

EFFECT OF SUB-ACUTE EXPOSURE TO BONNY LIGHT CRUDE OIL ON PLASMA BIOCHEMISTRY AND LIVER HISTOPATHOLOGY OF ALBINO RAT

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ABSTRACT

The study investigated the consequences of the effect of sub-acute exposure to Nigerian Bonny Light Crude Oil (BLCO) crude oil on the blood chemistry and integrity of the liver of male albino rats. A total of 20 male wistar rats were used for the study. Exposure to crude oil was achieved by oral administration of increasing doses (0.25, 0.50, 0.75 and 1.0 ml of BLCO/g body weight) to the rats every day for two weeks. The initial and final body weights were recorded. The toxic effects on the liver were accessed using commercial kits and histopathological studies were carried out using standard histopathological technique. The results revealed that liver cells were damaged due to the crude oil administered. There was significant increase ($p < 0.05$) in alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities and total and direct bilirubin concentrations, and a significant decrease ($p < 0.05$) in the total protein concentration as compared with the control group. Histopathological examinations indicated that crude oil caused severe pathological changes, it also revealed mild to severe disruption of the normal architectural structure of the liver accompanied by the death of many liver cells and the presence of pocket of blood within the liver parenchyma and cholangitis in the group treated with the highest dose (1.00 ml of BLCO /g body weight). The results therefore indicate that the sub-acute administration of the crude oil brought about impaired function of the liver which could lead to liver disease at very low doses and are such the use of the crude oil as a therapy to poisons, convulsion and other gastrointestinal disorders should be discouraged.

Keywords: Crude oil, Blood chemistry, Enzymes, Histopathology, Hepatocytes

INTRODUCTION

Pollution by crude oil is a widespread and common problem that can arise either accidentally or operational whether oil is produced, transported, stored, processed, or used at sea or on land (Orisakwe *et al.*, 2004).

In Nigeria, the exposure of crude oil in aquatic environment is on the increase following the several frequent spillages that have occurred in our coastal waters (Ordinioha and Brisibe, 2013). Oil spillage has caused destruction of food resources (Percival and Evans, 1997).

Animal species that are not directly in contact with the oil spillage can also be harmed via the food web as a result of lethal doses of toxicants. Predators that consume contaminated marine preys can be exposed to crude oil through the ingestion of prey.

Crude oil has been described as a complex mixture of over 6000 potentially different hydrocarbons and metals. Accidental exposure to crude oil or its complex chemical constituents can cause toxic effects in humans and other animal species (Sunmonu and Oloyede, 2007). Crude oil contains significantly 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. 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 will occur based on work, personal activities, age, diet, use of protective equipment and other factors. The toxic effect 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 Nigeria 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). The toxic chemicals present in the crude oil when ingested do affect tissues and organs and consequently influence growth and performance (George and Sese, 2012). There is a concern that workers and other individuals exposed to crude oil might have an increased incidence of organ damage. After absorption via pulmonary

or gastrointestinal routes, crude oil is transported in plasma initially bound to albumin and other large proteins to the liver (Orisakwe *et al.*, 2004).

The liver, for instances, is the fundamental organ in the metabolism and detoxification of xenobiotics (drugs/chemicals and toxins). Thus, these chemicals/drugs affect the liver more frequently than any other organ and this places the liver at greater risk for damage induced by toxic substances (Choumessi *et al.*, 2012). It is in the light of this regard that the present study was initiated to evaluate the effect of sub-acute exposure to undiluted Nigerian Bonny light crude oil (BLCO) on the blood chemistry and histopathology of the liver of 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 36 male albino rats 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 (Ladokun feeds) 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.

Lethal Dose of BLCO: Lethality studies to determine the LD₅₀ of the BLCO was performed according to the combined procedures described by Lorke (1983) and OECD guidelines-425 (OECD, 2001). It was assessed through oral route. Sixteen male albino rats with mean weight 185 ± 5.0 g were randomly assigned to

eight groups, with each group having two animals, the animals acted as replicates. After the period of acclimatization, they were then fasted overnight prior to the experiment. The animals were respectively treated with 1.4, 1.8, 2.2, 2.6, 3.0, 3.4, 3.8 and 4.2 ml of the BLCO per kg body weight (BW). The animals were then returned to their respective cages, allowed free access to pellets and drinking water 3 hours later. They were thereafter monitored for clinical signs, symptoms, behavioral change, feeding pattern and mortality within 24 hours of the experiment. Animals were observed individually once during the first 30 minutes after dosing, periodically during the 24 hours, with more attention during the first 4 hours. There was 100 % mortality with the group treated with 4.2 ml per kg body weight and 0 % mortality with the group treated with 3.8 ml per kg body weight after 24 hours. The lethal dose of the BLCO was calculated using the formula: $LD_{50} = \sqrt{D_0 \times D_{100}}$. Where D_0 = maximum dose that produce 0 % mortality, D_{100} = Maximum dose that produce 100 % mortality. The relative weights in grams were estimated as = initial weight \div final weight \times 100 and expressed in percentages.

Sub-lethal Toxicity of BLCO: Repeated dose toxicity study was carried out to determine the effects of BLCO on blood chemistry and liver of male albino rats. Twenty male rats with mean weights 130 ± 30 g that were not subjected to previous experiments were used for the study. The rats were randomly separated into five groups with four rats per group, each rat acted as a replicate. The control group was administered rat feed and water only, while the test groups (groups 1 to 4) were administered orally 0.25, 0.50, 0.75 and 1 ml of BLCO/kg BW respectively for two weeks.

Biochemical Assays: After 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 EDTA bottles. The blood samples were centrifuged at 4000 rpm for 10 minutes to obtain the plasma and then stored in a refrigerator at 4 °C. The plasma samples were used within 48 hours.

Determination of Liver 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).

Estimation of Total Protein, Total and Direct Bilirubin Concentrations: The concentrations of total plasma protein, total and direct bilirubin levels were estimated using sigma diagnostic kits (Sigma Diagnostic, USA).

Histopathological Examination: The animals were dissected under chloroform anesthesia and the liver recovered. The livers were carefully excised, trimmed of connective tissues, dried, weighted and then kept in a universal bottles containing 10 % buffered formalin for histopathological assay. Portions of the liver of all the animals in each group after sacrificed were weighed. The livers were first grossly examined for any observable lesions or tissue derangements before they were fixed in 10 % buffered formalin for 48 hours. The livers were then processed using an automatic tissue processor, embedded in paraffin wax and sections (5 μ m 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 baths 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 analysis of variance ($p < 0.05$). The results were expressed as mean \pm standard deviation. Statistical comparisons were performed using SPSS.

RESULTS

LD₅₀ of BLCO: The LD₅₀ of BLCO through oral route was determined as 4 ml/kg BW which can also be expressed as 16.95 g/kg BW or 16950 mg/kg BW. Oral administration of the BLCO cause negative behavioural changes in the animals and 100 % mortality was recorded within and after 24 hour from the group treated with 4 ml/kg BW. The animals were not active and physical activity was very slow and decreased appetite was observed.

Plasma Biochemistry: The effect of BLCO on body weight, absolute and relative weights of liver after 14 days of exposure to male rats indicated that there was a slight increase in the body weight of the control group and a decrease in the lower dose groups during the exposure period and a significant reduction ($p < 0.05$) in body weight in the high dose group (1 ml of BLCO/kg BW) compared to the control (Table 1). There were no significant differences ($p < 0.05$) in the absolute and relative liver weights of the animals in the experimental groups.

The data on liver enzymes showed progressive significant increase ($p < 0.05$) in ALT, AST and ALP activities of the treatment groups as compared with the control group (Table 2). There was also a significant increase ($p < 0.05$) in total bilirubin and direct bilirubin in a dose relative manner as compared with the control animals whereas there was a significant decrease ($p < 0.05$) in total protein of the different experimental groups in a progressive pattern as compared with the control group (Table 3).

Histopathological Effects of BLCO: The histopathological changes of the liver in the different treatment groups are as shown in Figures 1 to 5. The control group showed

normal liver with well-preserved lobular architecture, normal hepatocytes, normal central vein capsules with no sign of adhesion, inflammation or lesion. Low dose crude oil groups showed multiple of bile duct proliferation with foci of hepatic necrosis. In 0.75 ml of BLCO/kg BW group, there was also multiple bile duct proliferation with foci of hepatic necrosis and cholangitis. The highest dose group (1 ml of BLCO/kg BW) showed pocket of blood within the liver parachyma and cholangitis with hepatic necrosis. There was also duct proliferation.

DISCUSSION

The effect of sub-acute exposure of Bonny light crude oil (BLCO) on the blood chemistry and histopathology of the liver of albino rats was investigated. In the study, the weight of the rats exposed to BLCO decreased significantly ($p < 0.05$) in a dose dependent pattern. This may be due to the impairment caused by the crude oil in the digestive system of the rats orally exposed to it. This observation was in agreement with studies reported on guinea pigs, rats and other species (Mbadugha *et al.*, 2012; Raji and Hart, 2012; Azibalua *et al.*, 2014). Though the pattern and dosage of BLCO administration differs, there was reduction in body weight of the animals under investigation. There was no significant differences ($p < 0.05$) in the absolute and relative liver weights of the rats in the test groups. This observation confirmed the findings of Orisakwe *et al.* (2004).

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 (homeostatis). 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 compromised its integrity.

Table 1: Effect of 14 days sub-acute administration of varied concentrations of Bonny light crude oil on the body weight, and absolute and relative liver weights of male albino rat

Experimental group	Dose (ml of BLCO / kg BW)	Initial body weight (g)	Final body weight (g)	Absolute liver weight (g)	Relative liver weight (%)
Control	0.00	144.00 ± 36.43 ^b	150.00 ± 38.28 ^d	6.26 ± 2.02 ^d	4.16 ± 0.72 ^b
Group 1	0.20	137.25 ± 4.70 ^a	121.00 ± 3.50 ^a	4.68 ± 0.85 ^a	3.60 ± 0.63 ^a
Group 2	0.50	146.50 ± 5.69 ^c	141.50 ± 7.68 ^b	5.96 ± 0.57 ^b	4.12 ± 0.28 ^b
Group 3	0.75	160.00 ± 2.45 ^d	145.25 ± 5.72 ^c	6.04 ± 0.09 ^c	4.11 ± 0.14 ^b
Group 4	1.00	170.75 ± 6.95 ^e	149.00 ± 2.83 ^d	6.69 ± 0.63 ^e	4.52 ± 0.35 ^c

Values are expressed as mean ± standard deviation of three determinations, values with different alphabets superscript are significantly different ($p < 0.05$)

Table 2: Effect of 14 days sub-acute administration of varied concentrations of Bonny light crude oil on ALP, AST and ALT activities of male albino rat

Experimental group	Dose (ml of BLCO / kg BW)	ALP (mmol/l)	AST (mmol/l)	ALT (mmol/l)
Control	0.00	19.97 ± 0.23 ^a	16.00 ± 0.85 ^a	10.40 ± 1.13 ^a
Group 1	0.25	33.50 ± 0.70 ^b	18.85 ± 0.21 ^b	12.50 ± 0.71 ^b
Group 2	0.50	38.94 ± 0.47 ^c	22.2 ± 1.13 ^c	16.50 ± 0.71 ^c
Group 3	0.75	49.83 ± 0.47 ^d	23.60 ± 0.85 ^d	18.60 ± 1.13 ^d
Group 4	1.00	57.75 ± 2.33 ^e	27.00 ± 0.57 ^e	21.24 ± 0.79 ^e

Values are expressed as mean ± standard deviation of three determinations, values with different alphabets superscript are significantly different ($p < 0.05$)

Table 3: Effect of 14 days of sub-acute administration of varied concentrations of Bonny light crude oil on the total bilirubin, direct bilirubin and total protein of male albino rat

Experimental group	Dose (ml of BLCO / kg B.W)	Total bilirubin (µmol/l)	Direct bilirubin (mmol/l)	Total protein (µmol/l)
Control	0.00	15.07 ± 0.65 ^a	7.5 ± 0.87 ^a	27.65 ± 0.67 ^e
Group 1	0.25	16.84 ± 0.43 ^b	9.35 ± 0.70 ^b	24.99 ± 0.40 ^d
Group 2	0.50	20.62 ± 0.52 ^c	11.44 ± 0.52 ^c	23.47 ± 0.40 ^c
Group 3	0.75	24.70 ± 0.11 ^e	13.65 ± 0.87 ^d	19.57 ± 0.81 ^b
Group 4	1.00	22.57 ± 0.13 ^d	15.62 ± 0.52 ^e	17.77 ± 0.94 ^a

Values are expressed as mean ± standard deviation of three determinations, values with different alphabets superscript are significantly different ($p < 0.05$)

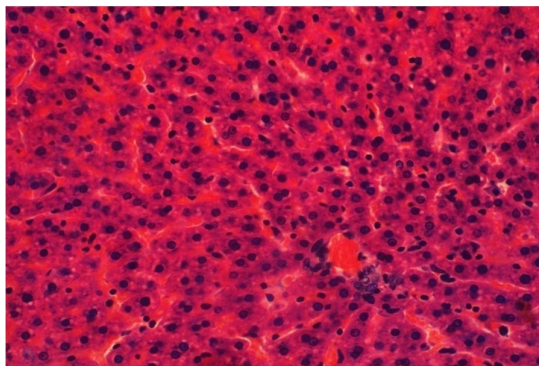


Figure 1: Photomicrograph of albino rat liver cells administered with water and feed only (control), H&E x400

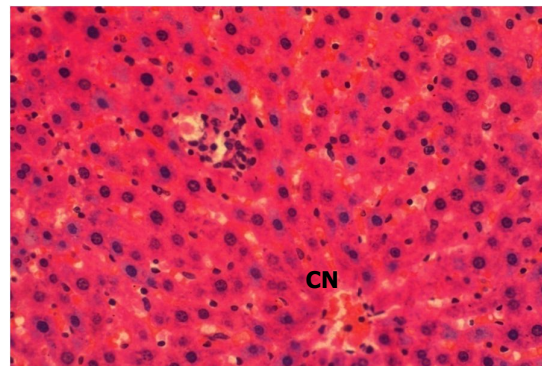


Figure 2: Photomicrograph of liver section in group 1 (administered 0.25 ml of BLCO/kg) showing mild centrilobular degeneration and necrosis of the hepatocytes (CN), H&E x400

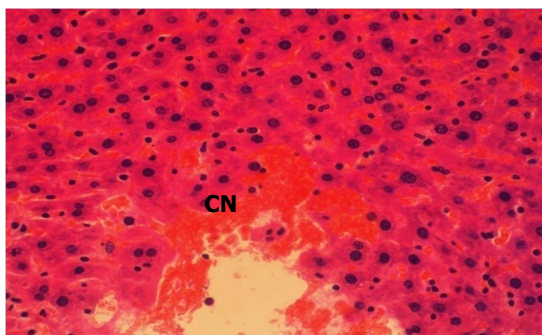


Figure 3: Photomicrograph of liver section in group 2 (administered 0.50 ml of BLCO/kg) showing sinusoidal congestion and centrilobular hepatic necrosis (CN), H&E x400

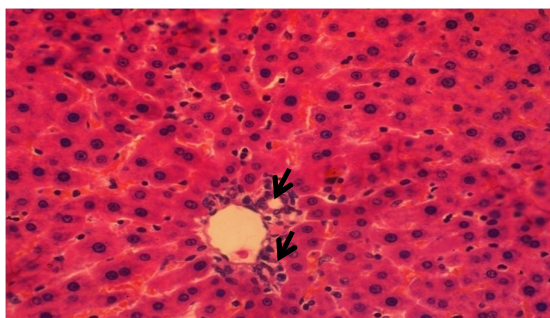


Figure 4: Photomicrograph of liver section in group 3 (administered with 0.75 ml of BLCO/kg) showing mild bile duct proliferation (arrows), H&E x400

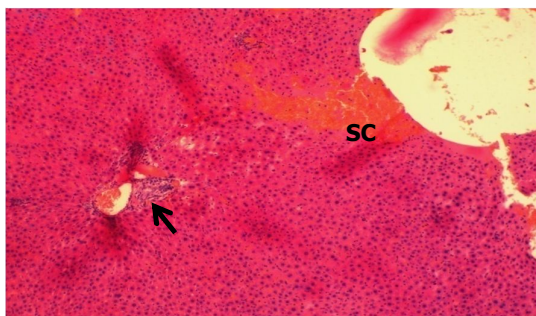


Figure 5: Photomicrograph of liver section in group 4 (administered with 1.00 ml of BLCO/kg) showing both bile duct proliferation and sinusoidal congestion, H&E x400

Serum alkaline phosphatase (ALP) is a sensitive detector in biliary cirrhosis, hepatitis and in diseases characterized by inflammation, regulation, intrahepatic and extrahepatic bile obstruction (Panthong *et al.*, 2003). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum are often associated with hepatocellular damage (Lyoussi *et al.*, 2004). After fourteen days of undiluted exposure of orally administered BLCO, the test

groups showed significant increase ($p < 0.05$) in ALP, AST and ALT activities in a dose dependent manner. The increased levels of ALP, AST and ALT activities are conventional indicators of liver injury (Shah *et al.*, 2011). This observation also corroborated with the findings of Patrick-Iwuanyanwu *et al.* (2013). These serum enzymes (ALT and AST) are largely used in assessment of liver damage by drugs or any other hepatotoxin (Ramaiah, 2011; Patrick-Iwuanyanwu *et al.*, 2012). The elevation of serum marker enzymes observed in the study may be attributed to severe hepatocellular injury irrespective of the experimental design that is dose pattern and period of exposure as recorded in other studies.

The rise in the enzymes AST and ALT activities observed in the study was in agreement with the findings of Crook (2006). Elevated AST activity is an indication of liver damage (Crook, 2006). Increased levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in the liver (Crook, 2006) even at very low doses and short term exposure as seen in other studies of high doses. This cellular leakage may be attributed to harmful metallic ions and dissolved hydrocarbons present in the BLCO which are capable of destroying cellular membranes (Patrick-Iwuanyanwu *et al.*, 2013).

The elevated total and direct bilirubin levels observed in the rats orally exposed to undiluted BLCO may be an indication of hepatobiliary disease. This is reported for the first time.

Total protein concentration in the rats orally exposed to 0.75 and 1mls of BLCO/kg BW were significantly reduced ($p < 0.05$) compared to that of the control groups. There was however no significant difference ($p < 0.05$) between total protein concentration of rats orally exposed to 0.25 and 0.50 ml of undiluted BLCO/kg BW and that of the control group. The dose dependent significant decreased in the total protein concentration most especially with the rats orally administered 1 ml of BLCO/kg BW compared to that of the control group might be due to hepatocellular toxicity caused by the dissolved fractions present in the crude oil (Odo *et al.*, 2012).

Histopathological examinations of the liver tissue of the treated rats indicated that sub-acute exposure of undiluted BLCO at very low doses 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 and cholangitis in a dose dependent pattern. This implies that the liver is one of the major target organs of BLCO dissolved fractions in the body system at very low doses.

Conclusion: BLCO at very low doses was found to have hepatotoxic effect and therefore demands that its tradomedical uses be discouraged or replaced with less or nontoxic agents with similar therapeutic potentials.

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