



## Hematotoxic Effect of Water Soluble Fraction of Bonny Light Crude Oil in Wistar Albino Rats

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### Authors' contributions

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### ABSTRACT

This study evaluated the Hematotoxic effect of water soluble fraction (WSF) of Bonny Light Crude Oil (BLCO) in Wistar Albino rats. After preparation of the WSF and a range finding test, the Wistar albino rats were administered three concentrations (25%, 50% and 100%) of WSF of BLCO for 30 and 60days. Data from the study showed that there was a significant decrease ( $p \leq 0.05$ ) in PCV values (30.2% in the control group to 17.2% in the 100% group), Hb levels decreased significantly from 9.25 g/dl in the control group to 5.27 g/dl in the 100% group. WBC count decreased significantly from 2932  $\text{mm}^3$  in the control group to 136  $\text{mm}^3$  in the 100% group and finally RBC count decreased significantly from 241.8E4  $\text{mm}^3$  to 567  $\text{mm}^3$  with increasing concentrations in the treatment groups after 60days administration. These results suggest that the oral consumption of the WSF of BLCO led to an onset of anaemia which indicates the presence of less than normal concentrations of PCV, Hb and RBC.

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## 1. INTRODUCTION

Oil spill is the inundation of the environment by liquid petroleum hydrocarbon resulting from anthropogenic activities. From January to August 2019 Shell Petroleum Development Company has reported 112 oil spills in the Niger Delta with estimated spill volume being about 8769.1 bbl [1]. Oil spill incidence arises from pipeline vandalism/sabotage of oil installations, blowout from old well heads, tanker/barge accidents etc and these affects humans by exposing us to either direct contact (skin absorption, inhalation, consumption of polluted water etc) or indirectly (consuming contaminated food, reducing economic impact). Spills on water spread widely because water is an excellent medium for dispersion because of wave action, the viscosity of the oil and the amount of oil spilled [2]. Although the fraction of oil that dissolves in water is relatively small in comparison with the total mass of oil but it is this fraction that is the major determining factor of oil toxicity. On land there can be percolation to the water table affecting groundwater especially in the absence of natural clay layers or anthropogenic barriers, pipelines layed beneath the ground will also affect groundwater as a result of vertical travelling distance being reduced. Oil residuals could be entrapped underground constituting a secondary source of groundwater pollution. The route for delivery of potential toxicants into the body's system includes ingestion of the crude oil orally or via eating polluted aquatic organism (fishery resources) and absorption via dermal contact.

The present study sought to ascertain the effect of the water soluble fraction of Bonny Light crude oil on haematological parameters of wistar albino rats.

## 2. MATERIALS AND METHODS

### 2.1 Collection of BLCO

Bonny Light Crude Oil was collected from the Nigerian National Petroleum Company Refinery at Eleme, Rivers State, Nigeria.

### 2.2 Preparation of the Water-Soluble Fraction (WSF)

The water soluble fraction (WSF) of Bonny Light crude oil was prepared using the method

adopted by Patrick-Iwuanyanwu et al. [3]. A measured volume (200 ml) of BLCO was mixed slowly with distilled water (600 ml) in a 1000 ml conical flask and covered with an aluminium foil. It was placed on an electric stirrer and stirred for 24 hours at 350 rpm. Then the mixture was allowed to stand for 6 - 9 hours in a separating funnel with a glass cork in other to obtain a phase separation of oil and water. The droplets of oil in the mixture settled in the upper layer while the pure and clear WSF was gotten at the lower part of the separating funnel and was then drained off into a dark coloured, screw capped Winchester bottle, and stored in a refrigerator at 0-4°C.

### 2.3 Procurement of Animals and Care

Wistar albino rats (male and female) weighing between 74 – 140 g were procured from the animal house of Department of Veterinary Medicine, University of Nigeria Nsukka, Enugu State. They were kept in a well-aerated cage with free access to rat feed and water *ad libitum* in the Animal House of the Department of Biochemistry, University of Port Harcourt, Rivers State and subjected to a well-ventilated natural 12 hour light-dark cycle. The acclimatization period lasted for 14 days before administration commenced. The animals were grouped with 5 rats each in plastic cages with wood shavings as beddings and maintained under normal laboratory conditions. The experiment was carried out after the experimental protocol was approved by the institutional animal ethics committee.

### 2.4 Range Finding Test (LD<sub>50</sub>)

A range-finding test also known as LD<sub>50</sub> was done to establish the dose with the ability to eliminate 50% of the laboratory animals and the maximum concentration that will not affect them. Four different concentrations (100, 30, 9 and 2.7) of the WSF of the bonny light crude oil were administered using a dilution factor of 0.3. The test animals were diligently observed for 7 days for physical changes like: discharges from the nose, eyes, hair loss, movement within the cage and changes in respiratory rate.

### 2.5 Experimental Design

This study was carried out using the True Experimental Design method in which the

experimental (treated) groups of animals were compared with the control (untreated) groups. After the period of acclimatizing, the rats were randomly selected into 8 groups consisting of 5 males and 5 females.

## 2.6 Sample Collection

Twenty four hours after the 30 days and 60 days administration respectively, the treatment and control rats of each sex were weighed, anaesthetized using chloroform, sacrificed and their blood collected from the jugular. The blood was then carefully transferred into EDTA containing bottles for haematological analysis.

The percentage packed cell volume was determined according to the hematocrit method of Alexander and Griffiths [4] while the blood haemoglobin concentrations in all samples were estimated according to the cyanomethaemoglobin method of Alexander and

Griffiths [5]. Total red blood cell and white blood cell counts were estimated according to the visual method of Dacie and Lewis [6].

## 2.7 Statistical Analysis

Data are expressed as mean  $\pm$  standard deviation while comparison between treated groups was made using students t-test for equal variants using JMP 10 Statistical Analysis System (SAS). Values of  $p \leq 0.05$  were considered as statistically significant.

## 3. RESULTS

### 3.1 Range Finding Test

After administration of four different concentrations (100, 30, 9 and 2.7) of the WSF of the bonny light crude oil and close monitoring of the test animals for 7days there were no deaths or physical changes like nose and eye discharge or loss of hair recorded.

**Table 1. Treatment groups used in the study**

Group	Treatment	Duration (Days)	Number of animals
I	Normal feed + H <sub>2</sub> O	30 & 60	20
II	Normal feed + H <sub>2</sub> O + 25% WSF	30	10
III	Normal feed + H <sub>2</sub> O + 50% WSF	30	10
IV	Normal feed + H <sub>2</sub> O + 100% WSF	30	10
V	Normal feed + H <sub>2</sub> O + 25% WSF	60	10
VI	Normal feed + H <sub>2</sub> O + 50% WSF	60	10
VII	Normal feed + H <sub>2</sub> O + 100% WSF	60	10
VIII	Normal feed + H <sub>2</sub> O+ topical application of 100% WSF	60	10

**Table 2. The PCV, Hb, WBC and RBC levels treated with WSF of BLCO (30 days)**

Treatments	PCV (%)	Hb (g/dl)	WBC (mm <sup>3</sup> )	WBC (mm <sup>3</sup> )
Control	29.9 $\pm$ 5.95 <sup>a</sup>	9.17 $\pm$ 1.83 <sup>a</sup>	6974 $\pm$ 1369 <sup>a</sup>	611E4 $\pm$ 121E4 <sup>a</sup>
25% WSF	26.2 $\pm$ 5.88 <sup>a,b</sup>	8.03 $\pm$ 1.81 <sup>a,b</sup>	2983 $\pm$ 748 <sup>b</sup>	554E3 $\pm$ 199E3 <sup>b</sup>
50% WSF	24.6 $\pm$ 6.07 <sup>b</sup>	7.54 $\pm$ 1.86 <sup>b</sup>	3283 $\pm$ 1884 <sup>b</sup>	401E3 $\pm$ 255E3 <sup>b</sup>
100% WSF	23.8 $\pm$ 5.05 <sup>b</sup>	7.29 $\pm$ 1.55 <sup>b</sup>	547 $\pm$ 150 <sup>c</sup>	397E2 $\pm$ 146E2 <sup>b</sup>

WSF = Water soluble fraction. The data are expressed as Mean  $\pm$  SD; (n=10). Concentrations not connected by the same letters are significantly different

**Table 3. The PCV, Hb, WBC and RBC levels treated with WSF of BLCO (60 days)**

Treatments	PCV (%)	Hb (g/dl)	WBC (mm <sup>3</sup> )	RBC (mm <sup>3</sup> )
Control	30.2 $\pm$ 3.42 <sup>a</sup>	9.25 $\pm$ 1.04 <sup>a</sup>	2932 $\pm$ 2331 <sup>a</sup>	241.8E4 $\pm$ 154.3E4 <sup>a</sup>
25% WSF	24.0 $\pm$ 2.78 <sup>b</sup>	7.36 $\pm$ 0.85 <sup>b</sup>	525 $\pm$ 533 <sup>b</sup>	500.26E2 $\pm$ 536.2E2 <sup>b</sup>
50% WSF	22.9 $\pm$ 1.44 <sup>b</sup>	7.02 $\pm$ 0.44 <sup>b</sup>	320 $\pm$ 116 <sup>b</sup>	114.40E2 $\pm$ 181.4E2 <sup>b</sup>
100% WSF	17.2 $\pm$ 1.81 <sup>c</sup>	5.27 $\pm$ 0.55 <sup>c</sup>	136 $\pm$ 30 <sup>b</sup>	567 $\pm$ 171 <sup>b</sup>
Dermal(100%WSF)	31.8 $\pm$ 2.58 <sup>a</sup>	10.11 $\pm$ 0.89 <sup>a</sup>	2498 $\pm$ 828 <sup>a</sup>	193.3E4 $\pm$ 170.6E4 <sup>a</sup>

WSF= Water soluble fraction. The data are expressed as Mean  $\pm$  SD; (n=10). Concentrations not connected by the same letters are significantly different

### 3.2 Effect of the WSF of BLCO on Haematologic Parameters

The PCV, Hb, WBC, RBC and Platelet levels in the treatment and control group were analysed and the results obtained are shown in Table 2 and Table 3. There was a significant difference  $p \leq 0.05$  in PCV, Hb, WBC and RBCs with increasing concentration of WSF of BLCO after 30 days and 60 days of administration in comparison to the control.

## 4. DISCUSSION

Hematotoxicity is the study of blood and blood forming tissues as a primary target for chemicals and drugs either in the environment or workplace [7]. It generates information on immunologic responses and hematopoietic system. They are considered during toxicity studies because blood cells are vulnerable to any agent that is administered into the blood either via injection or topical application, relatively easy accessibility of blood and a result of the likelihood to reflect varying range of local and systemic effects of chemicals. The hematotoxic effect of BLCO has been well documented [3,8,9,10,11] but only a handful have reported on the Hematotoxic effect of WSF of BLCO. Results obtained as shown in Tables 2 and 3 indicates a trend towards a decrease in PCV, Hb, WBC and RBC levels with dose-dependent increase in the concentration of the administered WSF. The % PCV decrease was 12.37, 17.73 and 20.89 for groups II, III and IV representing 25%, 50% and 100% WSF of BLCO after 30days and % PCV decrease of 20.52, 24.17 and 43 for groups V,VI and VII representing 25%, 50% and 100% WSF of BLCO after 60days, this general decrease in PCV could be linked to destruction of cells, loss of blood and failure of the bone marrow to produce more red blood cells. PCV is used as an indicator in anaemia, investigation of dehydration and even burns.

There were significant WBC and RBC decrease between the control and the other treatment groups after 30 and 60days administration but no significant decrease in group VIII (topical/dermal group). These results suggests that the oral consumption of the WSF of BLCO led to an onset of anaemia which indicates presence of less than normal concentrations of PCV, Hb and RBC which could be as a result of bone marrow hypoplasia, The observed decrease in RBC counts may be as a result of increase in hemolysis mediated through the hydrocarbon

components of WSF of BLCO or as a result of lipid peroxidation (LPO) due to their high content of polyunsaturated lipids [12] suggesting the RBC may have undergone oxidative damage. Furthermore, the low WBC concentration could be the resultant effect of stress triggered by the water soluble fraction of the crude oil leading to immunodeficiency or a gradual suppression of the immune system. Patrick-Iwuanyanwu et al. [3] observed a marginal decrease of PCV and Hb levels when compared with the control as a result of the effect of WSF (BLCO).

## 5. CONCLUSION

The findings in this research suggest that there is likelihood of varying blood disorder arising from long term exposure to WSF of BLCO. Decreased haematological parameters (PCV, Hb, WBC and RBC) could be attributed to the presence of heavy metals present in the administered WSF of BLCO. This establishes its hematotoxic nature.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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