



## **Nephrotoxicological 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 Nephrotoxic effect of water soluble fraction (WSF) of Bonny Light Crude Oil (BLCO). 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 Urea concentration increased significantly ( $p \leq 0.05$ ) with increasing dose of BLCO ranging from 14.71 mg/dl in the control to 35.28 mg/dl in the 100% group after 30days and 14.28 mg/dl in the control to 41.08mg/dl in the 100% group after 60days, Creatinine concentration increased significantly ( $p \leq 0.05$ ) from 0.22 mg/dl in the control to 0.82mg/dl in the 100% group after 60 days administration while electrolyte (Na, K, Cl) concentration increased significantly ( $p \leq 0.05$ ) with increasing dose of BLCO after 60days administration. Histopathological examination of the kidney was characterized by partial partitioning of the glomerular tufts, obliteration of the Bowman's capsule and distortion of the renal tubules. The findings in this research suggest that WSF of BLCO induced nephrotoxicity.

**Keywords:** *Water soluble fraction; bonny light crude oil; urea; creatinine; nephrotoxicity.*

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## 1. INTRODUCTION

The inundation of the ecosystem by oil spills has led to it being given a worldwide attention [1]. There are about 603 oil fields in the Niger Delta with over 55% of these onshore while the others are in the shallow waters (less than 500metres) and as such some spills have occurred from some of these oil fields in the Niger Delta region of Nigeria, like the Forcados Tank 6 terminal in Delta State that spilled 570,000 barrels of oil into the Forcados estuary in July 1979 and Funiwa No. 5 Well in Funiwa field that spilled an estimated 421,000 barrels of oil into the ocean [2]. There have been situations where portability of water has been altered from incidences of spills in the environment when crude oil gets into the water table and flows into boreholes during abstraction into homes, streams or rivers which serve as drinking water in some communities have also been impacted. Out of frustration and with no alternative means of getting potable water, the residents of these communities are left with consuming the polluted water in that state exposing themselves to the negative health effects of such use. Water Soluble Fraction is a single phase solution comprised of individually dissolved molecules and is free of any dispersed oil-in-water emulsions or colloidal emulsions [3]. The WSF of crude oil contains heavy metals such as lead, chromium, arsenic, mercury, cobalt, copper, zinc, cadmium, vanadium etc. and also includes a combination of polycyclic aromatic hydrocarbons [4].

Nephrotoxicity is an adverse effect when one is exposed to drugs or toxicants that damage the kidney. These toxicants include metals like lead, arsenic and mercury, some antibiotics, cancer drugs etc. The onset of nephrotoxicity impairs hormonal functions that promote red blood cell formation, inability to get rid of excess urine and waste, also, blood electrolytes like sodium, potassium and chloride becomes elevated [5]. A rise of the aforementioned suggests a development or predisposition of renal failure.

Hence, this study sought to ascertain the nephrotoxic effect of the water soluble fraction of Bonny Light crude oil in 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 Equipment

Laboratory Incubator (Techmel & Techmel, USA), Magnetic stirrer (PEC Medical, USA), Olympus CX31 Microscope, Laboratory Centrifuge (Sorvall Instruments), Electronic balance (Life Assistance Scientific Co, UK), Glass ware (Pyrex, Germany). Other equipment/instruments were of high quality from reliable companies.

### 2.3 Chemicals

Creatinine and Urea kits were purchased from RANDOX laboratories while sodium, potassium and chloride kits were obtained from Micropoint company (USA).

### 2.4 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 et al. [6] with little modifications. 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 collected 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.

### 2.5 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 into 5 male and female rats each in separate 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 University of Port Harcourt research ethics committee.

## 2.6 Range Finding Test

A range finding test was carried out to establish the dose with the ability to eliminate 50% of the laboratory animals and the maximum concentration that will have no effect on 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 [5]. The test animals were diligently observed for initial 48 hours and an extra 5 days for physical changes like: discharges from the nose, eyes, hair loss, movement within the cage and changes in respiratory rate.

## 2.7 Experimental Design

This study was carried out using the True Experimental Design method in which the experimental (treated) groups of animals was 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.

## 2.8 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 plain anticoagulant free bottles and the serum separated via centrifugation at 2500rpm for 15minutes and stored at 4°C in a refrigerator until use. Serum Urea was determined by Urease-Berthelot method [7], Serum Creatinine was determined by modified Jaffe method [8], Serum Sodium was determined by Colorimetric method from the Micropoint test kit while Serum Sodium was determined by Mercurous (II) thiocyanate method from the Micropoint test kit, Serum Potassium was determined by Colorimetric method from the Micropoint test kit.

## 2.9 Statistical Analysis

Data are expressed as mean ± 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 Tests

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 48hours and a further 5days 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 effect of oral administration of WSF of BLCO on urea and creatinine concentration levels (30 days)**

Treatments	Urea (mg/dl)	Creatinine (mg/dl)
Control	14.71 ± 2.03 <sup>d</sup>	0.23 ± 0.14 <sup>a</sup>
25% WSF	24.17 ± 1.71 <sup>c</sup>	0.25 ± 0.13 <sup>a</sup>
50% WSF	27.01 ± 2.28 <sup>b</sup>	0.29 ± 0.32 <sup>a</sup>
100% WSF	35.28 ± 2.95 <sup>a</sup>	0.31 ± 0.14 <sup>a</sup>

WSF = Water soluble fraction. The data are expressed as Mean ± SD; (n=10). Concentrations not connected by the same letters are significantly different at  $p \leq 0.05$

**Table 3. The effect of oral administration of WSF of BLCO on urea and creatinine concentration levels (60 days)**

Treatments	Urea (mg/dl)	Creatinine (mg/dl)
Control	14.28 ± 0.75 <sup>e</sup>	0.22 ± 0.06 <sup>d</sup>
25% WSF	31.00 ± 0.92 <sup>c</sup>	0.42 ± 0.11 <sup>c</sup>
50% WSF	35.51 ± 0.33 <sup>b</sup>	0.71 ± 0.08 <sup>b</sup>
100% WSF	41.08 ± 0.99 <sup>a</sup>	0.82 ± 0.07 <sup>a</sup>
Dermal (100% WSF)	15.39 ± 1.39 <sup>e</sup>	0.28 ± 0.02 <sup>d</sup>

WSF = Water soluble fraction. The data are expressed as Mean ± SD; (n=10). Concentrations not connected by the same letters are significantly different at  $p \leq 0.05$

**Table 4. The effect of oral administration of WSF of BLCO on sodium, potassium and chloride concentration levels (30 days)**

Treatments	Sodium (mEq/l)	Potassium (mEq/l)	Chloride (mEq/l)
Control	143.02 ± 1.00 <sup>c</sup>	5.31 ± 0.87 <sup>b</sup>	75.70 ± 3.42 <sup>b</sup>
25% WSF	145.46 ± 2.88 <sup>c</sup>	5.78 ± 0.76 <sup>a,b</sup>	77.43 ± 4.99 <sup>b</sup>
50% WSF	151.87 ± 4.37 <sup>b</sup>	6.07 ± 0.57 <sup>a</sup>	86.71 ± 2.33 <sup>a</sup>
100% WSF	154.78 ± 2.54 <sup>a</sup>	6.36 ± 0.94 <sup>a</sup>	87.37 ± 3.19 <sup>a</sup>

WSF = Water soluble fraction. The data are expressed as Mean ± SD; (n=10). Concentrations not connected by the same letters are significantly different at  $p \leq 0.05$

**Table 5. The effect of oral administration of WSF of BLCO on sodium, potassium and chloride concentration levels (60 days)**

Treatments	Sodium (mEq/l)	Potassium (mEq/l)	Chloride (mEq/l)
Control	143.96 ± 4.21 <sup>d</sup>	5.53 ± 0.32 <sup>b</sup>	78.65 ± 0.85 <sup>d</sup>
25% WSF	156.14 ± 2.83 <sup>c</sup>	6.19 ± 0.56 <sup>a,b</sup>	83.42 ± 3.61 <sup>c</sup>
50% WSF	162.11 ± 6.65 <sup>b</sup>	7.07 ± 2.16 <sup>a</sup>	95.71 ± 2.65 <sup>b</sup>
100% WSF	169.58 ± 6.69 <sup>a</sup>	7.13 ± 1.90 <sup>a</sup>	112.05 ± 3.29 <sup>a</sup>
Dermal (100% WSF)	145.00 ± 6.36 <sup>d</sup>	6.01 ± 0.89 <sup>a,b</sup>	83.36 ± 3.05 <sup>c</sup>

WSF = Water soluble fraction. The data are expressed as Mean ± SD; (n=10). Concentrations not connected by the same letters are significantly different at  $p \leq 0.05$

### 3.2 Effect of Oral Administration of WSF of BLCO on Urea and Creatinine Concentration

The Urea and Creatinine levels in the control and treatment group were analysed and the results obtained are shown in Table 2 and Table 3. There was a significant ( $p \leq 0.05$ ) increase in Urea concentration with increasing treatment concentration after 30 days administration. However, there was no significant difference between the dermal administration of 100% WSF of BLCO and control after 60 days.

### 3.3 Effect of Oral Administration of WSF of BLCO on Electrolyte Concentration

The Sodium, Potassium and Chloride levels in the treatment and control group were analysed and the results obtained are shown in Table 4 and Table 5. There was a significant increase ( $p \leq 0.05$ ) in sodium concentration when the 50% and

100% WSF groups were compared with the control after 30 and 60 days of administration but there was no significant decrease with the dermal WSF group.

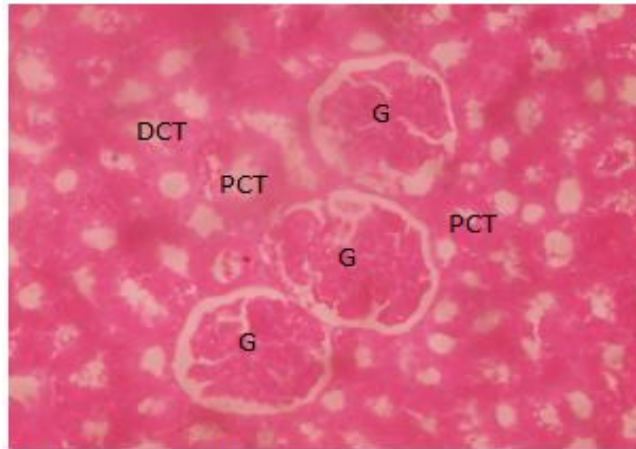
### 3.4 Effect of Oral Administration of WSF of BLCO on Histopathological Changes in the Kidney

Results of the histopathological examinations of the kidney are presented in Plates 1-8.

## 4. DISCUSSION

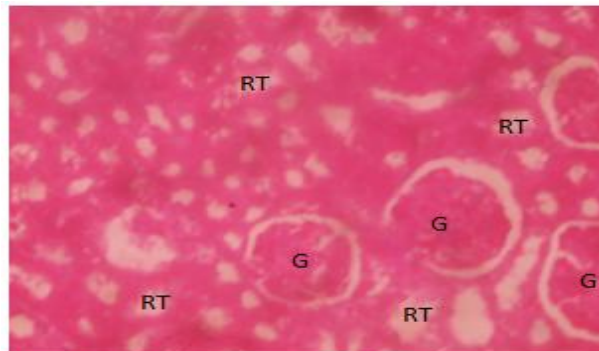
Nigerian Bonny Light Crude Oil is the most common crude oil brand found within the Niger Delta, because of this, most of the oil spill within the area usually involves it. After the incidences of spill, the focus is usually on the layers of oil on the surface of water bodies within the aquatic habitat instead of recognizing that varying fractions of crude oil have the capacity to dissolve in water. Most times, these water

bodies serve as the primary source of drinking water and domestic activities in the various localities in the region where this pollution takes place.



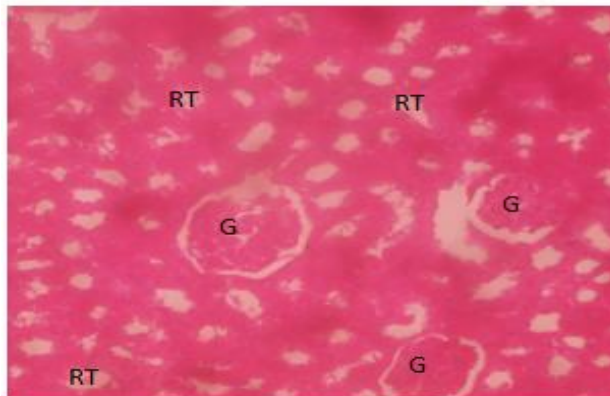
Kidney: Control

**Plate 1. A section of the rat kidney showing normal glomerular tuft (G) surrounded by patent Bowman's capsule spaces, Renal tubules (Distal Convoluted Tubules (DCT) and Proximal Convoluted Tubules (PCT))**



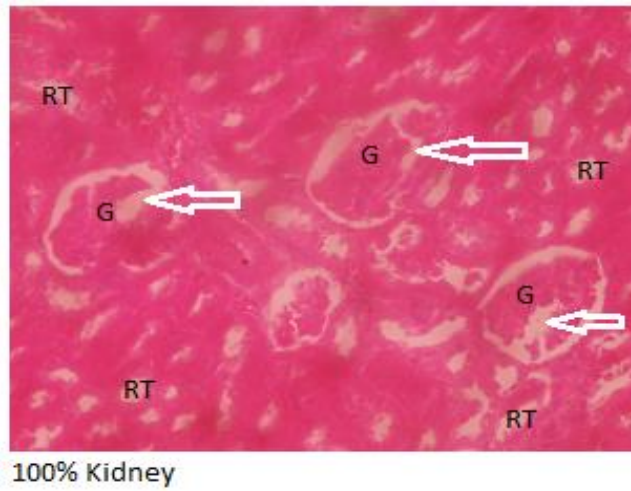
25% kidney

**Plate 2. A section of the rat kidney administered 25% WSF (BLCO) (30 days) showing normal Glomeruli (G) and Renal Tubules (RT)**

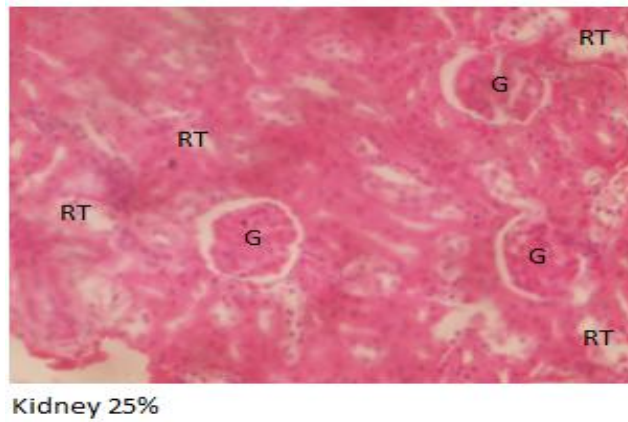


50% Kidney

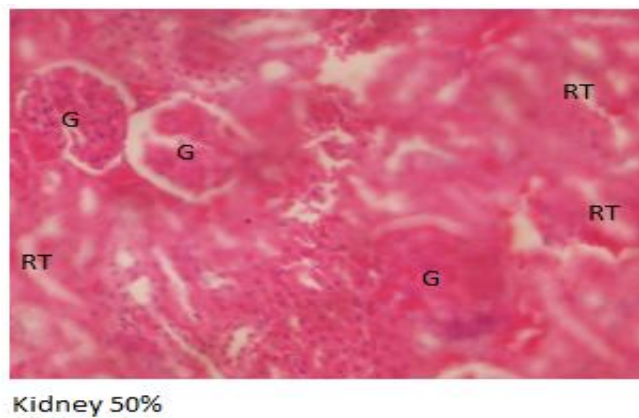
**Plate 3. A section of the rat kidney administered 50% of WSF (BLCO) (30 days) showing normal Glomeruli (G) and Renal Tubules (RT)**



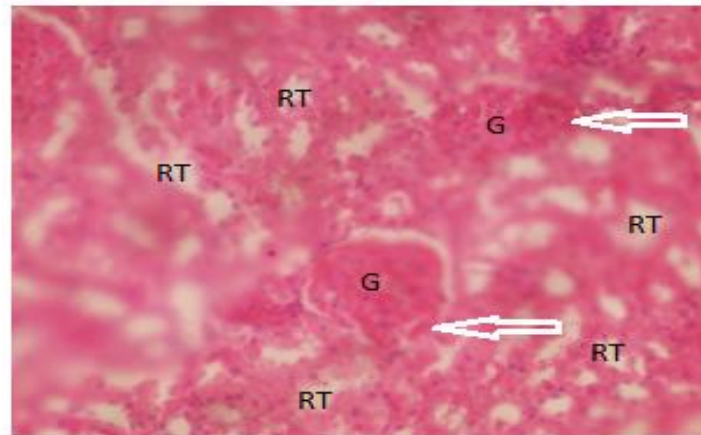
**Plate 4. A section of the rat kidney administered 100% WSF (BLCO) (30days) showing mildly distorted kidney tissues; partial partitioning of glomerular tufts arrowed (G) and normal Renal Tubules (RT)**



**Plate 5. A section of the rat kidney tissue administered 25% WSF (BLCO) (60 days) showing normal glomerular tuft (G) and renal tubules**

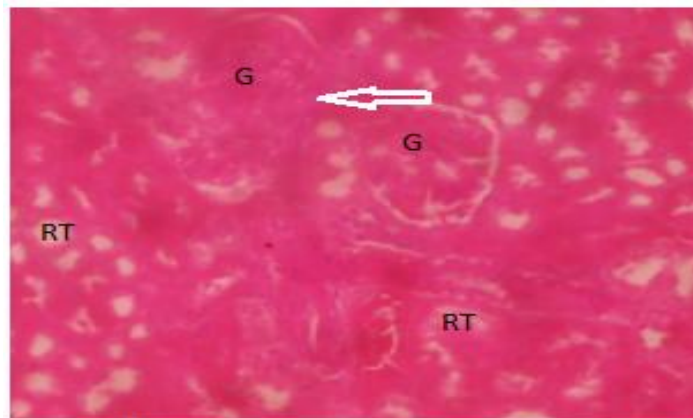


**Plate 6. A section of the rat kidney tissue administered 50% WSF (BLCO) (60days) showing normal glomerular tuft (G) and renal tubules**



Kidney 100%

**Plate 7. A section of the rat kidney tissue administered 100% WSF (BLCO) (60days) showing Glomeruli (G) with obliterated Bowman's capsule spaces arrowed and distorted Renal Tubules (RT)**



Dermal Kidney

**Plate 8. A section of the rat kidney tissue administered 100% WSF (BLCO) (60 days) dermally showing glomeruli (G) with obliterated Bowman's capsule spaces arrowed**

The effect of the WSF of BLCO on nephrotoxic biomarkers was analysed in this study. Urea and creatinine concentrations increased significantly ( $p \leq 0.05$ ) this is an indication that there was a dysfunction in renal activity which could be attributed to damage to the kidney from the oral administration of the WSF of BLCO. Diseases that impairs kidney function usually results in diminished glomerular filtration rate (GFR) which leads to retention of urea in the body. Production of urea occurs in the kidneys and hepatocytes and the amount of protein found in food determines the rate of production. During sweating, urea is excreted in minute amount from the skin but most of them leave the blood

through the kidney where urine formation takes place. The causes of elevated levels of urea are renal damage, dehydration, diet high in protein, ageing etc [6].

Elevation of serum creatinine in animals exposed to hydrocarbons have been reported by different authors [9,10]. Sodium, Potassium and Chloride levels increased significantly after 60 days administration between the treated groups and the control and this agrees with different authors which have shown that crude oil alters electrolyte concentration in the blood. The findings are in agreement with Ojo et al. [11] who showed that Nigeria Eket light crude oil increased plasma

sodium levels. Sodium ( $\text{Na}^+$ ) is the principal positive ion seen in the fluids outside of the cell. The elevation in sodium levels suggests that there was increased blood pressure in the treated groups. Potassium ( $\text{K}^+$ ) is the principal positive ion within the cells of the body and maintains the electric charge on the cell membrane. This charge enables signals to be sent between the nerves and the muscles thereby ensuring adequate transport of nutrients and waste into and out of the cell. Potassium has been reported to be a protective electrolyte that prevents hypertension [12]. Hyperkalemia occurs when  $\text{K}^+$  is in excess and this usually occurs when there is renal damage due to the inability to excrete excess potassium by the kidney. Elevated Potassium levels could be due to kidney disease, dehydration shock, adrenal insufficiency or presence of substances that reduces excretion of potassium from the body [13]. Chloride is an electrolyte responsible for maintaining anion-cation balance between the extra cellular and intra cellular fluids in the body. Increased chloride concentrations in plasma could be an indication of urinary obstruction or even dehydration. The significant increase ( $p \leq 0.05$ ) recorded after 60 days administration is an indication of renal impairment and these results were similar to that reported by Uhegbu et al. [14] who exposed male albino rats to Kerosene, Diesel and Petrol.

Histopathological examinations of the Kidney of the experimental rats suggests that exposure to the Water Soluble Fraction of BLCO affected the tissues and it was characterized by partial partitioning of the glomerular tufts, obliteration of the Bowman's capsule and distortion of the renal tubules implying that urine formation in the kidney would be compromised, preventing the body from excreting the chemical toxicants present in the WSF of BLCO. Environmental contaminants permeate through sweat glands and hair follicles of the skin via passive diffusion. The damage of the kidney in the group topically/dermally applied 100% of the WSF of BLCO suggests that there was absorption of heavy metals through the skin which may have led to the obliteration of the Bowman's capsule.

## 5. CONCLUSION

The findings in this research suggest that long term exposure to WSF of BLCO induced nephrotoxicity. The increase in nephrotoxic biomarkers could be due to the presence of

dissolved hydrocarbons, PAHs and heavy metals present in the prepared WSF of BLCO.

## ETHICAL APPROVAL

The experiment was carried out after the experimental protocol was approved by the University of Port Harcourt research ethics committee.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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