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Alterations in chromatin DNA and protein absorbance ratio in the liver of albino rats (*Rattus norvegicus*) treated with bonny light crude oil.

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ABSTRACT

Objective: To determine whether or not Bonny Light Crude Oil (BLCO), when administered to albino rats for six consecutive days at 48 hours interval would result in a dose-related alteration in chromatin structure as obtained in its absorbance ratio, and subsequent impairment of its function such as DNA synthesis.

Methodology and results: Twelve albino rats (*Rattus norvegicus*) were divided into four groups with group one serving as control, and group two to group four were administered with 2.5, 5.0 and 10.0 ml/kg bw of Bonny Light Crude Oil (BLCO) by intraperitoneal injection for six consecutive days. All the rats were sacrificed on the eighth day and their liver excised. The livers were all homogenized, and through differential and fractional centrifugation, the nuclei containing the chromatin were obtained. The chromatin DNA and protein absorbance ratio was determined at 260nm and 280nm by UV spectrophotometry. The results show that the chromatin ratio for control (untreated) rats was 0.95 while there were moderate increases in the ratio for treated rats. Significantly, the 260nm/280nm absorbance ratio increases occurred at 260nm and not 280nm, showing that chromatin DNA was more altered than chromatin protein.

Conclusion and application of findings: Bonny light crude oil probably induced DNA polymerization by unscheduled DNA synthesis in chromatin, which suggests genotoxicity especially carcinogenicity. This demonstrates probable adverse impact to human health on exposure to crude oil spillage and pollution in air, land and water bodies.

Key words: Bonny light Crude Oil (BLCO), Chromatin DNA and Protein absorbance Ratio, Carcinogenesis.

INTRODUCTION

Crude oil contains hundreds of hydrocarbons, comprised primarily of hydrogen and carbon e.g. simple straight chain paraffins, aromatic ring structures, and napthenes, with some sulphur, nitrogen, metals, and oxygen compounds. Crude oil composition varies slightly by its source, but its toxic properties are fairly consistent. Chemicals such as benzene and polycyclic aromatic hydrocarbons (PAHs) are very toxic components of crude oil and are of high concern, (Michael & Kathleen, 2011). Bonny light crude oil (BLCO) is a high grade of Nigeria Crude oil, with high America Petroleum Institute (API) gravity, produced in the Niger Delta Basin and named after the prolific region around the city of Bonny. BLCO has a higher content of aliphatic hydrocarbons (light fractions) than aromatic hydrocarbons (heavy fractions) in 80:20 ratio, thus it is referred to as 'light' crude oil (Nigeria National Petroleum Cooperation – NNPC, personal communication). Although divers toxicity studies have been reported by various geological crude oil like Bonny light and

Prudhoe Bay crude oil, such as effects to various enzymes in the liver , the brain, heart, kidney and their associated enzymes, mitochondria DNA, BLCO induced dose related increase in chromatin absorbance ratio has not been reported. For instance one study showed that BLCO, when given to adult male guinea-pigs by intraperitoneal injection at two varying doses of 2.5 ml/kg bw and 5.0 ml/kg bw, causing large dose-related increases in the concentrations of liver mitochondria DNA and cytoplasmic total hydrocarbon concentration (Oruambo & Adirimo, 2007). The activity of gamma glutamyl transferase reduced significantly in the liver of Sprague-Dawley rats treated with gossypol, possibly due to the presence of toxicants, e.g. polycyclic aromatic hydrocarbons in the system of the rats (Deoras *et al*. 2003).

A significant reduction in the activities of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) was observed in the liver of albino rats fed on diets containing catfish exposed to various concentrations of crude oil (Sunmonu and Oloyede, 2009). Cell membranes of all living cells have large hydrophobic or an oil-like composition. The lipid or naturally-oily components of cell membranes interact strongly with many of the toxic components of oil. Benzene, toluene, xylene, naphthalene are just a few of the chemical compounds of crude oil that can dissolve or deform cell membranes and cause cell death. Membranes contain enzymes and transport proteins that are critical to the cell. When the cell membrane is damaged or disrupted the working membrane may not work to transport or permit passage of molecules. Furthermore, damaged cell membranes permit critical cell molecules to leak out of the cell and this contributes to cell death. Benzene-ring compounds and other crude oil chemical components are known carcinogens — agents that can cause cancer. These compounds, if they reach the nuclear DNA, may cause mutation, cell malfunctions, or even cancers that are characterized by uncontrolled cell growth and multiplication. Polycyclic aromatic hydrocarbon (PAHs), component of crude oil reasonably anticipated to be human carcinogens (first published 1981, in the second annual Report on carcinogens, 1981).Carcinogenicity of PAHs was based on validated research done on experimental animals (International Agency for Research on Cancer (IARC). 1973, 1983, 1987**).** The cancers induced in these studies were of these types; papillomas, carcinomas, sarcomas and included tissues and organs of the skin, stomach, liver, bladder and kidney (Donald Reinhardt, 2010).

The World Health Organization /International Agency for Research on cancer (IARC) concludes that there is sufficient evidence that Benzo[a]pyrene, and other polycyclic aromatic hydrocarbons are carcinogenic in experimental animals and that BaP is probably carcinogenic in humans. Offspring of pregnant mice, who were injected with BaP during pregnancy, had an increased incidence of tumors, predominantly in lung, liver and ovaries during childhood (Urso & Gengozian 1982 ;1984; Wislocki *et al*, 1986; Turusov *et al,* 1990). In this study, as a follow-up to efforts to understand the probable molecular pathway(s) of BLCO potential genotoxicity or carcinogenicity, this study aimed to determine whether or not BLCO, when administered to albino rats at 2.5, 5.0 and 10.0 (ml/kg bw) for six consecutive days at 48 hours interval would result in a dose related increase in chromatin absorbance ratio.

MATERIALS AND METHOD

Crude Oil: Fresh Nigerian (Bonny) light crude oil (BLCO) was obtained from the Nigerian National Petroleum Corporation (NNPC) in Port Harcourt, Rivers State, Nigeria and brought to the Department of Chemistry laboratory, Rivers State University of Science and Technology in an amber bottle to prevent photo-oxidation.

Treatment of Animal: Twelve albino rats of the specie *Rattus norvegicus*, weighing approximately of 150g (0.15kg) were obtained from the University of Port Harcourt animal farm and transported to the Department of Chemistry, Rivers State University of Science and Technology Animal Room in a well-ventilated plastic basket. They were all fed with

commercial meal and given drinking water ad libitum throughout the period of the experiment. Prior to treatment with BLCO the rats were allowed an acclimatization period of five days in the Animal Room. The rats were divided into four groups with each group containing a total of three rats. The rats in group one served as untreated control, while the remaining groups were injected with BLCO through the intraperitoneal route. Rats in group two to group four were given 2.5 ml/kg bw, 5.0ml/kg bw and 10.0ml/kg bw dose of BLCO respectively. The treatment lasted for six consecutive days at an interval of 48hours. All the rats were sacrificed on the eighth day. After sacrifice, the animals were dissected and the liver excised and stored in a 0.5M potassium phosphate buffer of pH 7.4 and stored at 4^0C for further analysis.

Preparation of Liver Homogenate and Chromatin Extraction: The excised liver was then homogenized in 0.32M sucrose, 5mM $MgCl_2$ for nuclei isolation and subsequent chromatin preparation as reported elsewhere (Kasprzack *et al*, 1989).

Statistical Analysis: To ensure reproducibility of the results, each biochemical experiment, i.e. determination of chromatin absorbance at 260nm and 280nm was carried out in two sets of triplicates, and the Arithmetic Means with their corresponding standard deviations were calculated. The Results are expressed as Mean ± SD. The magnitude of change of each parameter under study over control was calculated in percentage and expressed as percentage increase or percentage decrease.

RESULTS

Table 1: Chromatin Absorbance at 260nm of Livers of rats Treated with 2.5 ml/kg bw, 5.0 ml/kg bw and 10.0 ml/kg bw BLCO by intraperitoneal route for six consecutive days at 48hours interval.

Doses (ml/kg bw)	Absorbance at 260nm	Percentage Change over control
Control (Nil)	2.15±0.0071	_
2.5	2.19±0.0071	0.94↑
5.0	2.23±0.01	3.18↑
10.0	2.23±0.0071	3.18↑

↑: Increase in percent change over control.

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DNA component of chromatin absorbs ultraviolet light maximally at 260nm From table 1, a moderate increase can be seen in treated rats when compared to untreated. There was a 0.94% slight increase at 2.5ml/kg bw of administered BLCO over control and a peak increase of 3.18% at a median dose of 5.0ml/kg bw

Table 2: Chromatin Absorbance At 280nm of Livers of rats Treated with 2.5 ml/kg bw, 5.0 ml/kg bw and 10.0 ml/kg bw BLCO by intraperitoneal route for six consecutive days at 48hours interval.

Doses (ml/kg bw)	Absorbance at 280nm	Percentage change over control
Control (Nil)	2.28±0.00	_
2.5	2.27±0.00	0.04↓
5.0	2.28±0.0071	0.04↑
10.0	2.27±0.0071	0.04↓

↓: Decrease in percent change over control.

Protein component of chromatin absorb ultraviolet light maximally at 280nm. In Table 2, a dose-related decrease in absorbance at 2.5 and 10 (ml/kg bw) and a zero absorbance percent change in 5.0ml/kg can be observed.

Table 3: Chromatin Absorbance ratio (260nm/280nm) of liver of rats Treated with 2.5 ml/kg bw, 5.0 ml/kg bw and 10.0 ml/kg bw BLCO by intraperitoneal route for six consecutive days at 48hours interval.

Doses (ml/kg bw)	Absorbance Ratio at 260nm/280nm	Percentage change over Control
Control (Nil)	0.95±0.00	_
2.5	0.96±0.00	1.04↑
5.0	0.98±0.00	3.09↑
10.0	0.98±0.00	3.09↑

In Table 3, a moderate dose related increase in chromatin absorbance ratio is observed in treated albino rats with BLCO, when compared with the untreated (control) albino rats. There was a 1.04% slight increase at 2.5ml/kg bw of administered BLCO over control and a peak increase of 3.09% at the median dose of 5.0 ml/kg bw.

DISCUSSION

From the result obtained above it can be observed that the protein component of chromatin is virtually unchanged. This shows that an alteration in chromatin DNA and protein absorbance ratio is not by the interaction of components in BLCO with histone proteins, thereby resulting in the exposure of DNA to the components. But rather, the interaction is directly with the DNA component. This is confirmed by the moderate increase in DNA absorbance at 260nm (Oruambo and Van Duuren, 1989). DNA wraps around an octomer of core histones to form nucleosomes blocking access to many potential DNA binding sites (Jeremy *et al,*2002), but during the process of cell division (mitosis) at interphase chromatin undergoes replication exposing its binding sites (David and Michael, 2005). Thus, making DNA vulnerable to attack by agents generated in-vivo such as BLCO toxic components resulting in the alteration of DNA structure and probably its functions, giving rise to unscheduled increase in DNA synthesis. Furthermore, this increase in chromatin DNA may also be contributed by DNA repair process assuming there had been damage to DNA. Results obtained in this research in addition to a growing body of evidence as seen in the literature on BLCO induced DNA synthesis, confirms the possible effect of toxicants in BLCO on chromatin DNA, suggesting possible genotoxicity of BLCO especially carcinogenesis. This result is in agreement with increase in DNA concentration in the liver of guinea pig treated by intraperitoneal route with BLCO, with peak increase occurring at the medium dose of 2.5 ml/kg bw BLCO. (Oruambo I.F *et al*, 2007**).** It also agrees in part with the finding of International Agency for Research on Cancer (IARC), which showed that crude oil given orally to male Charles River CD-1 mice at 5.0 ml/kg bw induced increase in nucleic acid. It also agrees with a published work, where it was determine that BLCO caused significant dose related increase in total cellular DNA and chromatin (nuclear) DNA in the liver of adult male guinea pigs treated through intraperitoneal route with 1.25, 250 and 5.0 (ml/kg bw) BLCO. (Oruambo *et al*, 2007). Chemicals such as polycyclic aromatic and mono aromatic hydrocarbons which are constituents of crude oil have not only been linked to DNA damage but also linked to the degree of damage with cancer risk. Many human cancers are associated with exposure to genotoxic chemicals. There is typically a long period of time between early events that include initial carcinogen exposure, the onset of DNA damage and fixation of mutations and the subsequent appearance of a tumor. (Miller J.A, 1970; Miller E.C *et al*, 1981; Yuspa S.H *et al*, 1988)

DNA damage though necessary, is not a sufficient step in the carcinogenic process because repair process usually steps in following damage. Chemical carcinogens such as PAHs in BLCO can cause the formation of carcinogen-DNA adduct or induce other modifications to DNA, such as oxidation damage and alterations to DNA ultrastructure (DNA-strand cross linking, DNA-strand breakage, chromosomal rearrangement and deletions). Although cells possess mechanism to repair many types of DNA damage, these are not always completely effective, and residual DNA damage can lead to the insertion of incorrect bases during DNA replication, followed by transcription and translation of the altered templates, ultimately leading to the synthesis of altered protein. Studies involving chemical induction of tumors in animal models are typically preferred using rats and mice, and hundreds of such studies attest to the essential role of DNA damage in the carcinogenic process. (Miller & Miller 1981; Yuspa & Poirier, 1988). In these models dose response curves for chemical exposure, DNA-adduct formation, mutagenesis and rate of tumor

formation are closely related. (Miller & Miller, 1981; Yuspa & Poirier, 1988; Poirier & Belland 1992; Poirier, 1996; Weston & Harris, 2003). This study further confirms the probable carcinogenicity of Bonny Light Crude Oil.

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