Biochemical evaluation of acute exposure to diluted Nigerian Bonny Light Crude oil in the Liver and Heart of Female Albino rats

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ABSTRACT

The study investigates the evaluation of some biochemical indexes in the liver and heart of female albino rats exposed to diluted Nigerian Bonny Light Crude Oil (NBLCO). A total of 28 female rats were used for the experiment. Exposure to crude oil diluted with olive oil was achieved by oral administration for 28 days. The rats were divided into four groups of seven rats each, 0.5ml of distilled water, 0.5ml/g body weight of olive oil, 1ml (0.5ml of NBCLO + 0.5ml of olive oil) mixture/g body weight and 1.5ml (1ml of NBLCO + 0.5ml of olive oil) mixture/g body weight. The effects of the diluted crude oil on the liver and heart homogenates and serum were investigated, and histopathological study of the organs were also carried out using standard histopathological technique. The total protein concentration was measured in the homogenates using Folin-Lowery C. method, aspartate aminotransaminase activity was assayed using kits from Randox Laboratory in the serum and heart homogenate while the anti-oxidant status (thiobarbituric acid reactive substances, reduced glutathione, catalase and superoxide dismutase activities) in the homogenates were determined using standard biochemical methods. The results of the study showed that the diluted crude oil significantly ($P<0.05$) affect all the parameters investigated in the liver and heart which brought about impaired function of the organs. Histopathological examinations indicate that the mixture caused death of many cells in the organs in a dose dependant manner especially with the groups treated with 1.0ml and 1.5 ml of mixture /g body weight as compared with the normal control group. The study therefore discourages the use of diluted crude oil as therapy for some diseases.

Key words: Crude oil, organs, enzymes, antioxidant status, histopathology, rat.

INTRODUCTION

Crude oil contains significantly high amount of toxic chemicals like polycyclic aromatic hydrocarbons, heavy metals etc. which can cause wide range of health effects in people and wildlife, depending on the level of exposure and susceptibility. Exposure of humans and animals to these chemicals is increasing in terms of the environmental level and the different usage of crude oil (Patrick-Iwuanyanwu *et al.*, 2011). Difference in exposure or contact usually occurs based on work, personal activities, age, diet, use of protective equipment and other factors. The toxic effect of crude oil can be acute, lethal, sub-lethal or both, depending on the level of exposure organism exposed and the dosage it is exposed to (Al-Balawi *et al.*, 2013). Niger delta is the richest part of Nigeria in terms of oil and gas resources as well as extensive mangrove forest along the West African coast line. In this area, more than 90 % of crude oil activities in N.geria take place (Asara *et al.* 2013).

It is important to note that majority of the people in the communities of the Niger Delta area ingest crude oil directly as a curative agent for anti-poisoning (snake venom antidotes), anti-convulsion and treatment of skin infection (Dede *et al.*, 2002). Bonny light crude oil is used in combination with olive oil in folklore medicine in some parts of Niger Delta region of Nigeria to treat burns, gastrointestinal disorders, witchcraft and poisoning (Orisakwe *et al.*, 2000; Dede *et al.*, 2002). Apart from Nigeria, some African countries like Kenya, Tanzania, Zimbabwe, Ghana and Tunisia use crude oil to treat ailments like stomach ache, diarrhoea, coma from respiratory diseases (Okoye, *et al.*, 2014). This practice has been on for a long time without due consideration to the bio-safety of the commodity. A lot of research works has been reported on crude oil either directly that is used as therapy or indirectly that is in terms of environmental pollution. Generally, crude oil presence in terrestrial and aquatic ecosystem occurs as a consequence of spillage which may result from natural seepage, exploration activities, equipment failure, leakage from oil wells or oil tankers, negligence, accidents and sabotage (Jackson *et al.*, 1989; Odiete, 1999; Osuji *et al.*, 2004; Igwe *et al.*, 2008). Ikanone *et al.*, 2017 reported that at very low dose NBLCO have hepatotoxic effect and disrupt plasma biochemistry. Okoye *et al.*, 2014 reported that Escravous crude oil could be considered as a potential endocrine disrupter which can affect the tissue architecture and the endocrine functions of the

energy. The potential toxic effects of crude oil are exerted on a variety of organs of living systems such as the skin, lungs, liver and kidney (Hunter, 1968; Eyong *et al*., 2004. Adedara *et al*., 2012; Ita *et al*., 2014. Okoye *et al*., 2014) There is paucity of information concerning the effect of this oil on the heart and also in female rats. It is in the light of this that the present research is designed to evaluate some biochemical parameters of acute exposure to Nigerian Bonny light crude oil in the liver and heart of female albino rats.

MATERIALS AND METHODS

Bonny Light Crude Oil (BLCO)

BLCO was obtained from the Niger Delta Petroleum Resources, Port Harcourt, Rivers State through the Department of Petroleum Resources (DPR), Lagos State, Nigeria

Animal

All procedures for maintenance and sacrifice (care and use) of 28 female albino rats weighing between 100 to 200g were carried out according to the criteria outlined by the National Academy of Science published by the National Institute of Health (NIH, 1985). All the animals were handled humanely, kept in plastic suspended cages placed in a well-ventilated and hygienic rat house under suitable conditions of temperature and humidity. They were provided rat pellets and served water *ad libitum* and subjected to natural photoperiod of 12 hour light and dark cycles. The animals were allowed two weeks of acclimatization prior to the commencement of the experiments using the animal model.

Table 1: Experimental design

Group	No of Albino rats	Dosage of NBLCO (g/kg body weight) – Olive oil in ml	Actual dosage in volume (ml/kg BW) – olive oil in ml
Group 1– Normal control	7	0.5ml of distilled water only	0.5ml of distilled water only
Group 2 – Positive control as Vehicle	7	0.5ml of olive oil only	0.5ml of olive oil only
Group 3	7	2.0 –0.5	0.5+0.5 (1ml mixture)
Group 4	7	4.0–0.5	1.0+0.5 (1.5ml mixture)

The mode of administration of the crude petroleum to the animals was through oral gavage. The dose of crude oil used for the study was adopted from previous research work by Ikanone, *et al*, (2017), the LD_{50} of Nigeria Bonny Light Crude Oil was determined to be 16.95g/kg BW or 16950 mg/kg BW or 4ml/kg BW through oral route of administration. The rats were randomly separated into four groups with seven (7) rats per group; no crude oil was administered to the positive and normal control groups. The positive control group was given 0.5ml/kg BW of Extra virgin olive oil only (Group 2) while the normal control group was given 0.5ml/kg BW of distilled water only (Group 1). The extra virgin oil serves as vehicle to drive the crude oil into the system. Rats of the test groups (group 3 and 4) were given crude oil every day, the quantity of crude oil and olive oil administered to the rats is as shown in table 1 above.

Biochemical Assays

At the completion of respective dosages, animals were allowed to fast overnight and anaesthetized in a desiccators pre-soaked with chloroform. All animals were sacrificed by cervical decapitation. During this study, no mortality was recorded. Blood samples were collected by cardiac puncture using 5 ml hypodermic needle and syringe into heparin bottles. The blood samples were centrifuged at 4000 rpm for 10 minutes to obtain the serum. The liver and heart were excised, weighed and multiplied by 10 to get the volume of the phosphate buffer (pH 7.4) that was used to homogenize the organs, in order to obtain the liver and heart homogenates. The homogenates and sera samples were stored in Eppendorf tubes at -5°C in a freezer for further biochemical analysis. The samples were used within 48 hours.

Determination of Serum Enzyme Activity

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated by the use of end point colourimetric diagnostic kit (Randox Laboratories Limited, England) according to the method of Reitman and Frankel (1957) Alkaline phosphatase (ALP) activity was determined by the use of Sigma diagnostic kits (Sigma Diagnostic, USA) according to the method of Englehardt (1970). AST activity was also estimated in the heart homogenate.

Estimation of Total Protein, Catalase (CAT) and Superoxide dismutase (SOD) activities, Glutathione (GSH) and Thiobarbituric reactive

[illegible] (TBARS) concentrations in the liver and heart homogenates.

The concentration of total plasma protein was estimated using sigma diagnostic kits (Sigma Diagnostic, USA). Catalase activity was estimated according to the method of Aebi (1984). Superoxide dismutase activity was determined according to the method of McCord and Fridovich (1969). Reduced Glutathione concentration was estimated according to the method of Ellman, (1959), while that of TBARS was done according to the method of Fraga and Dippel, (1988).

Histopathological Examination

The animals were dissected under an anesthesia and the liver and heart were recovered. The organs were carefully excised, trimmed of connective tissues, dried, weighed and then kept in a universal bottles containing 10 % buffered formalin for histopathological assay. Portions of the liver and heart of all the animals in each group after sacrificed were weighed. The organs were first grossly examined for any observable lesions or tissue derangements before they were fixed in 10 % formalin for 48 hours. The organs were then processed using an automatic tissue processor, embedded in paraffin wax and sections (5 µm thick) cut using a rotary microtome. The technique involved dehydrating the fixed tissues placed in tissue baskets with their respective labels and passing them through graded alcohol (70, 90, 95 and 100 %) solutions. The tissues were removed after dehydration and moved into xylene solution bath to clear the alcohol and facilitate molten wax impregnation. The sectioned tissues were stained with Haematoxylin and Eosin (H and E) and then examined microscopically using standard techniques of Arthur and John (1978).

Statistical Analysis

Data obtained from the different parameters of the study were subjected to test for the level of homogeneity and the Duncan multiple range test was used to separate means were heterogeneity occurred. P values at < 0.05 were considered significant using SPSS version 10.

RESULTS

Table 2 : Effect of diluted Nigerian Bonny Light Crude Oil on ALP, AST, and ALT activities in the serum of the rats after 28 days of oral administration

Group	ALP (u/l)	AST (u/l)	ALT (u/l)
1	13.340 ± 1.490^a	15.000 ± 1.410^a	62.472 ± 0.070^a
2	28.980 ± 1.870^b	24.000 ± 0.410^b	47.189 ± 2.135^b
3	9.660 ± 0.330^c	9.000 ± 0.010^c	56.434 ± 1.500^c
4	9.660 ± 0.330^c	32.000 ± 1.410^d	88.792 ± 0.930^d

Values are represented as Mean ± standard deviation for tw[illegible] o determinants, values with different alphabets superscript under the same column are significantly different at P<0.05.

Table 3: Effect of diluted NBLCO on the total protein concentration and antioxidant status of the liver homogenate after 28 days of oral administration

Group	Total Protein (g/g tissue)	Catalase activity (mg/g tissue)	SOD activity (mg g tissue)	GSH Conc (mmol/g tissue)	TBARS (mmol/g tissue)
1	2.650 ± 0.010^a	5.390 ± 0.050^a	0.029 ± 0.001^a	47.500 ± 0.354^a	0.021 ± 0.003^a
2	4.250 ± 0.021^b	2.290 ± 0.032^b	0.012 ± 0.001^b	36.830 ± 0.061^b	0.010 ± 0.003^a
3	1.662 ± 0.001^c	2.290 ± 0.032^b	0.051 ± 0.002^b	76.675 ± 0.236^c	0.010 ± 0.001^a
4	1.408 ± 0.010^c	5.390 ± 0.017^a	0.027 ± 0.001^a	45.000 ± 0.707^a	0.010 ± 0.001^a

Values are represented as Mean ± standard deviation for tw[illegible] o determinants, values with different alphabets superscript under the same column are significantly different at P<0.05

Table 4: Shows the activities of Superoxide Dismutase, Catalase and Aspartate Aminotransaminase for 28 days of oral administration of diluted NBLCO in the heart homogenate

Group	SOD activity (mg g tissue)	CAT activity (mg g tissue)	ALT (u/g tissue)
1	4.310 ± 0.500^a	5.050 ± 0.650^a	0.070 ± 0.001^a
2	4.610 ± 0.040^a	7.110 ± 0.350^a	0.700 ± 0.014^b
3	1.360 ± 0.090^b	6.420 ± 0.430^a	0.330 ± 0.010^b
4	0.720 ± 0.010^c	7.110 ± 0.350^a	0.830 ± 0.010^b

Values are represented as Mean ± standard deviation for tw[illegible] o determinants, values with different alphabets superscript under the same column are significantly different at P<0.05

Table 5: Shows the concentration of Total Protein, Thiobarbituric Acid Reactive Substances, and Reduced Glutathione for 28 days of oral administration of diluted NBLCO in the heart homogenate

Group	Total Protein (g/g tissue)	GSH Conc (mmol/g tissue)	TBARS (mmol/g tissue)
1	4.400 ± 0.610^a	0.770 ± 0.010^a	49.500 ± 1.130^a
2	3.174 ± 0.840^a	1.540 ± 0.010^b	83.000 ± 2.360^b
3	6.476 ± 0.039^b	1.540 ± 0.010^b	91.700 ± 2.465^c
4	3.429 ± 0.289^a	1.830 ± 0.020^c	44.670 ± 2.360^a

Values are represented as Mean ± standard deviation for two determinants, values with different alphabets superscript under the same column are significantly different at $P<0.05$

Histopathological Results

The histopathological changes of the liver in the different treatment groups are as shown in Figures 1 to 4. The normal control group (group 1) showed normal liver with well-preserved lobular architecture, normal hepatocytes, normal central vein capsules with no sign of adhesion, inflammation or lesion but for the case of the positive control group (group 2), there were mild proliferation of bile ductules. Low dose crude oil group (group 3) showed diffuse micro-hepatic vacuolation with distented sinusoid. Atrophy and necrosis of the hepatocytes were also evident.

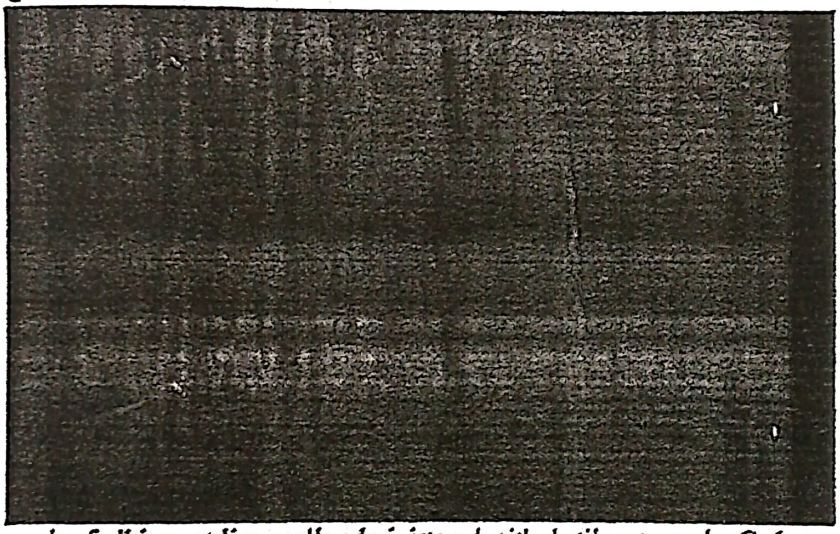


Fig 1: Photomicrograph of albino rat liver cells administered with distil water only, C, (normal control). H&E x400, showing no visible lesions.

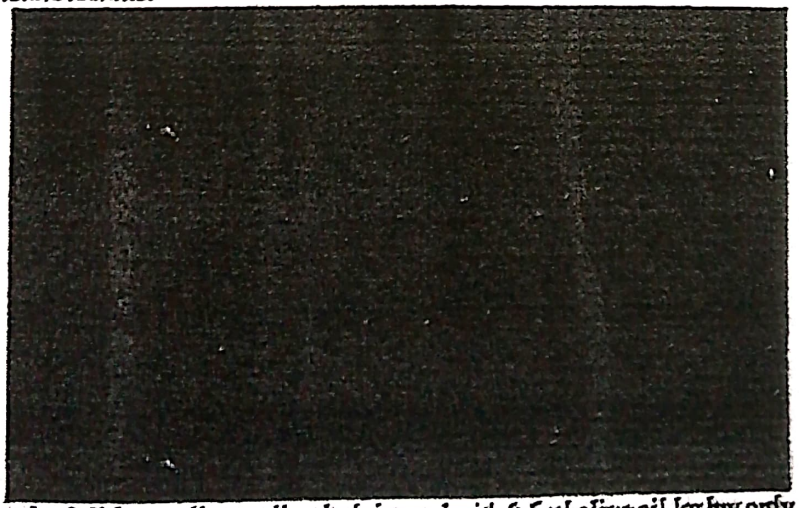


Fig 2: Photomicrograph of albino rat liver cells administered with 0.5ml olive oil/kg bw only, C (positive control), showing diffuse micro-hepatic vacuolations and showing mild proliferation of bile ductules (arrows). H&E x400 In group 4, there was focal area of hepatic necrosis with nuctrophilic infiltration.

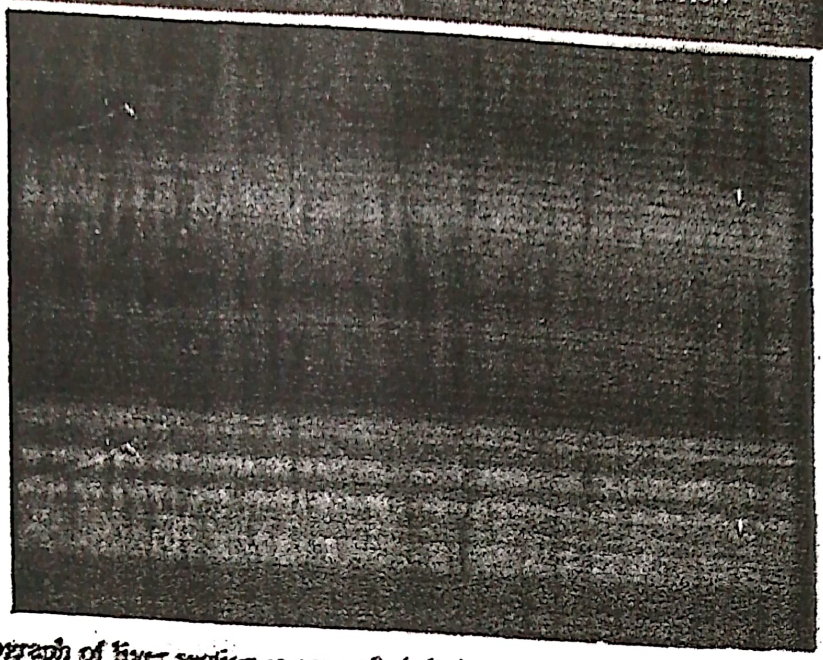


Fig 3: Photomicrograph of liver section in group 3. (administered 1.50 ml of PHF/kg + 0.50ml of Olive oil) showing diffuse micro-hepatic vacuolations (arrow) with inflamm amassed (). Atrophy and necrosis of hepatocytes were also evident. H&E x40)

Fig 4: Photomicrograph of liver section in group 4. (administered 1.0 ml of PHF/kg + 1.50ml of Olive oil) showing focal area of hepatic necrosis with neutrophilic infiltration (arrow). H&E x40)

The histopathological changes of the heart in the different treatment groups are as shown in Figures 5 to 8. The normal control group (group 1) showed normal heart with well-preserved ventricular architecture, normal cardiocytes, normal central vein capsules with no sign of adhesion, inflammation or lesion but in the case of the positive control group (group 2), severe vacuolar degeneration and necrosis of myofibrils with severe oedema fluid in the interstitium. Low dose crude oil group (group 3) dosed with 1.5ml of cows urine after 24 showed multiple foci of myocardial necrosis with oedema fluid in the interstitium while in the higher dose (group 3) dosed with 1.5ml of cows urine after 24 hrs showed severe diffuse oedema fluid within the interstitium with fibrin strands. There was multiple foci of myocardial necrosis.

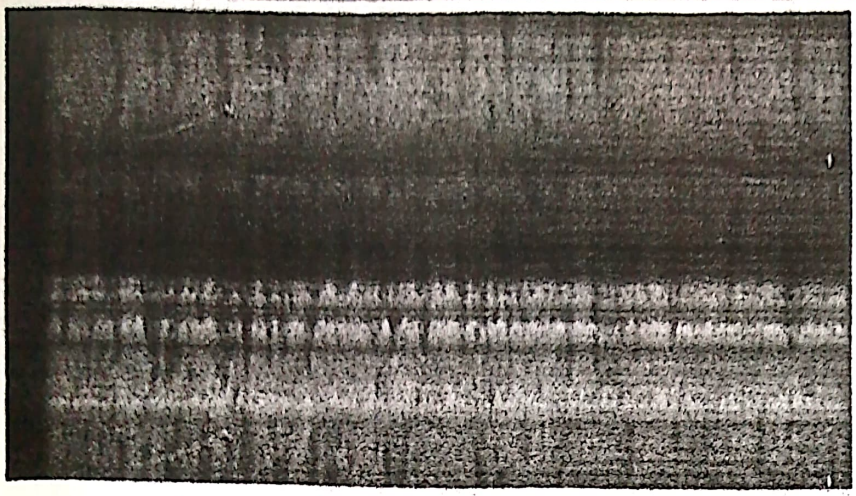


Fig 5 Photomicrograph of albino rat heart cells administered with 0.5ml olive oil/kg bw only, C_+ (positive control) H&E x400 showing severe diffuse vacuolar degeneration and necrosis of myofibrils (arrow) with severe oedema fluid in the interstitium (O).

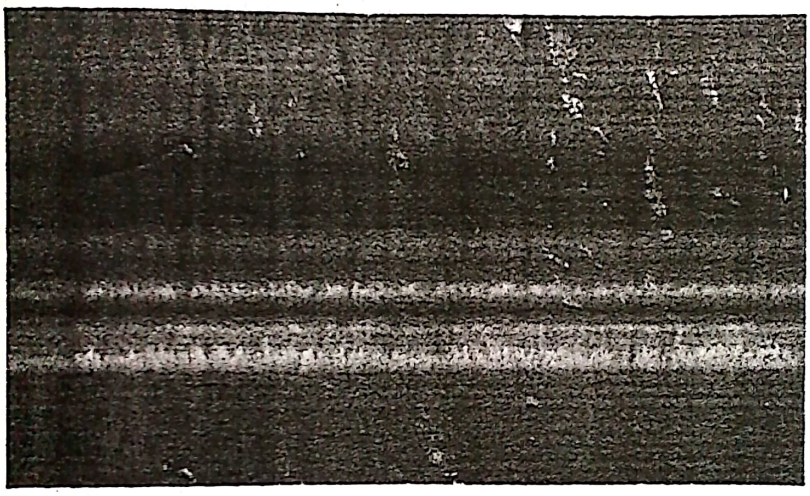


Fig 6 Photomicrograph of albino rat heart cells administered with distil water only, (normal control), H&E x400, showing no visible lesions.

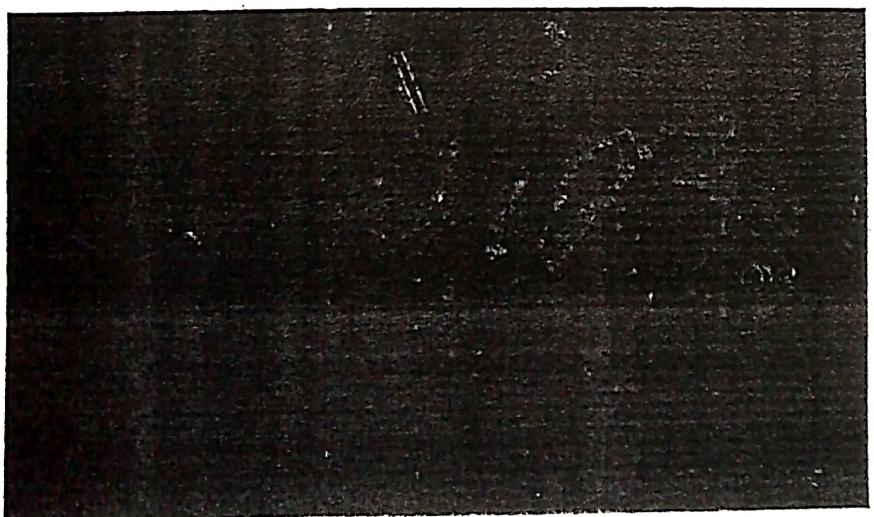


Fig 7 Photomicrograph of heart section in group 3, (administered 0.50 ml of BLCO/kg + 0.50ml of Olive oil) showing multiple foci of myocardial necrosis (arrow) with oedema fluid in the intenstitium (O) and mild mononuclear cells infiltration, H&E x400

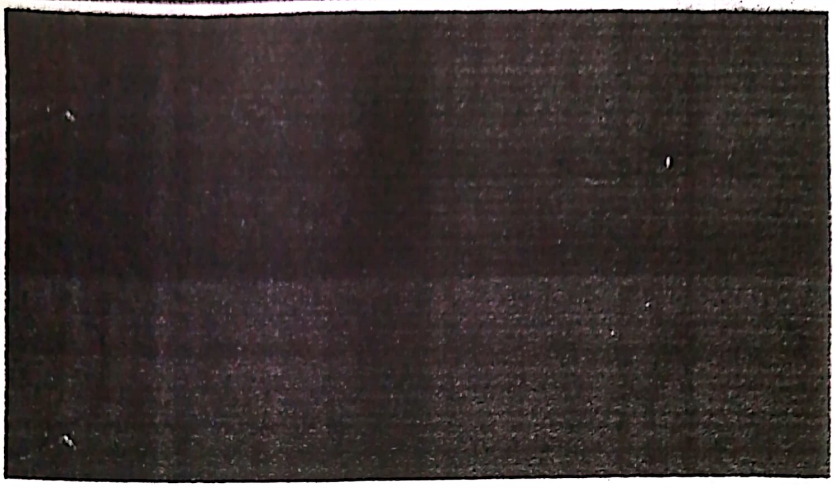


Fig 8: Photomicrograph of heart section in group 4. (administered 1.0 ml of BLCO/kg – 0.5ml of Olive oil) showing severe diffuse oedema fluid within the interstitium with fibrin strands (arrow). There was multiple foci of myocardial necrosis. H&E x400.

DISCUSSION

Crude oil is used in folkloric medicine in the Niger Delta area of Nigeria for treatment of various ailments including stomach upset, wounds and burns. It is used in combination with olive oil to treat gastrointestinal ailment, act as anti-venom for snake bite and for reversing the effect of poisons (Orisakwe *et al*., 2000). The route of administration is mostly oral and externally applied on various ailments such as burns and wounds. The Nigerian Bonny Light Crude Oil is classified as Class A, a light crude oil, with aromatic hydrocarbons accounting for up to 95% of the total hydrocarbons originally present in crude oil. It is expected that the potential of this light crude oil would have toxic effect.

During the period of the research, it was observed immediately after the oral administration of crude oil: the animals in the treated groups (2, 3, and 4) became less aggressive and were docile. This consequently led to loss of appetite and decreased locomotion. Soon after, they regained their appetite. These observations are in agreement with Ikenoue *et al*. (2017).

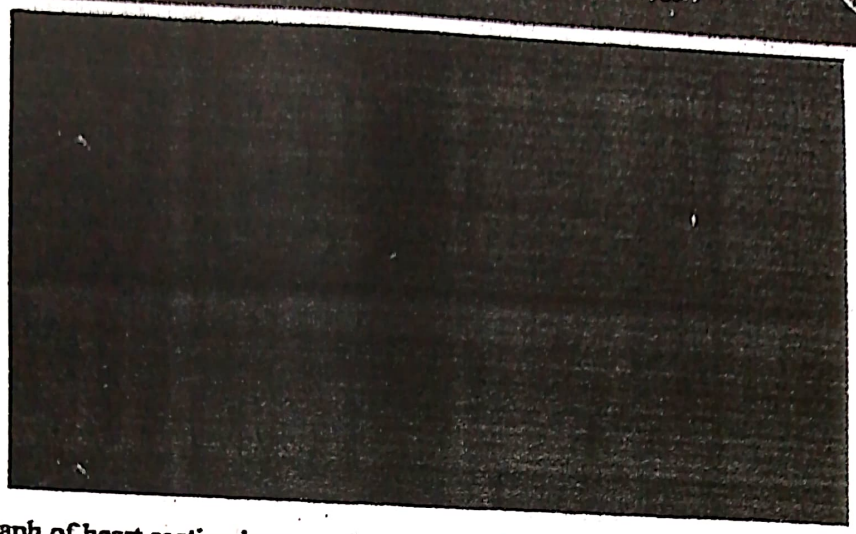
The biochemical indices monitored in the liver are useful markers for assessing the functional capacity of the organ exposed to toxicants. If these indices are altered, normal function of organ will be impaired (Afolayan and Yakubu, 2009).

The liver plays an important role in metabolism and also in the regulation of the internal body environment (homeostasis). Hepatic injury may be due to some toxicants found in polluted environments which may find their way into the individual's body either by consumption or during respiration, failure for the liver to eliminate these toxicants in the body may lead to malfunction and finally compromised its integrity.

The effect of diluted NBLCO in the heart of mammals has not been listed in literature. Considering the biochemical significances of the heart in the pumping of blood to all parts of the body, it is pertinent to evaluate its biomarkers as regard to crude oil therapy in order to find out if the diluted crude oil treatment is bio-safe.

The result of ALP activity in the serum is as presented in Table 2. Serum ALP activity is a sensitive indicator in biliary cirrhosis, hepatitis, and in disease characterized by inflammation regulation intrahepatic and extrahepatic bile obstruction (Panibong *et al*., 2003). Mean ALP activity of the rat in groups 3 and 4 were significantly low ($P<0.05$ when compared to the control groups (1 and 2). The result of AST and ALT activities are as presented in Table 2. AST and ALT in the serum are often associated with hepatocellular damage (Lynossi *et al*., 2004). After twenty eight (28) days of dose exposure of orally administered BLCO the rat groups (3 and 4) showed significant increase ($P<0.05$) in ALP, AST, and ALT activities in a dose dependent manner. The increased levels of ALP, AST, ALT activities are conventional indicators of liver injury (Shah *et al*., 2011). This observance is also in conformity with the findings of Ikenoue *et al*. (2017). These serum enzymes (ALT and AST) are largely used in assessment of liver damage by drugs or any other hepatotoxicants.

The result of total protein concentration in the liver homogenate is as presented in Table 3. The result shows that there was significant decrease ($P>0.05$) in total protein concentration of groups 3 and 4 when compared with the normal (group 1) and positive control groups (group 2) after the experimental period



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of twenty eight (28) days of exposure. The biochemical consequence of this decrease could be attributed to the fact that the toxic constituents (water soluble fractions) present in the Nigeria Bonny Light Crude Oil may have impaired protein synthesis which could ultimately lead to the decrease in total protein concentration. The result of this study is not in agreement with that reported by Ikanone *et al.*, (2017). The reasons for this are not far-fetched. This could be as a result of sex differences and duration of study. While in this study female rats were used as against male rats in Ikanone *et al.* (2017). Hormonal imbalance between the male and female albino rats, and the experimental design could have accounted for the total protein difference, hence, the disagreement.

The results of SOD and catalase activities in the liver homogenate are as presented in Table 3. The result shows a decrease in SOD activity in treated groups, 3 and 4 when compared to that of the control groups, 1 and 2 which appear to be slightly different from each other. This decrease in the enzyme activity is expected because the crude oil also contributed reactive oxygen species (ROS) (e.g. free radicals) to the liver apart from the ones the organ generates naturally thereby decreasing the activity of superoxide dismutase. Superoxide dismutase catalyzes the dismutation of superoxide anion to hydrogen peroxides which serves as a substrate for catalase enzyme. For catalase activity, the result shows an increase in the normal control group, and treated group, 3 when compared to positive control group and treated group, (group 3) with both groups having the same value. Catalase is expected to convert hydrogen peroxide generated from SOD to water and oxygen and the metabolic process continues. Catalase activity in the experimental group, (groups 3 and 4) is high because more hydrogen peroxide is being produced from SOD catalyzed reaction as a result of more ROS being generated from the constituents (hydrocarbons and heavy metals) present in crude oil. The result of Reduced Glutathione concentration in the liver homogenate is as represented in Table 3. The result shows that there was significant increase ($P<0.05$) in group 4 compared to the groups 2 and 3. The result of Thiobarbituric Acid Reactive Substances (TBARS) in the liver homogenate is as represented in Table 3. TBARS is a product of lipid peroxidation. The result shows that there was no significant increase ($P>0.05$) between the test groups when compared to control groups in the liver homogenate. The result of total protein concentration in the homogenate is as presented in Table 5. The result shows that there was a significant increase ($P<0.05$) in groups 2 and 3 when compared to the normal control group but there was a significant decrease ($P<0.05$) in group 3 when compared to group 1 after the experimental period of 28 days. This shows that there is an inverse relationship with the doses and the total protein concentration (that is, as the dose increases from group 1 to group 4 total protein concentration decreases).

The result of Thiobarbituric Acid Reactive Substances (TBARS) in the heart homogenate is as represented in Table 5. TBARS is a product of lipid peroxidation. The result shows that there was a significant decrease ($P<0.05$) in group 3 when compared to group 1 and a significant increase ($P<0.05$) in groups 2 and 3 when compared to normal control group in the heart homogenate. The biochemical consequence of this is that the lipid membrane in the cardiocytes has been damaged by the toxicant thereby making the cell membrane porous which invariably confirms that lipid peroxidation has occurred in the heart, hence, the increase.

The result of Reduced Glutathione concentration in the heart homogenate is as represented in Table 4. The result shows that there was no significant difference ($P>0.05$) in group 4 when compared to group 1 and there was a significant increase ($P<0.05$) group 3 when compared to group 4 but no significant difference ($P<0.05$) between groups 2 and 3.

The result of aspartate aminotransaminase activity in the heart homogenate and serum are as represented in Table 4. The result shows a significant increase ($P<0.05$) in activity of aspartate aminotransaminase in group 4 and in positive control group when compared to normal control group, but there was no significant difference ($P>0.05$) in groups 1 and 3 in heart homogenate. In the serum, the result shows no significant difference ($P>0.05$) in all the groups (both control and treated groups). AST is one of the markers used to ascertain the integrity and function of the heart cells (cardiocytes) apart from it being present in the liver. When there is myocardiac infarction, that is, injury to the heart as a result of toxicants or poison, the enzyme AST leak into the blood as a result of the damage done to the heart membrane. An increase of this enzyme in the serum is an indication of myocardiac infarction. Although aspartate aminotransaminase activity in the homogenate is higher than in the serum does not mean that damage has not been done to the heart cells. Some of the enzymes have leaked into the blood, hence, the enzyme activity in the serum was observed.

The results of catalase and superoxide dismutase activities in the heart homogenate are as represented in Table 4. For superoxide dismutase activity, the result shows a significant decrease ($P<0.05$) of the enzyme activity in treated groups 3 and 4 when compared to control groups 1 and 2. This decrease in the enzyme activity is expected because the crude oil

also contributed ROS to the heart cells apart from the one it generates naturally thereby decreasing the activity of superoxide dismutase. For catalase activity, the result shows no significant difference ($P>0.05$) in all experimental groups. The catalase enzyme is expected to convert hydrogen peroxide generated from SOD to water and oxygen.

Catalases activity in the experimental groups is high because more hydrogen peroxide is being produced from SOD catalyzed reaction as a result of more ROS being generated from the constituents (hydrocarbons and heavy metals) present in crude oil.

Histopathological examinations of the organ tissues of the treated rats indicated that acute exposure of diluted BLCO at very low doses can alter their metabolic functions. In the case of the liver, the mixture affected the structural integrity of the liver cells and compromised its function. This is characterized by multiple bile duct proliferation with foci of hepatic necrosis with nuctrophilic infiltration in a dose dependent pattern while that of the heart, shows multiple foci of myocardial necrosis within oedema fluid in the interstitium with fibrin strands also in a dose dependant pattern.

CONCLUSION

The study shows that acute exposure of Nigeria Bonny light crude oil diluted with extra virgin olive oil is hazardous to health. The outcomes of the research reveal that exposure to diluted NBLCO can damage the liver and heart cells and hence discourage the use of the crude oil in combination with olive oil as therapy.

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