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Impact of Crude Petroleum Oil and *Monodora myristica* on Membrane bound ATPases and Erythrocyte osmotic fragility *in-vivo*

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Abstract
This study examined haematological parameters, ATPases activities and osmotic fragility of erythrocyte in-vivo of rats fed crude petroleum oil (CPO) diet and Monodora myristica extracts. Thirty male albino Wistar rats were utilized for the study. They were acclimatized for 14 days and separated into six groups of five rats. Group 1 served as the control. Groups 2 and 3 were given crude petroleum oil contaminated catfish diet (CPO-CCD) only and CPO-CCD + tween 80, respectively. Groups 4 to 6 were given CPO-CCD and M. myristica water extract (MWE), M. myristica ethanol extract (MEE) and M. myristica diethyl ether extract (MDEE), respectively. Significant ($p < 0.05$) decrease in erythrocyte membrane adenosine triphosphatases (ATPases), packed cell volume (PCV), red blood cell (RBC), and haemoglobin (Hb) and increase in white blood cell (WBC), haemolysis of RBC in 0.0 %, 0.1 %, 0.5% and 0.9 % NaCl were observed in rats fed with CPO-CCD only compared to the control. Treatments with MWE, MEE and MDEE showed significant ($p < 0.05$) increase in erythrocyte membrane ATPases, PCV, RBC, Hb and decrease in WBC and haemolysis of RBC when compared with Groups 2 and 3. It could be inferred from the results that CPO-CCD toxicity revealed altered erythrocyte membrane ATPases and M. myristica extracts could reverse the adverse effect.

Keywords: *ATPases, Erythrocyte, Monodora myristica, Diet, Crude oil*

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1. Introduction

Oil spill is a threat to the biodiversity and land animals (Ekpenyong and Asuquo, 2017). Environmental degradation and destruction of aquatic lives through crude oil pollution have subjected inhabitants of the region to untold hardship (Atubi, 2009; Mauduit *et al.*, 2019). Exposure of experimental aquatic animals to crude petroleum oil (CPO) and its products can induce toxic symptoms (Johansen and Esbaugh, 2019). Crude petroleum oil causes harm to aquatic life through ingestion, inhalation, absorption and physical contact. Water polluted with CPO constitute a potential health risk to humans (Achuba, 2018) who may use water for domestic, drinking purposes and consumption of fish found therein (Jing *et al.*, 2016). Odor, taste and colour are usually present in oil polluted water (Johnson and Affam, 2019). Several homologous series of aromatic hydrocarbons with alkyl side chains that replace hydrogen atoms are present in CPO (Jing *et al.*, 2016). Crude petroleum oil naphtheno-aromatic compounds are toxic mixture of aromatic compounds and saturated cyclic rings (Jing *et al.*, 2016). The toxic responses of CPO may result from ingestion orally or inhalation that could lead to mortality (Sørensen *et al.*, 2019).

There is evidence that, due to oil exploration activity in the oil producing area, the water and therefore fish in such area could be polluted. The fishermen in such area could harvest fish from the polluted water for human consumption. The effect of CPO pollution on aquatic lives is therefore the concern of many scientists. Since most of the world's population especially Niger Delta area of Nigeria depend on aquatic animals for food. This dependency increases as the demand for food increases.

Crude oil and its distillates toxicity cause oxidative stress (Johansen and Esbaugh, 2019;

Okpoghono *et al.*, 2018 a; Okpoghono *et al.*, 2018 b; Okpoghono *et al.*, 2018 c) and changes in erythrocytes chemistry (Ita and Udofia, 2011). Crude oil has been implicated in the onset of anaemia and leucocytosis (Ita and Udofia, 2011). Aromatic compound such as benzene and its metabolites have high toxic effects on the hematopoietic system that leads to bone marrow suppression (Mauduit *et al.*, 2019). Haematology is an aspect of clinical services, which deals with diagnosis and treatment disorders relating to the blood (Blann, 2014). Haematological variables have become very useful for monitoring the health condition of humans (Kori-Siakpere and Ubogu, 2008). White blood cells (WBC) are cells of the immune system, which protect the body against foreign invaders and infectious disease. Haematocrit is the volume percentage of red blood cell (RBC) in blood (Heidenreich *et al.*, 2020). Decreased Hb in animals exposed to petroleum product has been reported (Okoro *et al.*, 2006). Therefore, the studies of blood parameters such as haemoglobin (Hb), packed cell volume (PCV), RBC, WBC, MCHC (mean corpuscular haemoglobin concentration), MCH (mean corpuscular haemoglobin) and MCV (mean corpuscular volume) are of biochemical and physiological interest. These may help to understand the relationship of blood characteristics to polluted environment and food consumption.

Crude petroleum oil has been reported to plays a significant role in damaging the RBC membrane (Mauduit *et al.*, 2019). These effects may alter membrane bound ATPases activities of the body. Therefore, there is need to find a means of ameliorating this effect. Spices are important in food preparation and are effective in the treatment and management of disease condition (George *et al.*, 2019; George *et al.*, 2012; Otuaga *et al.*, 2020a; Otuaga *et al.*, 2020b). *Monodora myristica* is a spice mainly used in the southern part of Nigeria as condiment in food due to its aromatic flavour. The spice is rich in natural antioxidant and the possible antioxidant properties have been reported (George *et al.*, 2015). Therefore, this study was undertaken to investigate if this spice could be used in ameliorating alteration of membrane ATPases, erythrocyte osmotic fragility and haematological status of rats fed with crude petroleum oil contaminated catfish diet (CPO-CCD).

2. Materials and methods

2.1 Crude petroleum oil and M. myristica

The CPO used for this study was obtained from the Nigerian National Petroleum Cooperation refinery, Warri, Delta State. The *M. myristica* seeds (Fig. 1) were obtained from Obiaruku main market, Delta State and were identified at the Forestry Research Institute of Nigeria Ibadan.

Fig. 1: *M. myristica* seeds

2.2 Extraction of *M. myristica*

The extraction was carried out following the method documented by George *et al.* (2012). About 100 g of spice powder was extracted with 500 ml of each solvent: hot water (60 ˚C), ethanol (95 % v/v) and diethyl ether (95 % v/v) for 72 hours. The extracts were concentrated using water bath at 40 ^{0}C.

2.3 Stimulation of CPO pollution

Fifty catfish of length 20-25 cm and weight 250-300 g were obtained from commercial farm, and allowed to acclimatized for 7 days for the experiment. The catfish was divided into two groups:

Group 1: control, contains twenty-five (25) catfish placed in 30 L plastic aquaria with borehole water for four weeks (Plate 1).

Group 2: contains 25 catfish placed in plastic aquaria containing (30 L borehole water) and then polluted with CPO (823.3 µl/l) (Okpoghono *et al.*, 2018a). (Plate 2). The harvested catfish was use as source of protein to prepare diet for the experimental rats (Okpoghono *et al.*, 2018a).

Plate 1. Colour of water (Group 1 "**A**") and catfish appearance (Group 1 "**B**") after 4 weeks.

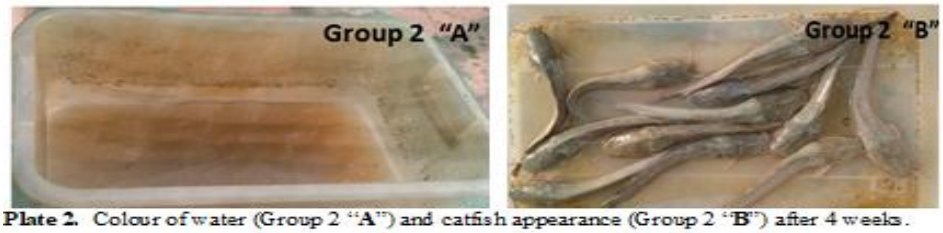


Plate 2. Colour of water (Group 2 "**A**") and catfish appearance (Group 2 "**B**") after 4 weeks.

Fig.2: Appearance of catfish that was used to formulate diet for the experimental rats.

2.4 Preparation of diet

At the end of the 4 weeks, the catfish were oven dried at 40 °C. The diets for each group were prepared by mixing the following food items: catfish (protein) (25 %), corn starch (52 %), oil (4 %), maize cob (4 %), granulated refined sugar (10 %) and vitamin/mineral mixture (Hebei Vsyong Pharmaceutical co. Ltd, China) (5 %). The food items mix was manually made into pellets to feed the rats.

2.5 Experimental design

Thirty Wistar rats (male) obtained from the animal house Delta State University (DELSU) Abraka were used for the study. Acclimatization was allowed for 14 days, during this period the rats had access to drinking water and standard laboratory pellet. Thereafter, they were divided into six Groups (of five rats in each Group) and allowed access to drinking water daily. The experiment was conducted for a period of four weeks. The six groups are shown below:

Group 1: Normal control,
Group 2: CPO-CCD only
Group 3: CPO-CCD +1 ml/kg b. wt. of 5 % tween 80,
Group 4: CPO-CCD + *M. myristica* water extract (MWE)
Group 5: CPO-CCD + *M. myristica* ethanol extract (MEE) and
Group 6: CPO-CCD + *M. myristica* diethyl ether extract (MDEE)

Rats in Group 1-5 were given water and pellet. At the end of the experiment, they were sacrificed by cervical decapitation. Their blood was collected using syringe and needle through the heart and then transferred to an anticoagulant tube. The blood samples were utilized for biochemical analyses. The protocols of the experiment complied with DELSU Animal Ethics Committee guidelines (Faculty of Science Animal Ethics Committee guidelines), as well as internationally accepted guidelines for care and use of experimental animals (NIH, 1978).

2.6 Determination of weight gain (WG) and relative organ weight (ROW)

The WG and ROW of experimental rats were calculated using Equations (1) to (4).

$$WG = \text{final body weight (FBW)} - \text{initial body weight (IBW)} \quad (1)$$

$$\text{Relative liver weight (g/100 g)} = \frac{\text{Total liver weight}}{\text{FBW}} x\ 100 \quad (2)$$

$$\text{Relative kidney weight (g/100 g)} = \frac{\text{Total kidney weight}}{\text{FBW}} \text{x } 100 \quad (3)$$

$$\text{Relative brain weight (g/100 g)} = \frac{\text{Total brain weight}}{\text{FBW}} \text{ x } 100 \quad (4)$$

2.7 Determination of PCV

PCV was determined using the method described by Thrall and Weiser (2002). Blood sample was allowed to flow into capillary tubes by capillary action. The capillary tubes were then sealed at one end and centrifuged at 2,500 rpm for 5 minutes to separate the blood cells from the plasma. The capillary tubes were removed from the centrifuge, and values were read using the haematocrit reader card.

2.8 Determination of Hb

Haemoglobincyanide (HiCN) method described by Bansal *et al.* (2016) was used for determination of Hb. Two millilitres (2 ml) each of Hb reagent were transferred into test tubes labelled blank, control and test. Ten microliters (10 µl) of samples were placed into the respective test-tubes, mixed and allowed to stand for 3 minutes at room temperature. Two millilitres (2 ml) of standard reagent were placed in the test tube labelled standard. The absorbance of all tubes was taken at 500 nm against the blank and recorded.

2.9 Determination of WBC

White blood count was determined following the method of Thrall and Weiser (2002). About 0.38 ml of the WBC reagent (Turk's fluid) was added into 20 µl of the blood sample**.** WBC was estimated using microscope and counting chamber.

2.10 Determination of RBC

Determination of RBC was carried out following the method provided by Thrall and Weiser (2002). The solution of formalcitrate was prepared by adding 10 ml of formalin (40% formaldehyde) into one litre of 31.3 g/L trisodium citrate solution. This fluid was then filtered and stored in a clean glass container until required. The blood sample was diluted by washing one microliter (1 µl) of blood taken into 4.0 ml of diluents to give a final dilution of 1 in 20. The samples were mixed, and then loaded in the haemocytometer. The cells were counted and recorded.

2.11 Determination of MCHC, MCH and MCV

The MCHC, MCH and MCV levels were calculated using Equations (5) to (7):

$$MCHC\ (g/dl)\ = Hb/PCV \quad (5)$$

$$MCH\ (pg) = Hbx10/RBC \quad (6)$$

$$MCV(ft) = PCV/RBC \quad (7)$$

2.12 Erythrocytes osmotic fragility (EOF)

Determination of EOF was done following the method of Mineo *et al.* (2005). About 0.02 ml of blood of rats were transferred into test tube containing 1 ml of increasing concentration of phosphate- buffer NaCl solution (0.0, 0.1, 0.5, and 0.9 %) at pH 7.4. The test tube was then gently mixed, incubated at 37 ^{o}C for 30 minutes and then centrifuged at 2000 rpm for 15 minutes. The supernatant of each test tube was decanted and determined at 540 nm using spectrophotometer. The percentage of RBC hemolysis in the NaCl concentration and in distilled water (0.0 %) as the maximum percentage was estimated.

2.13 Preparation of erythrocytes membranes

The erythrocyte membranes preparation was done as illustrated by the method of Dodge *et al.* (1963). After the separation of the erythrocytes from plasma, buffy coat, and washing of erythrocytes cells, they were haemolyzed with 40 volumes of hypotonic 5P8 buffer (5 mM Na_2HPO_4-NaH_2PO_4, 0.1 mM PMSF, pH 8.0) followed by centrifugation at 15,000g for 20 min refrigerated centrifuge to get the membrane fractions. The supernatant was colorless (white membranes). The membranes were prepared in Tris-HCl buffer for determination of ATPase activity,

2.14 Estimation of membrane-bound adenosine triphosphatase (ATPase)

Total ATPases

Total ATPases activity in tissues was measured using the method of Evans (1969). The ATPase activity in 0.1 mL of aliquot of the homogenates were measured in a final volume of 2 mL containing 0.1 ml of 0.1 M Tris-HCl ({pH 7.4), 0.1 ml of 0.1 M NaCl, 0.1 ml of 01 M $MgCl_2$, 1.5 ml of 0.1 M KCl, 0.1 ml of 1 mM EDTA and 0.1 ml of 0.01 M ATP. The reaction was allowed for 20 min followed by the addition of 1 mL of 10% TCA and then centrifuged (3000 rpm for 10 min) and the inorganic phosphorus (Pi) release was estimated in the supernatant.

Na^+/K^+-ATPase

The activity of Na^+/K^+-ATPase was assayed according to the procedure of Hesket *et al.* (1978). The reaction mixture (0.1 ml of buffer, 0.2 ml of $MgSO_4$, 0.2 ml of NaCl, 0.2 ml of KCl, 0.2 ml of EDTA and 0.2 ml of ATP) was incubated for 20 min at 37°C, then the reaction was initiated by adding 0.2 ml of sample. Thereafter, incubated at 37°C for 15 min. About 1 ml of 10% TCA was added after 15 min to arrest the reaction mixture. The supernatant Pi liberated was estimated.

Mg^{2+}-ATPase

The activity of Mg^{2+}-ATPase was assayed according to Ohinishi et al. (1982). Briefly, 0.1 ml of buffer, 0.1 ml of $MgCl_2$, 0.1 ml of ATP, 0.1 ml of water and 0.1 ml of sample were placed in test tube and then incubated at 37°C for 15 min. Thereafter, 0.5 ml of 10% TCA was added. The Pi free was estimated in the supernatant.

Ca^{2+}-ATPase

Ca^{2+}-ATPase activity was assayed according to Hjertan and Pan (1983) method. The reaction contained 0.1 ml of buffer, 0.1 ml of $CaCl_2$, 0.1 ml of ATP and 0.1 ml sample. The contents were incubated for 15 min at 37°C. The reaction mixture was arrested by adding 0.5 ml of ice cold 10% TCA. The free Pi of supernatant was determined.

Inorganic phosphate (Pi) estimation

The Pi liberated from total ATPases, Na^+/K^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase was determined according to the method of Henry (1974) by using Randox diagnostic kit. The activity of the enzyme in tissues was expressed as µg Pi release/mg protein.

2.15 Statistical analysis

The results were presented as mean ± standard deviation and mean bars. The group mean values were compared using least significant difference (LSD). The SPSS package (version 22.0) was used for the analysis.

3. Results and discussion

3.1 Catfish appearance, body weight and organs weight of rats administered CPO-CCD

The catfish appearance after four weeks contamination with CPO is illustrated in Fig.2. The catfish without CPO appears dark while CPO polluted catfish appears pale. There were no significant differences indicated in the initial weight of all the experimental groups (Fig. 3). However, a significant ($p<0.05$) increase was observed in the control (Group 1) final weight when compared with other groups. The results revealed significant ($p<0.05$) decrease in weight gain by Groups 2 and 3 when compared with Group 1. Interestingly, treatment with *M. myristica* extracts (Group 4, Group 5 and Group 6) showed significant ($p<0.05$) increase in weight gain when compared to Groups 2 and 3.

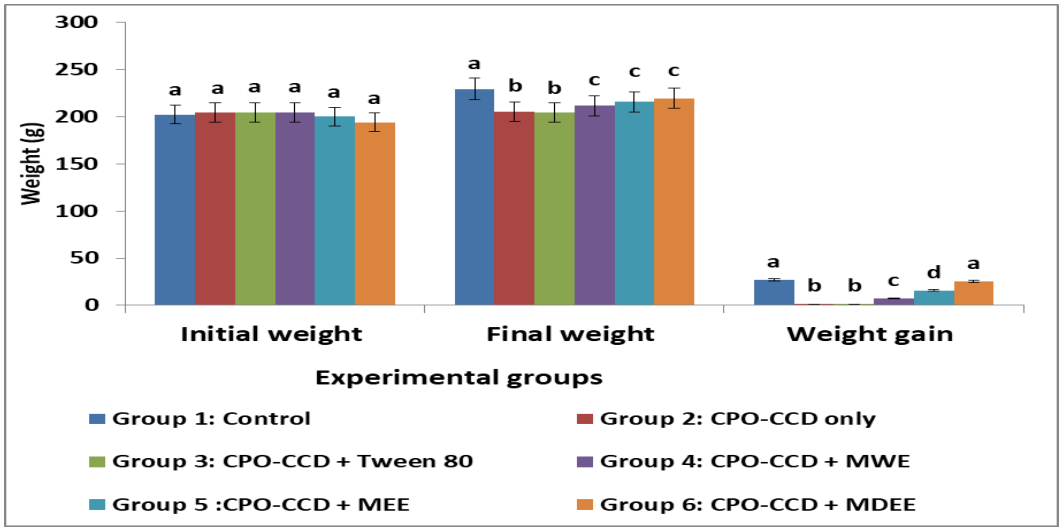
The absolute organ weight (AOW) and relative organ weight (ROW) of rats are shown in Table 1. The results indicated no significant differences in the organs (liver, kidney and brain) weights of rats in all the groups. The relative liver weight, kidney weight and brain weight of the rats also showed no significant difference in all the experimental groups. Change in weight is a marker for checking animal health status. Reduction in body weight is use as an indicator in ascertaining biochemical changes of experimental rats (Fadairo and Otite-Douglas, 2015). The significant ($p < 0.05$) decrease in weight gain of rats fed CPO-CCD (Fig. 3) could be associated with considerable tissue damage caused by CPO-CCD toxicity. This is also in line with the study of Fadairo and Otite-Douglas (2015). According to the study, alterations in body weight gain are usually seen as toxicity indices. The significant reduction in weight gain and organ body weight ratio in crude oil fed rabbits reveals the toxic nature of crude oil. The results of the study also align with the works of Timbell (1991) and Horiguchi *et al.* (1996) who demonstrated that Cd toxicity causes significant reduction in organs/ body weight ratio of rats. The escalating increase in weight gain of rats fed CPO-CCD treated with *M. myristica* extracts (with MDEE having the highest weight gain followed by MEE and MWE) when compared with the CPO-CCD control, may likely indicate the ameliorating effects of the *M. myristica* extracts.

The decrease observed in the liver weight of the rats given CPO-CCD only (Table 1) is in line with the results of previous study conducted by Eidi *et al.* (2012) who indicated that AOW decrease might be an indication of organ injury. In this study, the non-significant ($p > 0.05$) difference in the relative and absolute kidney weight of the rats may probably be due to the short duration of feeding the rats with the crude oil contaminated catfish diet. The relative brain weight was low variably when compared to the relative liver weight and relative kidney weight, this may be due to the fact that the brain is not considered to be affected or influenced by nutritional factors (Long *et al.,* 1998).

Table 1: Absolute organs weight and relative organs weight of rats fed CPO-CCD and extracts of *M. myristica*

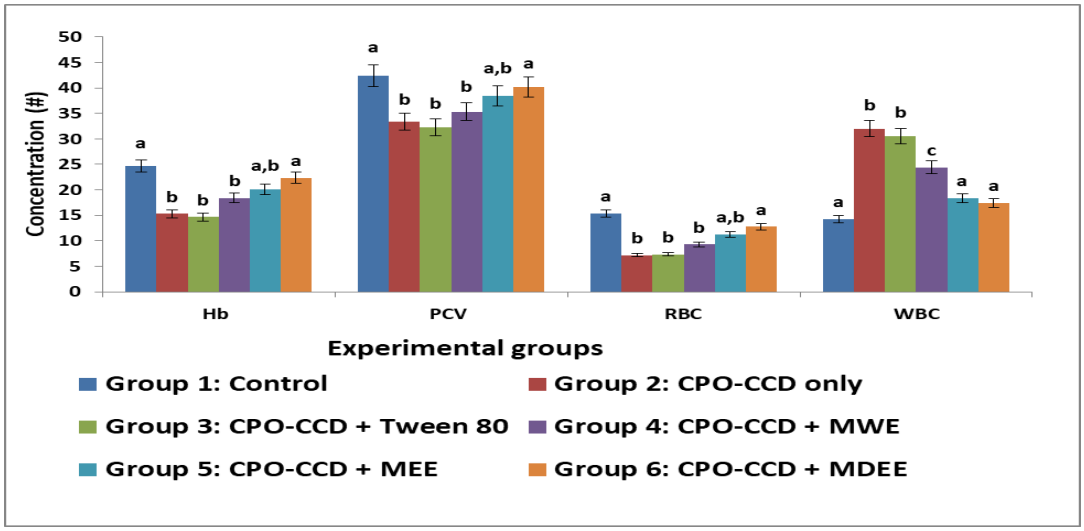
Groups	Liver weight (g)	Relative liver weight (g liver/100g) (%)	Kidney weight (g)	Relative kidney weight (g kidney/100g) (%)	Brain weight (g)	Relative brain weight (g brain/100g) (%)
1.	5.27 ± 0.42[a]	2.33 ± 0.35[a]	2.77± 0.66[a]	1.21± 0.31[a]	1.67±0.42[a]	0.72 ± 0.04[a]
2.	3.62± 0.53[a]	1.76 ± 0.24[a]	1.34± 0.24[a]	0.66± 0.17[a]	1.02± 0.15[a]	0.53 ± 0.02[a]
3.	3.16 ± 0.54[a]	1.70 ± 0.30[a]	1.25 ± 0.31[a]	0.58 ± 0.15[a]	1.08± 0.21[a]	0.58 ±0.01[a]
4.	4.16± 0.10[a]	1.97 ± 0.10[a]	1.47± 0.38[a]	0.70± 0.18[a]	1.26± 0.23[a]	0.60± 0.01[a]
5.	4.53± 0.44[a]	2.10 ± 0.16[a]	1.68 ± 0.56[a]	0.77 ± 0.22[a]	1.46± 0.22[a]	0.68± 0.01[a]
6.	4.81± 0.63[a]	2.19 ± 0.27[a]	2.21± 0.48[a]	1.00± 0.19[a]	1.49± 0.25[a]	0.69± 0.04[a]

Values are given in mean ± SD. n=5. Mean values with same superscript letter in the same column were not significant at p<0.05.



Bars represent mean values from five rats in each group. Bars with different superscript letter in the same column differ significantly at p<0.05.

Fig. 3: Body weight and weight gain of rats fed CPO-CCD treated with extracts of *M. myristica*



Bars represent mean values from five rats in each group. For each parameter, bars with different letter differ significantly at p < 0.05. # = Hb (g/dl), PCV (%), RBC (x10^{12}/L), WBC (x10^{9}/L).

Fig. 4: Haematalogical parameters of experimental rats fed CPO-CCD and extracts of *M. myristica*.

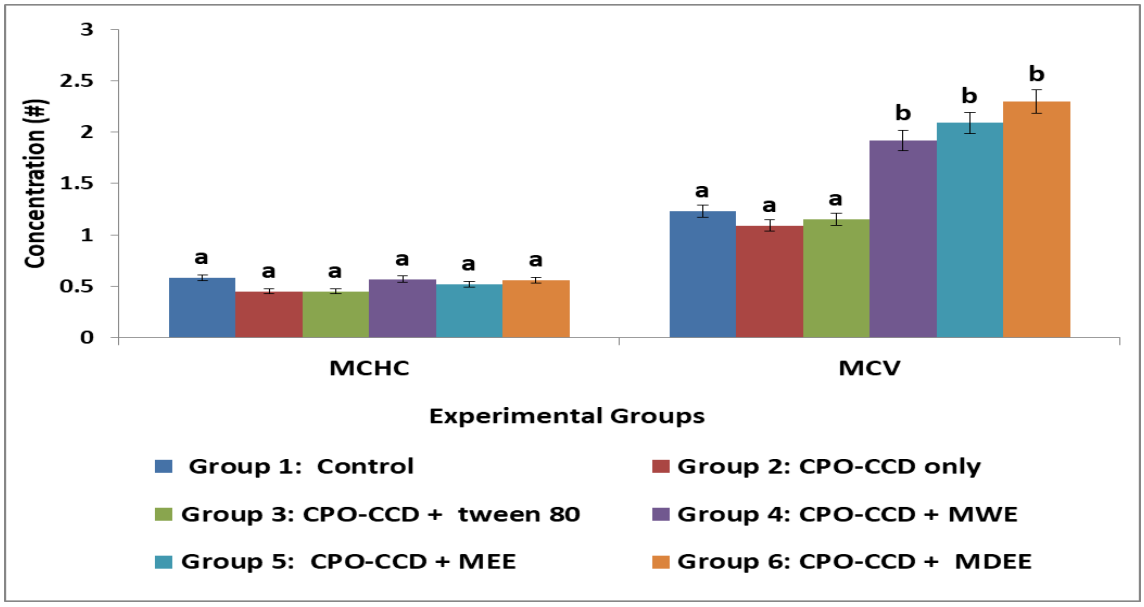
3.2 Hb, PCV, RBC and WBC of rats given CPO-CCD

The haematological changes of rats fed CPO-CCD and treated with different extracts of *M. myristica* are presented in Fig. 4. A significant ($p < 0.05$) decrease in the levels of Hb, PCV and RBC, and increased WBC level were observed in Groups 2 and 3 as compared with the control. Administration of *M. myristica* extracts (Group 4, Group 5 and Group 6) significantly ($p<0.05$) increases Hb, PCV, RBC and decrease WBC. The observed reduction in the levels of RBC, Hb and PCV suggest an anaemic condition of CPO-CCD induced haematotoxicity (Fig. 4). However, the significant reduction in RBC count could be epithetical to vanquishing of erythropoiesis and cytotoxic effect caused by constituents of the contaminated diet. This is in obedience to previous studies of Ita and Udofia (2011). The significant ($p<0.05$) increase observed in WBC of rats fed contaminated diet in this study was similar to the findings of previous studies conducted by Ikechukwu *et al*. (2011). The authors reported an increase in WBC of rats exposed to CPO. This increase in WBC suggests induction of the shielding mechanism of the immune system during CPO toxicity.

3.3 Changes in MCHC and MCV of rats fed CPO-CCD

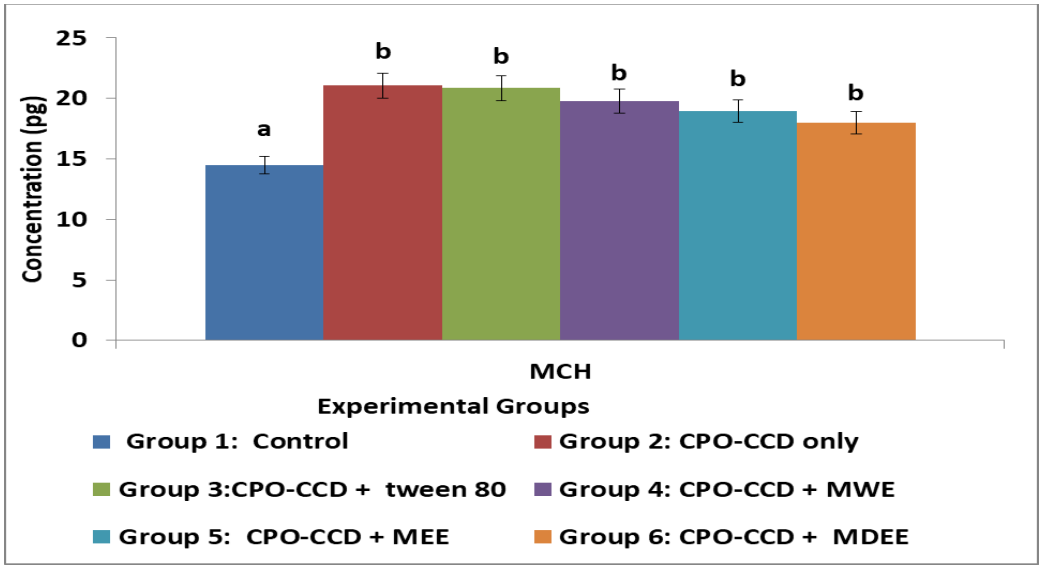
Effect of *M. myristica* extracts on MCHC and MCV levels of rats fed CPO-CCD are shown in Fig. 5. There were no significant differences in MCHC level of all the entire groups. Groups 2 and 3 had decrease in MCV level when compared with the Control, this was not significant. On the other hand, significant increase was observed in MCV levels of Groups 4, 5 and 6 when compared with Groups 2 and 3. The MCH level of various groups administered CPO-CCD and *M. myristica* extracts are presented in Fig. 6. The results illustrated that MCH level of Groups 2 and 3 were significantly higher in comparison with the Control. However, MCH level of Groups 4, 5 and 6 were not significant when compared with Groups 2 and 3. However, the decrease observed in MCHC and MCV of rats fed the CPO-CCD only, and CPO-CCD plus tween 80 in comparison with Control (Fig. 5), may probably be an indication of swelling of RBC and decrease in haemoglobin synthesis. The MCHC is an indicator of RBC swelling (Ita and Udofia, 2011). Fluctuation in the MCH indicates that the concentration of Hb in the RBC was lower in rats fed CPO-CCD compared to Control (as shown in Fig. 4), thus indicating anaemic condition. Nevertheless, the values of MCHC, MCH, and MCV are crucial in the diagnosis of anaemia. Changes observed in these haematological parameters (MCV, MCH and MCHC) in this study could point defense against the CPO-CCD through erythropoiesis stimulation. This is in accordance with the study of Okoro *et al.* (2006). The authors reported that decrease in MCV and Hb level is an indication of decrease in the RBC values, either as a result of hypoxia or microcytic anaemia.

Interestingly, treatment with *M. myristica* extracts reversed the anaemic condition. This may be evidenced by improvement of mean values of Hb, RBC and PCV as compared to the Control. Uboh *et al*. (2009) reported the effect of antioxidant vitamins A and E on petroleum product induced haematotoxicity. The authors also pointed that antioxidant vitamins reduce haematotoxicity following exposure of rats to gasoline vapours. The extracts of *M. myristica* are rich in antioxidants (Okpoghono *et al.,* 2018 b), which may have help in reduction of haematotoxicity. Antioxidants rich food substances are of importance in reduction of toxicological effect (Ekakitie *et al.,* 2021; Hossain *et al.,* 2020).



Bars represent mean values (n=5). Bars of same parameter with different alphabet differ significantly at $p < 0.05$. # = MCHC (g/dl), MCV (ft)

Fig. 5: Effect of *M. myristica* extracts on MCHC and MCV levels of rats given CPO-CCD



Bars represent mean values (n=5). Bars with different superscript letter (a, b) differ significantly at $p < 0.05$.

Fig. 6: MCH level of rats administered CPO-CCD and *M. myristica* extracts

3.4 Haemolysis of RBC, erythrocyte membrane and tissues ATPase activities

There were significant ($p<0.05$) increase in haemolysis of RBC in 0.0 %, 0.1 %, 0.5% and 0.9 % NaCl of rats given CPO-CCD only (Group 2) and CPO-CCD plus tween 80 (Group 3) compared to Control (Fig. 7). However, rats given *M. myristica* extracts (Groups 4, 5 and 6) had significant ($p<0.05$) decrease in haemolysis of RBC in 0.0, 0.1, 0.5 and 0.9 % NaCl compared to Groups 2 and 3. Erythrocyte osmotic fragility test refers to the propensity of erythrocytes to hemolyse when they are subjected to osmotic stress in a hypotonic solution. Administration of CPO-CCD resulted in significant increase in RBC osmofragility. This might be due to the increased lipid peroxidation mediated oxidative damage to the erythrocyte membrane. Increased oxidative stress and reduce antioxidant status may have led to increased erythrocyte deformability, RBC membrane lipoperoxidability (Ziegler *et al.*, 2017; Okpoghono *et al.*, 2018 c) and consequently, high osmotic fragility. This shows that erythrocytes are less stable in hypotonic solution and might be an

indication of membrane fluidity and intravascular hemolysis. Collectively, CPO-CCD intoxication might have led to anaemia. This could be consequence of either suppression in the activity of hematopoietic tissue and increased RBCs mechanical fragility (Ziegler *et al.,* 2017). *M. myristica* fed rats significantly reduced the RBC osmofragility due to the presence of tannins in *M. myristica* (Okpoghono *et al.*, 2018b; Tarahovsky, 2008). Tannins binds to the cell membrane surface and induce cluster and raft formation (Tarahovsky, 2008). Penu *et al*. (2020) reported that antioxidant medicinal plant may be of importance in the treatment of numerous health complications, such as membrane disorder and thrombolysis.

In the present study, a significant decrease in the activities of ATPases in the erythrocyte was observed in rats fed CPO-CCD only, and CPO-CCD plus as compared to Control (Fig. 8). Membrane-bound Na^+/K^+-ATPase and Ca^{2+}/Mg^{2+}-ATPase are responsible for the transfer of sodium/potassium and magnesium/calcium ions across the cell membranes at the expense of ATP by hydrolysis (Tang *et al.,* 2019). CPO-CCD induced free radicals' formation might have led to decrease in the activity of Na^+/K^+-ATPase and Ca^{2+}/Mg^{2+}-ATPase. In addition, Na^+/K^+-ATPase and Ca^{2+}/Mg^{2+}-ATPase are sulfhydryl (-SH) containing enzymes. These –SH groups are indispensable for their activities (Folabi *et al.,* 2016). Inhibition of ATPase activity by CPO-CCD could have been due to its binding with –SH groups at the active sites of the enzymes. Furthermore, either due to oxidation by free radicals or due to conjugation by reactive chemicals which produced deleterious effects on the membrane proteins. Treatment with *M. myristica* extracts (Groups 4, 5 and 6), significant ($p<0.05$) increase in RBC membrane Na^+, K^+ and Ca^{2+} -ATPase were observed in comparison to Groups 2 and 3 (Fig. 8). Administration of *M. myristica* extracts to CPO-CCD -intoxicated rats, may have sustained the activities of membrane-bound enzymes by preventing the oxidation of –SH groups. This might be through free radical quenching action of antioxidant molecules of *M. myristica* (George and Okpoghono, 2017; Okonta *et al.*, 2021).

In this study, no significant difference was observed in total ATPase, Na^+, K^+ and Ca^{2+} -ATPase in the tissues (liver, kidney and brain) of the entire experimental groups (Tables 2, 3 and 4). The ATPases are a critical sulfhydryl class in cells. Na^+/K^+-ATPase are the energy-requiring step in the development of the electrochemical gradients that drive solute and water transport in the proximal tubule. Inhibition of the Na^+/K^+-ATPase would be expected to impair solute and water reabsorption in the proximal tubule. Also, impair the transport of substrates for energy metabolism and synthesis in the tissues (e.g., amino acids, citrate, fatty acids, glucose and lactate). In the present study, a decrease in the activities of membrane bound total ATPases in the kidney was observed in rats given CPO-CCD, however this was not significant compared to Control. Na^+, K^+ and Ca^{2+}-ATPase are adenosine triphosphate (ATP) dependent. Mitochondria are the major site of ATP production, and mitochondrial dysfunction could lead to the fall in the ATP levels (Folabi *et al.,* 2016). Therefore, the resultant deficiency in cellular ATP levels as a result of mitochondrial dysfunction could leads to inhibition of the Na^+, K^+ and Ca^{2+} -ATPase. Treatment of CPO-CCD intoxicated rats with *M. myristica* extracts sustained the activities of membrane bound ATPases, but, no significant differences were indicated (Tables 2, 3 and 4). Elevated levels of Na^+ concentration resulted in depressed effects of Ca^{2+} and augment Ca^{2+} influx. This could be as a result of free radical quenching action of *M. myristica* extract thereby protecting sulphydryl groups against damage. Also, the membrane stabilizing property might have been due to the blocking of lipid peroxidation in cell membranes by *M. myristica* extract. In so doing, abolished the CPO-CCD-stimulated elevation of intracellular free Ca^{2+} concentration. Also, restored the CPO-CCD-induced inhibition of Na^+, K^+, Mg^{2+} and Ca^{2+}-ATPases activities. This may be concerned with protective effect of *M. myristica* extract on mitochondrial function and potent efficacy in scavenging intracellular reactive oxygen species.

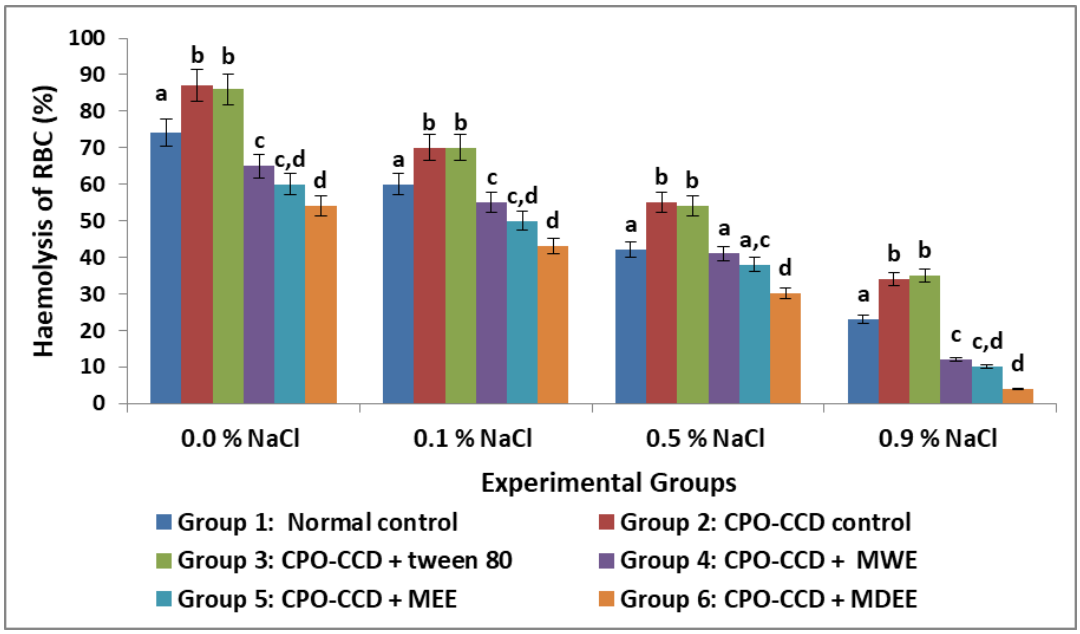


Fig. 7: Changes in erythrocyte osmotic fragility of experimental rats and control.

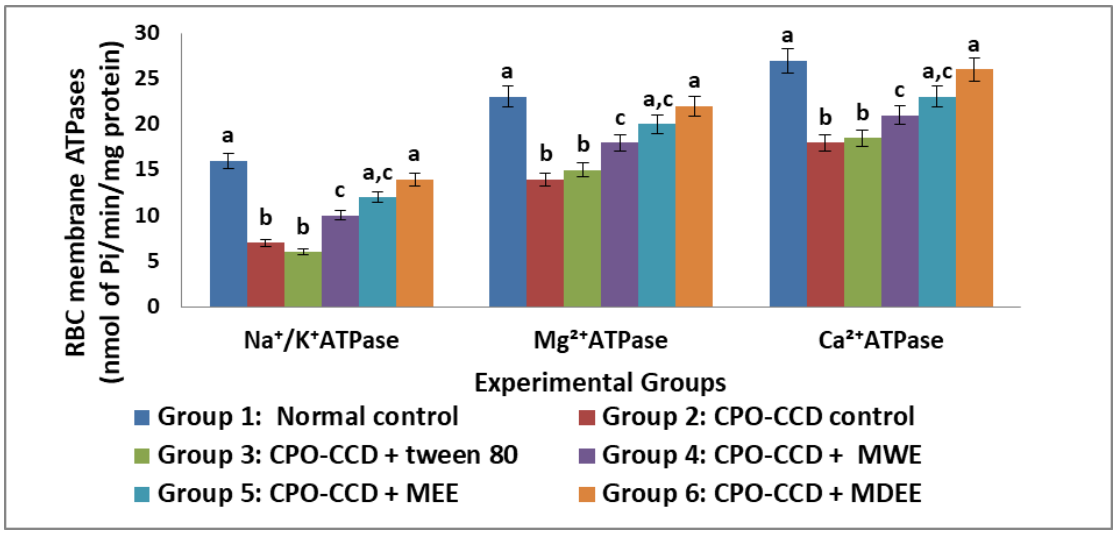


Fig 8: Effect of *M. myristica* RBC membrane bound ATPases of experimental rats and control

Table 2: Changes in hepatic ATPases of experimental rats and control

Groups	Total ATPases*	Na^+/K^+ATPase*	Mg^{2+}ATPase*	Ca^{2+}ATPase*
1.	1.93 ± 0.07[a]	0.45 ± 0.01 [a]	0.74 ± 0.11 [a]	0.43 ± 0.06 [a]
2.	1.54 ± 0.02 [a]	0.15 ± 0.07 [a]	0.36± 0.04 [a]	0.23 ± 0.03 [a]
3.	1.55 ± 0.01 [a]	0.16 ± 0.05 [a]	0.37 ± 0.01 [a]	0.22 ± 0.01 [a]
4.	1.62 ± 0.01[a]	0.22 ± 0.04 [a]	0.45 ± 0.01 [a]	0.35 ± 0.01 [a]
5.	1.64 ± 0.04[a]	0.28 ± 0.05 [a]	054 ± 0.01 [a]	0.39 ± 0.08 [a]
6.	1.73 ± 0.01 [a]	0.37 ± 0.01 [a]	0.63 ± 0.03[a]	0.45 ± 0.01 [a]

Values are given in mean ± SD. n=5. Mean values of parameters with same superscript letter in the same column had no significant at $p<0.05$.
*µg pi liberated/min/mg protein

Table 3: Changes in renal membrane bound-ATPases of experimental rats and control

Groups	Total ATPases*	Na^+/K^+ATPase*	Mg^{2+}ATPase*	Ca^{2+}ATPase*
1.	1.73 ± 0.03[a]	0.26 ± 0.03 [a]	0.32 ± 0.03 [a]	0.29 ± 0.01[a]
2.	1.34 ± 0.04 [a]	0.12 ± 0.01 [a]	0.14± 0.01 [a]	0.11 ± 0.01 [a]
3.	1.35 ± 0.01 [a]	0.12 ± 0.01 [a]	0.14 ± 0.02 [a]	0.12 ± 0.01 [a]
4.	1.42 ± 0.02 [a]	0.18 ± 0.05 [a]	0.18 ± 0.02 [a]	0.22 ± 0.05 [a]
5.	1.54 ± 0.02 [a]	0.21 ± 0.01 [a]	0.21 ± 0.02 [a]	0.25 ± 0.01 [a]
6.	1.63 ± 0.03 [a]	0.23 ± 0.01 [a]	0.34 ± 0.06 [a]	0.30 ± 0.08 [a]

Values are given in mean ± SD. n=5. Values with same superscript alphabet in the same column do not differs significant at p<0.05. *µg pi liberated/min/mg protein

Table 4: Changes in brain-ATPases of experimental rats and control

Groups	Total ATPases*	Na^+/K^+ATPase*	Mg^{2+}ATPase*	Ca^{2+}ATPase*
1.	1.63 ± 0.03[a]	0.25 ± 0.05 [a]	0.44 ± 0.03 [a]	0.34 ± 0.09 [a]
2.	1.24 ± 0.04 [a]	0.11 ± 0.04 [a]	0.15± 0.05 [a]	0.12 ± 0.06 [a]
3.	1.25 ± 0.01 [a]	0.12 ± 0.05 [a]	0.14 ± 0.01 [a]	0.11 ± 0.05 [a]
4.	1.32 ± 0.01 [a]	0.14 ± 0.05 [a]	0.19 ± 0.02 [a]	0.24 ± 0.07 [a]
5.	1.34 ± 0.01[a]	0.15 ± 0.05 [a]	0.24 ± 0.05 [a]	0.28 ± 0.05 [a]
6.	1.53 ± 0.02 [a]	0.23 ± 0.05 [a]	0.43 ± 0.06 [a]	0.33 ± 0.04 [a]

Values are given in mean ± SD. n=5. Values with same superscript letter in the same column were not significant at p<0.05. *µg pi liberated/min/mg protein

4. Conclusions

It is possible to suggest that CPO-CCD induced toxicity implicated the alteration of erythrocyte membrane, which results to different haematological abnormalities in function. CPO-CCD induced free radicals' formation resulted in decrease in the activity of total ATPase, Na^+/K^+-ATPase and Ca^{2+}/Mg^{2+}-ATPase in the erythrocyte membrane and tissues. Nevertheless, this effect was not significant. However, at concentration of 0.0 % to 0.9 % NaCl, RBC osmofragility significant increase in rats given CPO-CCD only compared to control. Interestingly, the administration of *M. myristica* extracts showed a significant renewal of haematological abnormalities and reduction of RBC osmofragility. This study has provided evidence that *M. myristica* extracts could reverse the adverse effect of petroleum polluted diet prompted erythrocyte membrane alterations.

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