HEPATOTOXIC EFFECTS OF THE DERMAL EXPOSURE OF ALBINO RATS TO BONNY LIGHT CRUDE OIL

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Abstract
The effect of consistent exposure of the inhabitants in the Niger Delta region of Nigeria to crude oil may induce undesirable biochemical changes in the biota. Alterations in some hepatic function indices of male and female albino rats exposed to Bonny light crude oil (BLCO) by skin application were investigated. Sub lethal dose (500mg/kg body weight) of the BLCO was applied to shaved portion of the dorsal skin of the exposed rats while controls were similarly exposed to a placebo. After 30 days intervals sets of rats were weighed, sacrificed and their blood collected for serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and serum albumin (SA) assay. The rat liver was also carefully excised, weighed and examined for histological changes. Results show that the relative liver weight decreased by 66.97% after 90 days in the males and increased by 9.25% after 90 days in females. SGOT and SGPT levels increased significantly (p< 0.05) in both male and female rats during the study period. Changes in SGPT levels were more pronounced in the females compared to the males. The SA content decreased by 15.4% after 30 days and increased significantly (p< 0.05) by 52.1% after 90 days for the males. In the females, SA content increased by 12.5% after 30 days and decreased by 5.3% after 90 days. Histological examination of the liver cells of rats exposed for 90 days showed vacuolar degeneration, macrovisicular steatosis of hepatocytes and areas of necrosis compared to controls. These effects were more pronounced among the female rats compared to the males. These results suggest that the prolonged dermal exposure of albino rats to BLCO which leads to absorption into the blood stream and subsequent uptake by the liver may exert damaging effect on hepatocytes thereby causing hepatotoxicity.

Keywords: BLCO, Hepatotoxicity, SGOT, SGPT, Dermal exposure, Albino rats

INTRODUCTION
Bonny light crude oil (BLCO) is one of the major types of crude oil found in the Niger Delta region of Nigeria. It is classified as light crude oil (SG <0.82) and contains relatively low sulfur content in addition to asphaltenes, aromatic hydrocarbons, heavy metals and other compounds shown to exert varying degrees of toxicity (Adedara et al, 2011; Igwe, et al, 2016). The frequent dermal contact of the local population in the Niger Delta with BLCO is therefore a source of concern due to frequent oil spills and inadequate remediation of impacted areas as well as its traditional use in the treatment of convulsion, poisoning, witchcraft, burns, foot rot, leg ulcers, eczema, rashes, arthritis and gastrointestinal disorders among other ailments (Oriakwe, et al., 2000; Otaigbe and Adesina, 2005).

In animals, the liver plays a central role in the metabolism of xenobiotics. It is involved in the transformation and clearing of chemical agents and is therefore susceptible to the toxins from these agents. Exposed chemical substances may experience detoxification and inactivation in
the liver and become less harmful to the system or hepatic cell damage may occur as a result of their metabolism through the process of oxidation, reduction, hydroxylation and conjugation (Zakrzewski, 2002). Abnormalities associated with liver function (hepatotoxicity) can be seen from altered activities of some serum transaminase enzymes, liver weight changes and histological distortions among other indicators (Orisakwe et al., 2005; Orish et al., 2007; Igwe et al., 2017).

Previous studies on BLCO show that it has hepatotoxic potentials. Oruambo and Aderimo (2007) reported that BLCO induced alterations in the liver mitochondria DNA content, cytoplasmic total hydrocarbon and calcium ion content in adult guinea pigs exposed by intraperitoneal injection. Exposure of rats to BLCO by oral gavage altered the antioxidant systems of rats in a dose-dependent manner via induction of oxidative stress (Farombi et al., 2010). BLCO also altered the ratios of redox NADH/NAD⁺ in rat liver cytosol and mitochondria fractions and slowed down the availability of glucose to liver tissues for the production of adenosine triphosphate (ATP) via glycolysis (Oruambo and Dokubo, 2008). Increases in liver function enzymes (serum transferases) following intraperitoneal administration of crude oil have been reported (Ayalogu et al., 2001). Oral administration of BLCO on albino rats also caused a significant dose-dependent increase in serum liver function enzymes as well as induced liver histopathological changes in the forms of necrosis and oedema (Orisakwe et al., 2005).

There is paucity of information regarding hepatotoxicity and biochemical changes associated with BLCO exposure via the dermal route. Therefore, the present study was conducted to determine hepatotoxic effects associated with prolonged dermal contact of adult albino rat to BLCO.

**MATERIALS AND METHODS**

**Bonny Light Crude Oil**

Bonny Light Crude Oil sample was obtained from the Research and Development (R&D) laboratory of the Nigerian National Petroleum Corporation (NNPC) in Port Harcourt, Nigeria. The samples were collected in amber glass bottles and transferred to the Biochemistry Research Laboratory of the Rivers State University, Port Harcourt, Nigeria. The samples were stored in the dark at room temperature before use.

**Animal Protocol**

Thirty (30) male and 30 female albino rats weighing between 150 g to 200g were obtained from the Department of Biochemistry, University of Port Harcourt and transferred to the animal unit of the Biochemistry Research Laboratory, Rivers State University, Port Harcourt. The rats were housed in metal cages, fed with standard rodent feed and water *ad libitum and maintained under*
well ventilated 12-hour light-dark cycle condition (Adedara et al., 2011). The rats were allowed to acclimatize to the new laboratory condition for 7 days before treatment commenced. The rats were separated into groups (5 rats in each group) according to sex and duration of exposure. The exposed group received sub-lethal dose (500 mg/kg body weight) of the BLCO by topical skin application on 2cm² shaved portion of the dorsal skin while the control groups received similar dose of distilled water on the shaved portion of the dorsal skin. This treatment was repeated every 48 hours throughout the duration of the study. All the rats were examined daily for physically and behavioral changes. The investigation was carried out based on approved institutional guidelines for the care and handling of laboratory animals as stipulated by the National and institutional guidelines for the protection of animal welfare during experiments (Public Health Service, 1996)

Sample Collection

At the end of every 30 days, sets of rats (designated as Treated and Control) of each sex, were weighed, anaesthetized, sacrificed and their blood collected by cardiac puncture using sterilized syringe and needle. The blood was carefully transferred into clean dry plain bottles and left at room temperature to clot. Afterwards they were centrifuged at 4000 rpm for 5 minutes. Separated serum was carefully transferred into fresh clean dry bottles and stored at 5°C for enzyme assay. The rats were further dissected and their liver carefully excised, cleaned of any attached strands and weighed before being processed for histopathology.

Biochemical Assay

The serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and serum albumin (SA) levels were determined by spectrophotometric using the respective Randox test kits (Randox, 2003) and following the manufacturer’s instructions.

Histopathology Investigation

A portion of the excised rat liver was fixed in 10% formalin, washed, dehydrated in isopropanol, rinsed with xylene and embedded in paraffin wax. The paraffin sections were prepared and stained with haematoxylin and eosin. Thin sections of the liver were made into permanent slides and examined under microscope with photographic facility and photomicrographs were taken. The micrographs were analyzed and interpreted by the pathologist. The processing, sectioning, staining, microscopic examination and interpretation were carried out as described by Toros et al., (2013)

Data Analysis

Results from the investigation were expressed as mean of triplicate determinations ± standard
deviation. Comparison between treated and corresponding control were made using SPSS computer software for t-test of equal variants to determine the “p” value. A “p” value <0.05 was considered statistically significant.

RESULTS

Relative Liver Weight and Liver Function Enzyme Activity

Results of the relative liver weight, SGOT and SGPT activity of the albino rats following treatment with BLCO are presented in Tables 1 and 2. There was general decrease in the relative liver weight of the male rats when compared to that of the controls except for the result of the sixty-day treatment where a 14.65% increase was observed (Table 1). The female rats showed general increase in relative liver weight compared to the controls (Table 2). Elevated levels of SGOT and SGPT were recorded for the treated male rats compared to the controls throughout the 90 days period except for the 30-day sample where a 14.3% decrease was recorded for SGPT (Table 1). The increases after 90 days exposure were significant (p<0.05). The female rats also had elevated SGOT and SGPT levels among the treated group compared to controls except the 30-day samples where 16.1% and 56.9% decreases were recorded (Table 1). The elevation of SGOT and SGPT in the 60 days and 90 days samples was significant (p<0.05).

Table 1 Levels of Liver Function Indicators in Male Albino Rats Exposed to BLCO

<table>
<thead>
<tr>
<th>Duration of Exposure (Days)</th>
<th>Relative Liver Weight Control</th>
<th>Treated</th>
<th>Relative Liver Weight Difference</th>
<th>SGOT Activity Control (IU/L)</th>
<th>Treated (IU/L)</th>
<th>SGPT Activity Control</th>
<th>Treated (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>3.03 ±0.00</td>
<td>2.67 ±0.02</td>
<td>41.30±4.82</td>
<td>54.30±3.24</td>
<td></td>
<td>18.67 ±1.15</td>
<td>16.00 ±1.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(-11.88)</td>
<td>(31.4)</td>
<td></td>
<td></td>
<td>(14.3)</td>
</tr>
<tr>
<td>60</td>
<td>2.73 ±0.01</td>
<td>3.13 ±0.01</td>
<td>49.00±2.16</td>
<td>59.00±0.82</td>
<td>25.00</td>
<td></td>
<td>27.33±1.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(-14.65)</td>
<td>(20.4)</td>
<td>±0.00</td>
<td></td>
<td>(9.3)</td>
</tr>
<tr>
<td>90</td>
<td>3.33 ±0.03</td>
<td>1.10 ±0.03</td>
<td>47.00±1.41</td>
<td>84.67±7.51</td>
<td>28.50</td>
<td></td>
<td>32.00±1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(-66.97)</td>
<td>(71.9)</td>
<td>±0.71</td>
<td></td>
<td>(14.3)</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation of 5 rats. The values in brackets represent % change compared to control. * Significantly different from control (p<0.05)
Table 2 Levels of Liver Function Indicators in Female Albino Rats Exposed to BLCO

<table>
<thead>
<tr>
<th>Duration of Exposure (Days)</th>
<th>Relative Liver Weight (g)</th>
<th>SGOT Activity (IU/L)</th>
<th>SGPT Activity (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
</tr>
<tr>
<td>30</td>
<td>3.53 ± 0.01</td>
<td>3.73 ± 0.04 (5.67)</td>
<td>76.30 ± 2.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.48 ± 0.01 (4.50)</td>
<td>46.00 ± 1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.37 ± 0.03 (9.25)</td>
<td>47.50 ± 0.50</td>
</tr>
<tr>
<td>60</td>
<td>3.33 ± 0.01</td>
<td>3.48 ± 0.01 (4.50)</td>
<td>46.00 ± 1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>4.00 ± 0.02</td>
<td>4.37 ± 0.03 (9.25)</td>
<td>47.50 ± 0.50</td>
</tr>
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<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Values are mean ± standard deviation of 5 rats. The values in brackets represent % change compared to control. * Significantly different from control (p<0.05)

Figure 1 Levels of Liver Function Indicators in Male Albino Rats.
Figure 2 Variations in the Levels of Liver Function Indicators of Male Albino Rats.

Figure 3 Levels of Liver Function Indicators in Female Albino Rats.
Figure 4 Variations in the Levels of Liver Function Indicators of Female Albino Rats.

**Serum Albumin**

The results of serum albumin levels of the male and female albino rats are contained in Table 3. There was a 15.4% decrease in serum albumin level of the treated group when compared to that of the control after 30 days of treatment for the male albino rats. The female rats showed a 12.5% increase in albumin after 30 days exposure to BLCO. After 60 days and 90 days exposure there were increases in albumin levels of the male treated group when compared to that of the control. The female rats however, showed the same trend as the males after 60 days but a 5.3% decrease after 90 days exposure

Table 3 Serum Albumin Levels of Male and Female Albino Rats Exposed to BLCO

<table>
<thead>
<tr>
<th>Duration of Exposure (Days)</th>
<th>Serum Albumin Levels (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Control</td>
<td>Male Treated</td>
</tr>
<tr>
<td>30</td>
<td>1.10 ± 0.70 (-15.4)</td>
</tr>
<tr>
<td>60</td>
<td>3.60 ± 0.57</td>
</tr>
<tr>
<td>90</td>
<td>3.55 ± 0.15</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation of 5 rats. The values in brackets represent % change compared to control.  

*Significantly different from control (p<0.05)*
Figure 5 Serum Albumin Content of Male Albino Rats

Figure 6 Serum Albumin Content of Female Albino Rats
Histopathology

Results of the histopathological examinations are summarized in Table 4

Table 4 Histopathology of the Liver of Albino Rats Exposed to BLCO

<table>
<thead>
<tr>
<th>Duration of Exposure (Days)</th>
<th>Description of Male Control</th>
<th>Hepatocytes Male Treated</th>
<th>Nuclei of hepatocytes are vesicular and show prominent nucleoli</th>
<th>Hepatocytes show macrovesicular steatosis. There are areas of necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>Show well preserved architecture. The kupfer cells are prominent</td>
<td>The hepatocytes show degeneration (fatty changes). There are areas of necrosis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Plate 1 Histomicrograph of Male Albino Rats Liver after 90 Days Exposure to BLCO
DISCUSSION

BLCO has been reported to contain toxic poly aromatic hydrocarbon (PAH) compounds and heavy metals which are known to have the potential of exerting varying toxic effects on animals (IARC, 1989; Adedara et al., 2011; Igwe, et al., 2016). In the present study, the intrinsic biochemical changes observed can be attributed to the possible effects of dermal absorption of the crude oil.

Relative liver weight generally increased among the female treated rats and decreased after 90 days treatment among the male treated rats, compared to the controls. This suggests potential hepatotoxicity of the xenobiotics found in BLCO since changes in relative organ weight is a widely accepted toxicity indicator (Khan et al., 1987). Levels of the liver function enzymes SGOT and SGPT increased significantly (p < 0.05) among the treated rats compared to controls. This clearly indicates the damaging effect of the xenobiotics to hepatocytes leading to release of the liver enzymes into circulating blood. Though SGOT and SGPT are found in muscles and other cells, a rise in blood levels is attributed to inflammation or necrosis of liver cells (Jeffries, 1979). Ayalogu et al., (2001) had reported similar rise in serum transferase activity following intraperitoneal administration of crude oil and other petroleum products.
After 90 days exposure period, there were some observed changes in the morphology of the excised liver of the treated rats, such as black dots all over the liver and swollen feet and ankles. These are possible manifestations of hepatotoxicity arising from the dermal exposure to BLCO.

An important biochemical index also used to assess the status of the liver is serum albumin. Result of the analyses of serum albumin levels of the rats revealed a decrease in concentration after 30 days of treatment for the male rats (Figure 5) and a decrease after 90 days of treatment for the female rats (Figure 6). The liver is responsible for the synthesis of plasma albumin and detoxification of xenobiotics (Zakrzewski, 2002). The decrease in serum albumin levels of these rats exposed to BLCO may be related to the biochemical activities occurring in the liver. Inhibition of liver enzymes leading to decreased liver synthesis of albumin and/or increased coupling of the serum albumin with the xenobiotics could cause the observed reduction in serum albumin. These observations are similar to the changes in serum proteins in rats orally administered with crude oil as reported by Orisakwe et al., (2005). As treatment progressed, there were significant increases (p< 0.05) in serum albumin levels for the male rats after 90 days exposure (Figure 5). This may be attributed to adaptive response to the absorbed components of the crude oil which may have elicited the production of more albumin molecules. Albumin, being a transport protein, binds to xenobiotics and transports them to the liver for detoxification (Zakrzewski, 2002; Orisakwe et al., 2005).

The histopathological examination results (Table 4) show indication of distortion in the cell structure of hepatocytes following treatment with BLCO. Uptake of these xenobiotics is probably causing morphological changes, inflammation and necrosis of the cells of the liver, as observed from the histomicrographs. Chronic dermal exposure to BLCO had resulted in the absorption of its components into the blood stream and subsequent uptake by the liver. This has been shown to induce damaging effects on hepatocytes causing hepatotoxicity.

CONCLUSIONS

Chronic dermal exposure of sub lethal doses of Bonny light crude oil on adult albino rats resulted in the absorption of its components by the skin and incorporation into the systemic circulation. This produced deleterious alterations in the architecture and morphology of liver cells as well as dose dependent and time related changes in the relative liver weight, SGOT, SGPT and serum albumin levels. The magnitude of effects varied with duration of exposure and sex of the animals. Evidence of inflammatory infiltrates and tissue necrosis of hepatocytes were also recorded. These findings suggest an obvious distortion in liver function parameters and potential hepatotoxic effect of the dermal exposure. Therefore, continuous dermal contact with BLCO should be discouraged as it has the potential to induce hepatic toxicity.
REFERENCES


and kidney function tests amongst paint factory workers in Nkpor, Nigeria. *Journal of Toxicology and industrial Health.* 23(3): 161 - 165


