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Abstract

The harmful effects of folkloric uses of Nigerian bonny light crude oil (BLCO) in ailments management may outweigh the expected beneficial effects. We investigated the levels of heavy metal concentrations in the tissues as well as the effect of BLCO on the antioxidant status of erythrocytes of rats after oral exposure to 0, 200 and 800 mg/kg BLCO for 7 days. Analysis of heavy metal concentrations in BLCO showed that Zn > Fe > Pb > Cu > Ni. The trend of accumulation of the metals in the tissues is blood—Fe > Pb >Zn whereas Cu and Ni levels were not affected; Liver—Ni > Zn > Fe > Cu > Pb and Testes—Ni > Cu > Pb > Zn > Fe. The order of concentration of the metals in the tissues is as follows: iron—blood > liver > testes; zinc—liver > blood > testes; lead—blood > liver > testes; copper—testes > liver > blood; nickel—liver > testes > blood. Activities of the antioxidant enzymes of erythrocytes such as superoxide dismutase, catalase, glutathione S-transferase and glutathione peroxidase increased significantly in a dose-dependent manner with significant elevation in hydrogen peroxide and malondialdehyde levels, whereas glutathione level was not significantly decreased in BLCO-treated animals. Collectively, the results showed that BLCO induces oxidative damage to erythrocytes of rats.

Keywords

Nigerian bonny light crude oil (BLCO), oxidative damage, blood, liver, testes, erythrocytes, rats

Introduction

Bonny light crude oil (BLCO) is classified as light crude oil with aromatic hydrocarbons accounting for up to 45% of the total hydrocarbons (Orisakwe et al., 2004a). It has been reported to contain vanadium, nickel, asphaltenes, nitrogen, and low sulfur content, all of which have been shown to be toxic (Akintonwa and Ebere, 1990). The folkloric uses of BLCO by the local population of Niger Delta in the Southern part of Nigeria includes dermal application for burns, foot rot and leg ulcers, poisoning and witchcraft, ingestion in the treatment of gastroinstestinal disorders and reproductive capacity.

Previous studies on BLCO revealed that it is hepatotoxic and hematotoxic (Orisakwe et al., 2005). A case of crude oil poisoning in a 2-year-old child following ingestion and dermal application of BLCO for treatment of a febrile convulsion with sequel of shock, acute renal failure, mechanical intestinal obstruction, extensive epidermolysis, conjunctivitis, mucositis, esophagitis and chemical pneumonitis has been reported (Otaigbe and Adesina, 2005). It has been shown to induce alterations in the liver mitochondria DNA concentrations, cytoplasmic total hydrocarbon and calcium concentrations in adult guinea pigs exposed by intraperitoneal injection for 2 days (Oruambo and Jones, 2007). The nephrotoxicity of BLCO

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characterized with alterations in serum electrolytes, urea and creatinine and pathological changes in kidney biopsies have also been reported (Orisakwe et al., 2004b). BLCO exposure adversely affects male fertility and results in severe impairment of testicular functions including degenerative changes in seminiferous tubules and Leydig cells in rats (Orisakwe et al., 2004a) and alteration of antioxidant systems in a dose-dependent manner via induction of oxidative stress following oral gavage at 200, 400 and 800 mg/kg for a week in rats (Farombi et al., 2010).

There is paucity of information regarding biochemical disturbances associated with BLCO hematotoxicity. Cellular toxicity is a function of the balance between the rate of formation of reactive metabolite and the rate of their removal. Although many heavy metals when in trace amounts are essential for various metabolic processes in organisms, they create physiological stress leading to the generation of free radicals when in high concentration. Stress in turn induces the production of reactive oxygen species ([ROS] Choudhary et al., 2007). Elevated levels of heavy metals can induce impairment and dysfunction in systems including the blood and cardiovascular, nervous, eliminative pathways (colon, liver, kidneys and skin), endocrine, energy production pathways, gastrointestinal, enzymatic and reproductive. Erythrocytes are vulnerable to lipid peroxidation (LPO) due to their high content of polyunsaturated lipids (Puppo and Halliwell, 1998). The erythrocyte has several cellular defence mechanisms to prevent the buildup of ROS and collectively protect it from oxidative damage. It is possible that BLCO transported through blood to the liver for metabolism may produce cellular damage to erythrocytes. The present study was therefore undertaken to determine the tissue concentrations of heavy metals as well as the effect of BLCO on the antioxidant system in erythrocytes of rats after acute exposure to BLCO.

Material and methods

Bonny light crude oil

BLCO was obtained from the Nigerian National Petroleum Corporation (NNPC), Ekpan, Warri, Delta State, Nigeria.

Chemicals

Epinephrine, glutathione (GSH), 5,5′-dithiobis(2-nitrobenzoic acid), hydrogen peroxide (H_2O_2), thiobarbituric acid and 1-chloro-2,4-dinitrobenzene were purchased from Sigma Chemical Co. (USA). All other reagents were of analytical grade and were obtained from the British Drug Houses (UK).

Animal protocol

Thirty healthy adult male Wistar rats weighing approximately 140 $\pm$ 10 g obtained from the Department of Biochemistry, University of Ibadan (Ibadan, Nigeria) were randomly assigned to three groups of 10 animals per group. They were housed in plastic suspended cages placed in a well-ventilated rat house, provided rat pellets and water ad libitum and subjected to natural photoperiod of 12-hour light:dark cycle. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. The ethic regulations have been followed in accordance with National and institutional guidelines for the protection of animal welfare during experiments (Public Health Service, 1996). BLCO was dissolved in corn oil and administered orally at doses of 200 and 800 mg/kg body weight per day for 7 days (Farombi et al., 2010). Corresponding group of animals were administered with corn oil alone and served as control. Twenty-four hours after the last treatment, all the rats were sacrificed by cervical dislocation. The testes and liver were quickly removed and weighed. The body weights of rats were taken before exposure to various treatments and prior to sacrifice.

Determination of heavy metals in the tissues

The testes and liver were washed in ice-cold 1.15% KCl solution, blotted and weighed. They were then homogenized in 4 volumes of homogenizing buffer (50 mM Tris-HCl mixed with 1.15% KCl and pH adjusted to 7.4), using Teflon Homogenizer. The resulting homogenate was centrifuged at 10,000 g for 15 min in a Beckman L5-50B centrifuge at 0–4°C and the supernatants collected for determination of heavy metal levels (Farombi et al., 2007). The blood (5 mL) obtained from the retro-orbital venous plexus of the animals and the tissue supernatants were digested according to the method described by Hoenig and de Kersabiec (1996). The levels of Cu, Zn, Ni, Pb and Fe in the filtrates from each digested sample were then determined with the aid of Atomic Absorption Spectrophotometer A Analyzer 200 (USA). Heavy metal analysis of the crude oil was

Table 1. Antioxidant status of erythrocytes of rats following BLCO treatment for 7 days.[a]

Parameters	Control	200 mg/kg BLCO	800 mg/kg BLCO
SOD	0.93 ± 0.15	1.34 ± 0.58	1.37 ± 0.28[b]
CAT	1029.78 ± 93.62	1417.9 ± 387.29[b]	2212.48 ± 330.09[b]
H_2O_2	146 ± 15.58	181 ± 13.85[b]	210.67 ± 19.14[b]
GSH	44 ± 8.94	38.4 ± 2.19	37.2 ± 1.10
GST	0.29 ± 0.03	0.47 ± 0.16[b]	0.48 ± 0.20[b]
GPX	299.62 ± 16.81	385.38 ± 16.21[b]	589.42 ± 17.52[b]
LPO	4.05 ± 0.47	5.19 ± 0.99[b]	6.46 ± 1.48[b]

BLCO: bonny light crude oil, SOD: superoxide dismutase, CAT: catalase, H_2O_2: hydrogen peroxide, GSH: glutathione, GST: glutathione *S*-transferase, GPx: glutathione peroxidase, LPO: lipid peroxidation, CDNB: 1-chloro-2,4-dinitrobenzene.
[a]Values are expressed as mean ± SD of 10 rats. SOD activity (Units/mg protein), CAT activity (μmole H_2O_2 consumed/min/mg protein), H_2O_2 generation (μmole H_2O_2 produced/min/mg protein), GSH (μmole/mg protein), GST activity (μmole CDNB-GSH complex formed/min/mg protein), GPx activity (Units/mg protein), LPO (μmole MDA formed/mg protein).
[b]Significantly different from control, $p < 0.05$.

also determined. An acetylene air mixture was used as the flame. The working standard for each of the metals were aspired into the flame in the order of 0.0, 0.2, 0.4, 0.8 and 1.6 ppm before the tissues were aspirated into the flame.

Erythrocytes preparation

Five milliliters of the blood was drawn from the retro-orbital venous plexus of the animals, before killing, into vials containing heparin as an anticoagulant. The erythrocytes were sedimented by centrifugation at 4000*g* for 10 min at 4°C within 1 hour of collection and the plasma was removed. The erythrocytes were washed three times with ice-cold phosphate buffer saline (PBS; 145 mM NaCl, 1.9 mM NaH_2PO_4 and 8.1 mM Na_2HPO_4) and centrifuged. The erythrocyte pellets were resuspended in PBS (0.1 M, pH 7.4) at 1:9 dilutions to be used for antioxidant indices.

Estimation of erythrocytes antioxidant status

The antioxidant status was estimated in the erythrocyte pellets. Superoxide dismutase (SOD) was assayed by the method described by Misra and Fridovich (1972). Catalase (CAT) activity was determined using H_2O_2 as substrate, according to the method of Clairborne (1995). H_2O_2 generation was assessed by the method of Wolff (1994). Glutathione peroxidase (GPx) activity was determined by the method of Rotruck et al. (1973) with some modifications, which is based on the reaction between GSH remaining after the action of GPx and 5′,5′-dithiobis(2-nitrobenzoic acid) to form a complex that absorbs maximally at 412 nm. Glutathione *S*-transferase (GST) was assayed by the method of Habig et al. (1974). Reduced GSH was determined at 412 nm using the method described by Jollow et al. (1974). Protein concentration was determined by the method of Lowry et al. (1951). LPO was quantified as malondialdehyde (MDA), according to the method described by Farombi et al. (2000) and expressed as micromoles of MDA per gram tissue.

Statistical analysis

Statistical analysis was carried out using one-way analysis of variance to compare the experimental groups followed by the Student's *t* test using SPSS (student version 7.5, SPSS Inc, UK) and values less than 0.05 were considered statistically significant.

Results

Antioxidant status of the erythrocytes

The erythrocytes antioxidant status of BLCO-treated rats is presented in Table 1. A significant ($p < 0.05$) increase in the activities of antioxidant enzymes SOD, CAT, GPx and GST were observed in the erythrocytes of all BLCO-treated groups. Compared to the control animals, the changes in the SOD activity accounted for 44.1% and 36.6%, whereas that of CAT were 37.7% and 114.8% at 200 and 800 mg/kg body weight (bw), respectively. The observed increases in GPx activity were 28.6% and 96.7%, whereas GST activity increased by 62.1% and 65.5% at BLCO doses of 200 and 800 mg/kg bw, respectively, when compared with the control animals. The level of GSH content of the erythrocytes was not significantly decreased in all BLCO-treated groups. However, H_2O_2 and MDA were significantly elevated in all BLCO-treated groups. The percentage increases were 24% and

Table 2. Levels of heavy metals in the blood of rats following BLCO treatment for 7 days.[a]

Heavy metals (ppm)	Crude oil	Control	200 mg/kg BLCO	800 mg/kg BLCO
Zn	0.78 ± 0.19	0.57 ± 0.08	0.86 ± 0.02[b] (50.9)	0.87 ± 0.01[b] (52.6)
Pb	0.36 ± 0.02	0.05 ± 0.01	0.34 ± 0.02[b] (58.0)	0.34 ± 0.03[b] (58.0)
Fe	0.67 ± 0.03	0.54 ± 0.08	6.05 ± 0.11[b] (1020.4)	6.13 ± 0.87[b] (1035.2)
Cu	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.02
Ni	0.02 ± 0.01	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001

BLCO: bonny light crude oil.
[a]Values are expressed as mean ± SD of 10 rats.
[b]Significantly different from control, $p < 0.05$. The values in parentheses are percentage change when compared with the control.

Table 3 Levels of heavy metals in the liver of rats following BLCO treatment for 7 days.[a]

Heavy metals (ppm)	Crude oil	Control	200 mg/kg BLCO	800 mg/kg BLCO
Zn	0.78 ± 0.19	0.91 ± 0.07	1.48 ± 0.18[b] (62.6)	2.0 ± 0.02[b] (119.8)
Pb	0.36 ± 0.02	0.29 ± 0.01	0.37 ± 0.02 (27.6)	0.39 ± 0.01 (34.5)
Fe	0.67 ± 0.03	2.23 ± 0.07	3.14 ± 0.40[b] (40.8)	4.70 ± 0.56[b] (110.8)
Cu	0.04 ± 0.01	0.12 ± 0.02	0.16 ± 0.01 (33.3)	0.19 ± 0.01[b] (58.3)
Ni	0.02 ± 0.01	0.02 ± 0.001	0.04 ± 0.001[b] (100)	0.06 ± 0.003[b] (200)

BLCO: bonny light crude oil.
[a]Values are expressed as mean ± SD of 10 rats.
[b]Significantly different from control, $p < 0.05$. The values in parentheses are percentage change when compared with the control.

Table 4. Levels of heavy metals in the testes of rats following BLCO treatment for 7 days.[a]

Heavy metals (ppm)	Crude oil	Control	200 mg/kg BLCO	800 mg/kg BLCO
Zn	0.78 ± 0.19	0.47 ± 0.01	0.61 ± 0.08 (29.8)	0.63 ± 0.02[b] (31.3)
Pb	0.36 ± 0.02	0.24 ± 0.02	0.30 ± 0.04 (25.0)	0.31 ± 0.01 (29.2)
Fe	0.67 ± 0.03	0.63 ± 0.08	0.67 ± 0.01 (6.3)	0.73 ± 0.02 (10.0)
Cu	0.04 ± 0.01	0.02 ± 0.001	0.04 ± 0.001[b] (100.0)	0.04 ± 0.001[b] (100.0)
Ni	0.02 ± 0.01	0.03 ± 0.001	0.05 ± 0.003[b] (66.7)	0.09 ± 0.001[b] (200)

BLCO: bonny light crude oil.
[a]Values are expressed as mean ± SD of 10 rats.
[b]Significantly different from control, $p < 0.05$. The values in parentheses are percentage change when compared with the control.

44.3% for H_2O_2, whereas those of MDA were 28.1% and 59.5% at 200 and 800 mg/kg bw, respectively, when compared with the control animals.

Heavy metal levels

The heavy metal composition of BLCO and their levels (with their percentages) in the blood, liver and testes of rats compared to the control after BLCO treatment are shown in Tables 2–4, respectively. Analysis of heavy metal composition of the BLCO sample showed that Zn > Fe> Pb> Cu > Ni. These metals were accumulated in the tissues of the rats to varying extent. The trend of accumulation of the metals in the organs is as follows: blood—Fe > Pb >Zn. Cu and Ni levels were not affected. Liver—Ni > Zn > Fe > Cu > Pb. Testes—Ni > Cu > Pb > Zn > Fe. The order of concentration of the metals in the tissues is presented in Figure 1: Iron—blood > liver > testes; zinc—liver > blood > testes; lead—blood > liver > testes; copper—testes > liver > blood; nickel—liver > testes > blood.

Discussion

Heavy metals are taken into the body via inhalation, ingestion and skin absorption. If heavy metals enter and accumulate in body tissue faster than the body's detoxification, pathways can dispose them of, a gradual buildup of these toxins will occur. Many redox-active and nonredox-reactive metals are known to cause oxidative stress, as indicated by LPO and

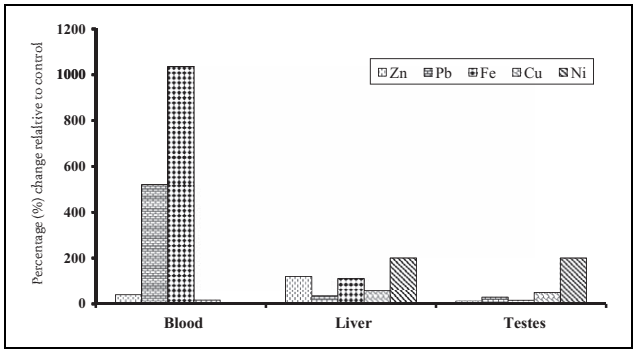

Figure 1. Relative percentage change in metal concentrations in the tissues of rats exposed to bonny light crude oil.

H_2O_2 accumulation in the cells (Schutzendubel et al., 2001). Iron is a redox-active transition metal known to catalyze hydroxyl radical production via Fenton chemistry. Furthermore, zinc and lead are redox-inactive metals which disrupt the metabolic balance and thus enhance the load of ROS (Briat, 2002; Stohs and Bagchi, 1995). The present study demonstrated that administration of BLCO produced a marked oxidative impact as evidenced by significant increase in MDA formation. The level of MDA in the tissue is considered a measure of LPO status. LPO is linked to the production of superoxide radical. Thus, the increased level of MDA suggests that metal ions stimulate free radical-generating capacity in the erythrocytes of BLCO-treated rats. Our present result corroborates with the induction of LPO by BLCO in the testes of rats (Farombi et al., 2010). The induction of oxidative damage by BLCO might be due to its lipophilicity, whereby they could penetrate the cell membrane easily. Orisakwe et al. (2004a) previously postulated biological membranes as the target sites where BLCO adverse effect occur.

SOD, the first line of defence against oxygen-derived radicals is responsible for the dismutation of superoxide radicals to H_2O_2, whereas CAT metabolically removes H_2O_2 from the intracellular environment, thereby further reducing the H_2O_2 and hydroxyl radical generation. We observed a dose-response increase in the activities of SOD and CAT in the erythrocytes of BLCO-treated rats. The increase in activities of these enzymes indicates enzyme induction. An increase in SOD activity has been reported to be beneficial in the event of increased free radical generation (Yen et al., 1996). A simultaneous increase in CAT activity is essential for overall beneficial effect of increase in SOD activity (Schaefer et al., 1998). H_2O_2 molecules are freely dissolved in aqueous solution and can easily penetrate biological membranes. Their deleterious chemical effects can be divided into the categories of direct activity, originating from their oxidizing properties, and indirect activity in which they serve as a source for more deleterious species, such as hydroxyl radicals and hypochlorous acid (Kohen and Nyska, 2002). Our results also showed that BLCO treatment resulted in significant elevation of H_2O_2 levels in the erythrocytes of rats. The increased levels of H_2O_2 mediate toxic effects through the formation of hydroxyl radical a potent activator of LPO (Saradha and Mathur, 2006). Hydroxyl radical could be formed by the interaction of superoxide radical with H_2O_2 through Haber–Weiss reaction. Furthermore, the accumulation of transition metals such as copper or iron also favour enhanced generation via Fenton-type chemistry which largely depends on the availability and location of metal ion catalyst. The acute toxicity of these heavy metals in the erythrocytes may be due to its faster uptake from the intestine. This result is consistent with the induction of H_2O_2 generation in the testes and spermatozoa of rats previously reported (Farombi et al., 2010).

GSH, a thiol group-containing molecule, is known for its effective antioxidant property by scavenging oxidative stress-inducing molecules. The main protective roles of GSH against oxidative stress include scavenging of hydroxyl radicals and singlet oxygen directly, detoxifying H_2O_2 and lipid peroxides by the catalytic action of GPx and also in regenerating antioxidant vitamin (i.e., vitamins C) back to its active form (Masella et al., 2005). GST is a family of isoenzymes that catalyze the conjugation of GSH with a wide variety of organic peroxides (including lipid peroxides) to form more water-soluble compound products that are readily excreted from the system. GPx has been postulated to protect erythrocytes from damage by H_2O_2, and it is responsible for reduction of lipid hydroperoxides (Jee and Kang, 2005). Our data showed that while the activities of GPx and GST significantly increased, the level of GSH was not significantly depleted in BLCO-treated rats. The increase in the activities of antioxidant enzymes in erythrocytes may indicate an adaptive response to neutralize BLCO-induced oxidative stress. In addition, the present result probably suggests the efficiency of the GSH cycle in the erythrocyte to reduce the disulfide (oxidized) form of glutathione (GSSG) to the sulfhydryl form (GSH) in an attempt to decrease BLCO toxicity. Metal stress has been reported to enhance the

sulfur reduction pathways and cysteine synthesis by affecting not only the sulfur uptake and transport but also by inducing the enzymes of the pathway (Rausch and Wachter, 2005; Srivastava et al., 2006). Our present result is inconsistent with testicular and spermatozoa GSH content (Farombi et al., 2010). The discrepancy can be attributed to tissue differences.

The concentrations of the five metals analyzed in tissue samples revealed that heavy metals accumulated differentially in the blood, liver and testes. The concentrations of Pb and Fe were highest in the blood, whereas they are least in the testes. However, Zn and Cu accumulated in the liver than in the blood and testes. We observed high level of Ni accumulation in the liver and testes than in the blood. Fe toxicity results when the amount of circulating iron exceeds the amount of transferin available to bind it (Oyagbemi et al., 2010). Pb affects the red blood cells (anemia and other effects on the hemopoietic system are the commonest effects) and causes damage to organs including the liver, kidneys, heart and male gonads, as well as affects the immune system. Elevated blood level of Pb may indicate accumulation, whereas that of Fe may indicate both accumulation from BLCO ingestion and its excessive release from the heme during oxidative damage of the red cells.

Zn and Cu are essential metals for structural and catalytic properties of many enzymes. The importance of zinc and copper are illustrated by their roles as cofactors for SOD. Zinc is also very important in protein, nucleic acid and energy metabolism, and copper is required in the synthesis of catacholamines. They are also required during tissue repair, regeneration and cell proliferation. The high levels of Zn and Cu in the liver may possibly reflect storage and also due to the fact that the liver being a target and center for metabolism may concentrate heavy metals. However, excess Cu can be toxic, particularly when associated with a deficit in Cu excretion (Bremner, 1998). The high level of Ni in the testes and liver observed in this study probably suggests the role of Ni in testicular and hepatic toxicities in BLCO-treated animals previously reported (Farombi et al., 2010). LPO has been shown to be a contributing factor in Ni-induced cellular oxidative stress (Chen et al., 2003a, 2003b). Nickel was shown to induce apoptosis via pathways including generation of ROS and activation of caspase proteins (Au et al., 2006).

In summary, the present study revealed that BLCO-induced erythrocytes toxicity may be due to the induction of oxidative stress. The unequal distribution of heavy metals in different target tissues may provide insights into the potential multiple organ toxicity of BLCO in individuals using it for management of ailments.

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