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## Original Article

### Hepatotoxicological evaluation of water-soluble fraction (WSF) of Bonny Light crude oil (BLCO) in Wistar albino rats

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**ABSTRACT:** Background: It is common practice to pay more attention to the clearing of visible surface petroleum spills in streams and rivers which serve as the main source of drinking water in polluted sites in the Niger Delta area rather than taking cognizance of dissolved aromatic hydrocarbons and metallic ions which are major components of petroleum products. For this reason, the toxicological effect of the water soluble fraction (WSF) of Bonny light crude oil (BLCO) was evaluated. Methods: The range finding test was determined to be higher than 100% and showed no mortality or physical changes after 7 days. Wistar albino rats were exposed to three different concentrations (25, 50 and 100%) of WSF (BLCO) for a period of 28days. Results: Data from the study showed a significant ( $p \leq 0.05$ ) increase in liver marker enzymes [aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP)] and biochemical parameters (cholesterol, urea, total and direct bilirubin) in rats exposed to WSF (BLCO). Generally, the increase in the level of biochemical parameters was concentration dependent with rats in the group treated with 100% concentration showing the highest activity when compared with control. There was a marginal decrease in the level of packed cell volume (PCV) and haemoglobin (Hb) in rats exposed to WSF (BLCO). White blood cell (WBC) of rats exposed to 25 and 50 % WSF (BLCO) increased marginally whereas a significant ( $p \leq 0.05$ ) increase was observed in the group exposed to 100% of WSF (BLCO). The histological examination of rats exposed to different concentrations (25, 50 and 100%) of WSF (BLCO) were characterized by fatty change, inflammation of the cell whereas rats in the control group showed normal architecture. Conclusion: The findings of this study highlights the deleterious and toxicological effects of exposure to water polluted by dissolved aromatic hydrocarbons probably present in WSF (BLCO).

**KEYWORDS:** toxicology; amino acid transferases; crude oil; liver damage.

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## INTRODUCTION

Crude oil is a naturally occurring substance found in certain rock formations on the earth. It is a dark, sticky liquid classified as a hydrocarbon. It is a complex mixture and vary widely in composition. It is highly flammable and can be burnt to create energy. The water environment experiences many dynamic changes induced by various events such as the spillage of toxic chemical that may have significant impact on life (Camougis, 1981). The severity of the effects depends on the organisms' exposure, the concentration of the components and mode of exposure (Overton *et al.*, 1994). Water and oil are usually considered to be non-miscible. However crude oil contains a very small soluble fraction (Kavanu, 1964). The water soluble fraction (WSF) constituents are dispersed particulates oil, dissolved hydrocarbons and soluble contaminants such as metallic ions (Kauss and Hutchinson, 1975). The components of crude oil that go into solution make up the water soluble fraction. They

are taken up by living cells and are metabolized (Ali and Mai, 2007). This is ecologically important because in event of oil spill into aquatic habitat, this is absorbed by living organisms with serious effects on the ecosystem. The toxicity of crude oil has been reported by Overton *et al.*, (1994) to be due to the fraction of the presence of toxic components like xylene, naphthalene, benzene and toluene. The water-soluble fraction (WSF) of crude oil and their derivatives products contains a mixture of polycyclic aromatic hydrocarbons (PAHs), monoaromatic hydrocarbons such as benzene, toluene, ethylbenzene and xylenes; phenols and heterocyclic compounds, containing nitrogen and sulfur (Saeed and Al-Mutairi, 1999), and also heavy metals. Some petroleum-derived hydrocarbons are toxic to a wide spectrum of marine animals because they preferentially accumulate in lipidic compartments like cellular membrane (Di Toro *et al.*, 2001), disturbing the physicochemical and physiological membrane properties (Sikkema *et al.*, 1994). Gunlacks and Hayas (1977) reported that the growth rate and biomass turnover of

some aquatic macrophytes have been adversely affected by the water soluble fraction of crude oil. However, the presence of harmful metallic ions in WSF has been reported (Kauss and Hutchinson, 1975; Winter *et al.*, 1976; Noyo *et al.*, 2007; Noyo *et al.*, 2008). Accumulation of these ions such as  $\text{Na}^+$  and  $\text{Ca}^{2+}$  may result in several stress problems capable of destroying plant cell wall and membranes (Hernandez *et al.*, 1995). The combination of these ions with  $\text{Cl}^-$  in solution to form  $\text{NaCl}$  and  $\text{CaCl}_2$  may cause leakage of cell contents and eventual death of cell (Hoagland, 1972). One of the major problems of the inhabitants of the Niger Delta region of Nigeria is the contamination of water and aquatic lives by crude oil. This contamination may not necessarily lead to outright mortality but may have significant effects which can lead to physiological stress and dysfunction in animals. The severity or degree of the problems in the inhabitants of the area is dependent upon the point of contact with the polluted water. Hence, the need for the preparation of different crude oil concentrations. Limited information on the impact of exposure of terrestrial animals to dissolved hydrocarbon such as water soluble fraction of crude oil was the motive for the present study. However, this study is an attempt to evaluate the toxicological effects of the water soluble fractions (WSF) of Bonny light crude oil on Wistar albino rats.

## MATERIALS AND METHODS

### Collection of Samples

Fresh samples of Bonny light crude oil (BLCO) were collected from the N.N.P.C Refinery at Eleme, Rivers State, Nigeria.

### Preparation and preservation of the WSF (BLCO).

The water-soluble fraction was prepared according to the method of Anderson *et al.*, 1974 with slight modification as described by Ogali *et al.*, (2007). Briefly, A sample of bonny light crude oil (BLCO) (150 ml) was slowly mixed with distilled  $\text{H}_2\text{O}$  (450 ml) in a 1000- ml conical flask. The flask was covered with Aluminum foil and held tightly with a rubber band. The flask was fastened to an electric stirrer, and shaken for 24 h as recommended by Parker *et al.*, (1976) and adopted by Patrick-Iwuanyanwu *et al.*, 2010. Then, the mixture was left standing for 3 h to obtain a clear phase separation between crude oil and  $\text{H}_2\text{O}$ . The mixture was then poured into a separating funnel (with glass stopper) and allowed to settle overnight. The pure and clear WSF obtained at the lower part of the funnel was collected into a dark-colored, screw-capped Winchester bottle as 100% WSF stock. The stock was further diluted with distilled water to give 50 and 25% concentration WSF and stored in a dark-colored, screw-capped Winchester bottle in a refrigerator (0–4 °C) until required for use.

### Range Finding Tests

Range finding tests to determine the lowest dose of WSF of Bonny light crude oil capable of eliminating 50% of the test animals and the highest concentration that will not have any effect on the animals were first carried out. Five different concentrations (100, 30, 9, 2.7 and 0.81) of the WSF of the crude oil were used based on a dilution factor of 0.3. Animals were closely monitored for 7days for observational changes

such as discharges from the eyes, nose, hair loss, tremors, changes in respiratory rate and movement within the cage.

### Animals

Thirty two matured Wistar albino rats weighing between 170–180g used in this experiment were obtained from the Animal House of the Department of Biochemistry, University of Port Harcourt, Nigeria. They were housed and kept under laboratory conditions with free access to a standard diet and water for seven (7) days of acclimatization. The experiment was performed after the experimental protocol was approved by the institutional animal ethics committee

### Experimental Protocol

After the acclimatization period, the rats were randomly selected into four groups comprising of eight animals each. Rats in group I were fed with normal feed and water only (Control group) whereas rats in group II were fed with normal feed, water and 1ml of 25% of WSF (BLCO) orally daily for 28 days while rats in group III were fed with normal feed, water and 1ml of 50% WSF (BLCO) orally daily for 28 days and Group IV was treated with normal feed, water and 1ml of 100% WSF (BLCO) orally daily for 28 days.

### Sample collection

Twenty four hours after the 28 days of oral administration of WSF (BLCO), the rats were anaesthetized in a chloroform-saturated chamber after which the animals were sacrificed using cervical dislocation method. Blood samples were obtained by cardiac puncture from each rat by means of a 2 ml hypodermic syringe and needle. The blood samples were introduced into clean dry bottles (EDTA bottles) for haematological parameters while the blood samples used for biochemical parameters were collected in an anticoagulant free bottle. Serum was separated by centrifugation at 2500rpm for 10 minutes and stored in a refrigerator at 4 °C until use. The levels of biochemical parameters (ALT, AST, ALP, total and direct bilirubin, cholesterol and urea) were estimated using the Humazym MUV test kits. The white blood cells (WBC) were estimated using the improved Neubauer counting chambers as described by Dacie and Lewis (1991). The haemoglobin (Hb) concentration was determined by the Cyameth-haemoglobin method while the Packed Cell Volume (PCV) was determined by the micro method as described by Dacie and Lewis (1991).

**Table 1: Treatments groups used in this study**

Group	Treatment	Duration	No of rats
Group (I)	Normal feed + $\text{H}_2\text{O}$	28 days	8
Group (II)	Normal feed + $\text{H}_2\text{O}$ + 25% WSF	28 days	8
Group (III)	Normal feed + $\text{H}_2\text{O}$ + 50% WSF	28 days	8
Group (IV)	Normal feed + $\text{H}_2\text{O}$ + 100% WSF	28 days	8

### Histopathological Examination

A portion of the liver of all the rat groups was fixed in 10% buffered neutral formalin for 48 hours followed by bovine solution for 6 hours and then processed for paraffin embedding. By using a microtome, sections of 5  $\mu\text{m}$

thickness were taken, processed in alcohol-xylene series and were stained with alum-haematoxylin and eosin (Galigher and Kayloff, 1971) and subjected to histopathological examination.

#### Statistical analyses

The results are expressed as mean  $\pm$  standard error of the mean (SEM). One-way analysis of variance (ANOVA) was employed for between and within group comparison while student's t-test was used for paired comparison. 95% level of significance ( $p \leq 0.05$ ) was used for the statistical analysis.

## RESULTS

The results of the range finding test showed no mortality or physical changes such as discharges from the eyes, nose, hair loss, tremors, changes in respiratory rate and movement within the cage after 7 days. The range finding test was however determined to be higher than 100% after 7 days. The results of the effect of oral administration of the Water soluble fraction (WSF) of bonny light crude oil (BLCO) at 25, 50 and 100% on liver enzymes are shown in Table 2. Results from the study showed significant ( $p \leq 0.05$ ) increases in AST, ALT and ALP activities in the treated groups with rats in the group administered 100% showing the highest activity when compared with control (Table 2). Administration of the WSF (BLCO) at 25, 50 and 100% strength to experimental rats significantly ( $p \leq 0.05$ ) increased total and direct bilirubin with rats treated with 100% WSF showing the highest values ( $28.30 \pm 0.33$  and  $14.40 \pm 0.03$  U/L) when compared with control ( $13.10 \pm 1.76$  and  $6.50 \pm 0.83$  U/L) for total and direct bilirubin respectively. The effect of the WSF on cholesterol and urea are shown in Table 2. Results of the study showed a significant ( $p \leq 0.05$ ) increase in the level of cholesterol and urea in groups treated with 25, 50 and 100% of the WSF when compared with control. The result of the effect of the WSF (BLCO) on haematological parameters is presented in Table 3. The PCV and Hb level in the treated groups showed marginal decrease when compared with the control. However, there was a marginal increase in WBC of rats treated with 25 and 50% of the WSF (BLCO) whereas rats treated with 100% of the WSF (BLCO) showed a significant ( $p \leq 0.05$ ) increase when compared with control. Results of the histopathological examination of the liver are shown in Figures 1-4. The result of the study on the liver of rats in the control group showed normal architecture of hepatocytes whereas hepatocytes of rats in the groups administered 25, 50 and 100% WSF (BLCO) were characterized by fatty change, inflammation of the cells around the portal tract (Portal Trinitis) and apoptosis of cell (Figures 2-4).

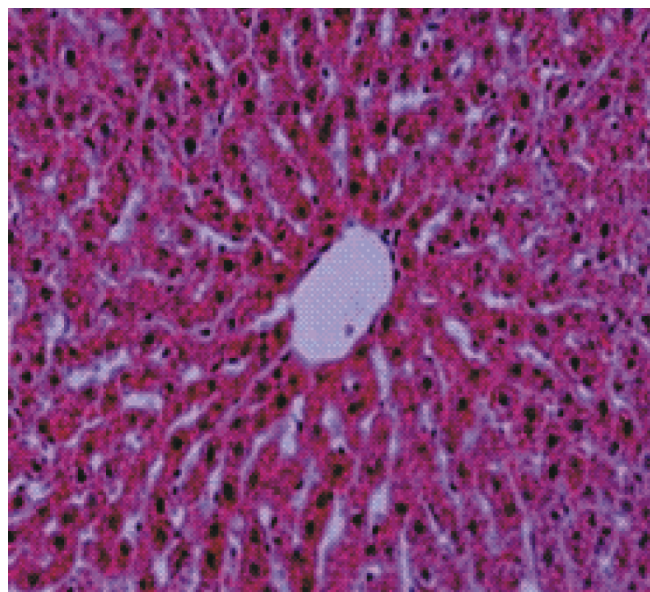
## DISCUSSION

One of the major problems of the inhabitants of the Niger Delta region of Nigeria is the contamination of water and aquatic lives by crude oil spills. This contamination may not necessarily lead to outright mortality but may have significant effects which can lead to physiological stress and dysfunction in animals. The toxicity of a petroleum fraction is related to its hydrophobicity (Freedman, 1995) because lipid solubility is an important factor in the passage of petroleum components through the plasma membrane of cells, as well as the degree of membrane disruption. The result in this study clearly indicate that oral administration of different concentrations

**Table 2: Effect of oral administration of WSF (BLCO) on serum biochemical parameters of rats**

	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	7.33 $\pm$ 0.88	6.33 $\pm$ 0.67	15.67 $\pm$ 1.20
25%	15.33 $\pm$ 0.67 <sup>a</sup>	16.00 $\pm$ 1.53 <sup>a</sup>	24.00 $\pm$ 0.58 <sup>a</sup>
50%	19.00 $\pm$ 2.00 <sup>b</sup>	17.67 $\pm$ 1.20 <sup>a</sup>	25.67 $\pm$ 0.33 <sup>b</sup>
100%	25.33 $\pm$ 0.67 <sup>c</sup>	22.67 $\pm$ 0.88 <sup>b</sup>	27.33 $\pm$ 0.33 <sup>b</sup>

%WSF	Total bilirubin ( $\mu$ mol/L)	Direct bilirubin ( $\mu$ mol/L)	Cholesterol (Mmol /L)	Urea (Mmol/L)
Control	13.10 $\pm$ 1.76	6.50 $\pm$ 0.83	2.40 $\pm$ 0.10	5.30 $\pm$ 0.14
25%	18.70 $\pm$ 0.65 <sup>a</sup>	9.60 $\pm$ 0.27 <sup>a</sup>	2.50 $\pm$ 0.11 <sup>a</sup>	6.00 $\pm$ 0.14 <sup>a</sup>
50%	24.60 $\pm$ 2.40 <sup>b</sup>	12.00 $\pm$ 1.07 <sup>b</sup>	6.80 $\pm$ 0.03 <sup>b</sup>	6.80 $\pm$ 0.03 <sup>b</sup>
100%	28.30 $\pm$ 0.33 <sup>c</sup>	14.40 $\pm$ 0.03 <sup>c</sup>	2.90 $\pm$ 0.03 <sup>b</sup>	8.10 $\pm$ 0.85 <sup>c</sup>



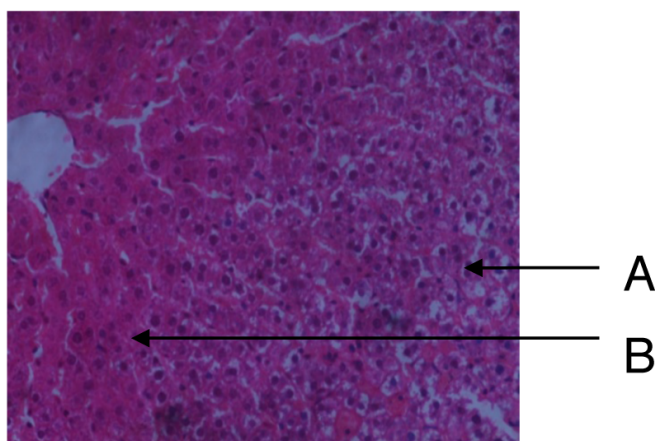
**Figure 1: A section of the rat liver tissues showing normal architecture in the control rats.**

(25, 50 and 100%) of the water soluble fraction (WSF) of Bonny Light Crude Oil (BLCO) for 28 days resulted to a significant ( $p \leq 0.05$ ) increase in the levels of biochemical parameters. The increased levels of aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) are conventional indicators of liver injury (Shah *et al.*, 2011). These serum enzymes (ALT



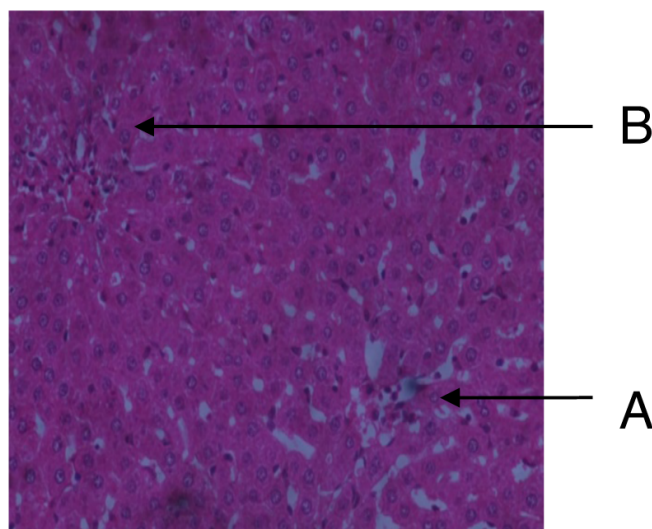
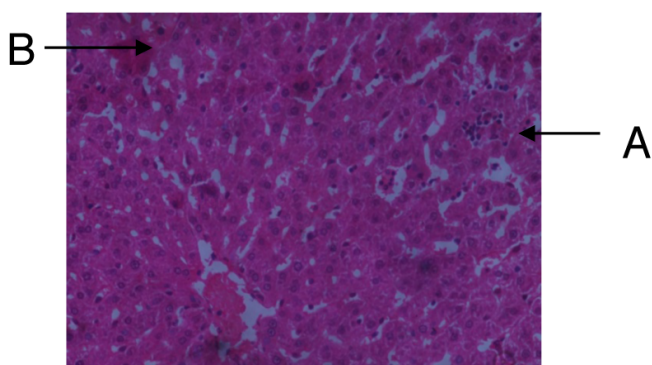
**Table 3: Effect of oral administration of WSF (BCLO) on haematological parameters of rats**

%WSF	PCV (%)	HB (g/dl)	WBC (cell mm <sup>-3</sup> )
Control	31.80±1.11	9.14±0.29	5200±70.71
25%	29.75±0.28	8.73±0.09	5250±76.32
50%	28.50±1.03	8.43±0.48	5275±85.93
100%	27.25±1.44	8.20±0.64	5425±95.74

**Figure 2: A section of the rat liver administered with 25% WSF (BLCO) showing fatty change (A) and cells round the portal tract spared (B).**

and AST) are largely used in the assessment of liver damage by drugs or any other hepatotoxin (Rahmaiah, 2011; Patrick-Iwuanyanwu *et al.*, 2012). The elevation of serum marker enzymes observed in this study may be attributed to severe hepatocellular injury.

The rise in the enzyme AST with a corresponding increase level of ALT observed in this study corroborates the findings of Sallie *et al.*, 1999. High AST level is an indicator of liver damage (Crook, 2006). Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in the liver (Drotman and Lawhan, 1978). This cellular leakage may be attributed to harmful metallic ions and dissolved hydrocarbons present in WSF (BLCO) (Kauss and Hutchinson, 1975; Winter *et al.*, 1976) which are capable of destroying cellular membranes (Hernandez *et al.*, 1995). The elevated conjugated bilirubin level observed in rats treated with different concentrations of WSF (BLCO) may be an indication of hepatobiliary disease. The increase in the level of urea observed in this study probably indicates that the WSF (BLCO) interfered with the renal function capacity to excrete this metabolite. This further indicates that renal integrity of rats treated with WSF (BLCO) may have been adversely affected. The increase in

**Figure 3: A section of the rat liver administered with 50% WSF (BLCO) showing fatty change, inflammation of cells round the portal tract (Portal Trinitis) (A) and Apoptosis-Death of cells (B).****Figure 4: A section of the rat liver administered with 100%WSF (BLCO) showing mild fatty change, mild inflammation (A) of the Cell round the portal tract and Apoptosis of cell (B).**

cholesterol levels may probably be an indication of liver damage. Haematological indices such as haemoglobin (Hb), packed cell volume (PCV) and white blood cell (WBC) provide information on the general state of the blood of an organism at a particular time. They are often associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health (Patrick-Iwuanyanwu *et al.*, 2007). The result from the present study showed a dose dependent decrease in Hb and PCV in rats treated with WSF (BLCO). This finding is similar to the report by Ovuru and Ekweozor (2004) in rabbits, Leighton *et al.*, (1985) in young Herring gulls and Atlantic Puffins. The result from this study has demonstrated that long-term exposure to WSF (BLCO) samples induces anaemia. The resulting anaemia is in accordance with the report of Krishna and Veena (1980) who reported the suppressive effect of petroleum samples on erythropoiesis. Reports of Sudakov (1992) and Marieb (1995) have shown that the toxic components especially those in

petroleum products change blood chemistry and hence induce anaemia by causing bone marrow hypoplasia and interfered with platelets production in the animals, hence the reduced values of Hb and PCV in rats treated with WSF (BLCO). The decrease in Hb and PCV levels in the treated rats is an indication that the WSF (BLCO) was capable of eliciting haemolytic toxicity of the blood cells in condition of long-term exposure. This may be attributed to cytotoxic effect and suppression of erythropoiesis caused by constituents of the WSF (BLCO). Crude oil fraction present in the WSF may be responsible for the serious consequences on hematological parameters in the experimental rats. The white blood cell (WBC) functions primarily in body defense against foreign bodies. This is achieved by leucocytosis and antibody production (Robins and Angell, 1976; Marieb, 1995). However, the increase in the level of WBC may be attributed to the defensive mechanism of the immune system (Hoeney, 1985).

Histopathological examinations of the liver tissues of the experimental rats indicate that exposure to WSF (BLCO) affected the structural integrity of the liver cells. This is characterized by the presence of fatty change, inflammation of cells round the portal tract (portal trinitis) and Apoptosis (death of cells). This implies that the liver is one of the major target organs of WSF (BLCO) -induced injury. The cumulative oxidative damage is therefore likely to be one of the underlying mechanisms responsible for the hepatotoxic effects of WSF (BLCO) as observed in the study.

### Conclusion

In conclusion, the results of this work suggest that repeated exposure to WSF (BLCO) may elicit an increase in serum enzyme activities and biochemical parameters (cholesterol, urea, total and direct bilirubin). This may be attributed to the toxicity of dissolved hydrocarbons and metallic ions present in WSF (BLCO). It then implies that long term exposure to WSF (BLCO) may be hepatotoxic, nephrotoxic and haematotoxic.

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