circ}$	$248.40 \pm 10.14^{\circ}$	$283.61 \pm 61.10^{\circ}$

Values are given in mean \pm SD, n=5. Mean values with different superscript letter in the same column differ significantly at $p<0.05$.

Key: CPO-CCD=crude petroleum oil contaminated catfish diet, MWE=M. myristica water extract. MEE=M. myristica ethanol extract. MDEE=M. myristica diethyl ether extract.

	$HDL-C$ (mg/dl)		
Groups	Liver	Kidney	Brain
1: Control		210.41 ± 18.11^{a} 200.58 ± 21.74^{a}	$230.39 \pm 30.02^{\text{a}}$
2: CPO-CCD only		$160.37 \pm 9.28^{\mathrm{b}}$ $150.43 \pm 15.01^{\mathrm{b}}$	$180.38 \pm 10.04^{\mathrm{b}}$
3: CPO-CCD +Tween 80		$163.39 \pm 27.22^{\mathrm{b}}$ $151.30 \pm 36.10^{\mathrm{b}}$	$182.28 \pm 7.81^{\mathrm{b}}$
4: $CPO-CCD + MWE$		$178.48 \pm 17.19^{\circ}$ $165.91 \pm 12.98^{\circ}$	$296.86 \pm 15.89^{\circ}$
$5:$ CPO-CCD + MEE		188.07 ± 13.30 ^d 173.34 ± 24.37 ^c	200.32 ± 16.99 ^d
$6:$ CPO-CCD + MDEE		$199.89 \pm 40.40^{\circ}$ $185.55 \pm 19.42^{\circ}$	$215.27 \pm 14.97^{\circ}$

Table 6: High density lipoprotein-cholesterol (HDL-C) level in the serum, liver, kidney and brain of rats fed CPO-CCD treated with different extracts of M. myristica

Values are given in mean \pm SD, n=5. Mean values with different superscript letter in the same column differ significantly at $p<0.05$.

Table 7: Low density lipoprotein-cholesterol (LDL-C) level in the serum, liver, kidney and brain of rats fed CPO-CCD treated with extracts of M. myristica

Values are given in mean \pm SD, n=5. Mean values with different superscript letter in the same column differ significantly at $p<0.05$.

Table 8: Atherogenic index in the serum of rats fed CPO-CCD treated with different extracts of M. myristica

Groups		Atherogenic index (mg/dl)		
	1: Control	0.11 ± 0.09 ^a		
	2: CPO-CCD only	$0.35 \pm 0.05^{\text{ a}}$		
	3: $CPO-CCD + Tween 80$	$0.36 \pm 0.06^{\text{ a}}$		
	4: $CPO-CCD + MWE$	0.24 ± 0.01 ^a		
	$5:$ CPO-CCD + MEE	0.23 ± 0.03 ^a		
	$6:$ CPO-CCD + MDEE	$0.12 \pm 0.01^{\text{a}}$		

Values are given in mean \pm SD, n=5. Mean values with different superscript letter in the same column differ significantly at $p<0.05$.

Figure 1. Schematic Representation for the Anti-Atherosclerotic Effect of M. myritica Extracts. M. myristica extracts contains antioxidants (flavonoids, saponin, tannin, alkaloids) and bioactive compounds, its consumption may also exert inhibitory effect on development of aortic atherosclerotic lesions and LDLs oxidation. Triacylglycerol (TAG), High Density Lipoprotein-Cholesterol (HDL-C), Low Density Lipoprotein- Cholesterol (LDL-C). CPO-CCD= Crude Petroleum Oil Contaminated Catfish Diet. Positive effect (+).

Discussion

It could be hypothesized from the results in Tables 1, 2, 3 and 4 that the observed growth of little new hairs may be due to the antioxidant property of M . *myristica* extracts. The roughness of hairs observed in the rats could be due to oxidative stress from high dose of the spice extracts, which is in line with previous studies (Iyawe and Onigbinde, 2012; Halliwell, 2008), which stated that high intake of antioxidant, may impose oxidative stress.

Hydrocarbon present in crude petroleum oil may result in fluctuations in serum, liver, kidney and brain lipid profile via a mechanism dependent on oxidative stress, increasing lipid peroxidation and decreasing enzymatic antioxidant defense mechanism (Azeez et al.,

2013). Petroleum hydrocarbon-induced oxidative stress on liver tissues, renal tissues and brain tissues is associated with hepatotoxicity (Orisakwe et al., 2004), nephrotoxicity (Uboh et al., 2009) and neurotoxicity (Kato et al., 1996). Significant ($p<0.05$) increase in T-Chl, TAG, LDL-C and decrease HDL-C in the serum and tissues (liver, kidney and brain) were observed in rats fed CPO-CCD alone and CPO-CCD plus tween 80 as when compared to control (Tables 4, 5, 6 and 7). This may indicate tissue damage due to oxidative stress and dysregulation of low lipoprotein receptor (LDLR). The results are line with the study conducted by Zhang et al. (2016), which stated that the dysregulation of LDLR expression results in abnormal lipid accumulation. This accumulation of lipid occurs in cells and tissues, such as hepatic cells, renal

mesangial cells (cells that regulate blood flow of the glomerular capillaries by their contractile process), renal tubular cells, podocytes (cells in the Bowman's capsule which wrap around the capillaries of glomerulus) and brain glial cells. George et al. (2013) have also reported abnormal lipid accumulation due to oxidative stress in the brain of streptozotocin induced diabetic rats.

The observed elevated levels of serum cholesterol and triglyceride in the fed CPO-CCD alone (Table 5 and 6) indicates their hyperlipidemia potentials. These increases have been implicated as the primary risk factor for cardiovascular heart disease (Ajiboye et al., 2015).

The increased LDL-C level in rats fed CPO-CCD only in the serum and the various tissues may likely due oxidative stress and oxidation of LDLs. This is in line with the previous study of Brunzel et al. (2008) which states that increased concentration of serum LDL-cholesterol signified oxidative stress and damage of LDL receptor binding the tissues of the liver.

Treatments of rats with the extracts of M. *myristica* resulted a significant improvement in the tested lipid profile parameters, which could be attributed to increase inhibition of intestinal absorption of cholesterol, interference with lipoprotein production, increased expression of hepatic LDL receptor and their protection.

Atherogenic indices are strong indicators of the risk of heart disease. The higher the value, the higher the risk of developing cardiovascular disease and vice versa. Low atherogenic indices are indications of protections against coronary heart disease (Ikewuchi and Ikewuchi, 2009). From the results obtained in this study, the oral administration of M. myristica seeds extracts, lowered the atherogenic index was (Table 8), indicating the likely cadioprotective properties of the extracts. M. myristica extracts possess anti-antigenic effect as shown in figure 1 above. The anti-lipidaemic and anti-atherogenic effects of *M. myristica* found in this study might be due to the high levels of antioxidants and bioactive compounds present in the M. myristica seeds extracts.

Conclusion

It can be concluded from the present data, that the levels of T-Chl, TAG, LDL-C is raised with a concomitant decrease HDL-C in rats fed CPO-CCD. These value were normalized with M. *myristica* extracts treatments. M. *myristica* extracts may have anti-lipidemic and antiatherosclerosis effects and hence may be utilized for prevention of atherosclerosis in patent with hypercholesterolemia.

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