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Lack of recovery from hepatic oxidative damage in rats treated with Nigerian bonny light crude oil

Isaac A. Adedara and Ebenezer O. Farombi*

Drug Metabolism and Toxicology Research Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria

The use of Nigerian bonny light crude oil (BLCO) in the treatment of gastrointestinal disorders, burns, foot ulcers and reproductive capacity is a common practice in the southern part of Nigeria. Towards understanding the mechanism and the reversibility of hepatotoxicity induced by BLCO, adult male Wistar rats were orally administered with BLCO at 0, 50, 100 and 200 mg kg^{-1} for 21 days. One-half of the rats were sacrificed on day 22, whereas the remaining half stayed for an additional 21 days without treatment. Whereas the activities of antioxidant enzymes such as superoxide dismutase, catalase, glutathione S-transferase were significantly ($p < 0.05$) increased, gamma glutamyl transferase activity was significantly decreased in a dose-dependent manner. The levels of glutathione, hydrogen peroxide and malondialdehyde were significantly elevated in BLCO-treated animals. In addition, hepatic degeneration was accompanied with elevation in serum aminotransferases activities without affecting bilirubin levels. Whereas most of the above-mentioned parameters were consistent in animals from withdrawal experiment, both total and conjugated bilirubin levels were significantly increased after 21 days of BLCO-treatment withdrawal. Taken together, BLCO-induced hepatotoxicity could be due to increased oxidative stress which was not reversible upon withdrawal of treatment within the time course of investigation in male rats. Copyright © 2012 John Wiley & Sons, Ltd.

KEY WORDS—bonny light crude oil (BLCO); antioxidant; oxidative stress; recovery; hepatotoxicity; rat

INTRODUCTION

The harmful effects of unorthodox remedies may outweigh the expected beneficial effects. A case of crude oil poisoning in a 2-year-old child following ingestion and dermal application of Nigerian bonny light crude oil (BLCO) for treatment of a febrile convulsion with sequel of shock, acute renal failure, mechanical intestinal obstruction, extensive epidermolysis, conjunctivitis, mucositis, oesophagitis and chemical pneumonitis has been reported.[1] Characterizing the effects of exposure to BLCO as a result of increase incidence of oil spillage in Niger Delta region of Nigeria and the use of BLCO in folklore medicine is not only of interest to clinicians but has become of significant issue to those in the environmental community and an actual subject of interest of many researchers.

Studies that demonstrate detrimental effects of BLCO in different organs and animals exist. Oral administration of BLCO has been shown to be hematotoxic with severe pathologic liver changes in the forms of necrosis and oedema.[2] It has also been reported to be nephrotoxic.[3,4] Intraperitoneal injection of adult guinea pigs with BLCO at 2.50 and 5.0 ml kg^{-1} body weight for 2 days increased the availability of crude oil hydrocarbons in the liver cells with subsequent induction of unscheduled mitochondrial DNA synthesis and alteration of mitochondrial/endoplasmic reticulum Ca^{2+} sequestration or Ca^{2+}-concentration gradient, leading to inhibition of Ca^{2+} influx into the cytosol as well as increased regenerative DNA concentration in partially hepatectomized rat liver.[5] BLCO dose-dependently increased the binding of nickel to chromatin proteins and changes to DNA concentration in the liver of guinea pigs.[6] BLCO exposure adversely affects male fertility and results in severe impairment of testicular functions including degenerative changes in seminiferous tubules and leydig cells[7] 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^{-1} for a week.[1,8]

Till date, the use of BLCO in the treatment of gastrointestinal disorders, burns, foot ulcers and reproductive capacity among adults is common in the southern part of Nigeria. The liver plays a major role in xenobiotic metabolism and is consequently the primary target of most toxic responses. Liver injury does not only depend on the type of chemical agent involved but also on the period of exposure.[9] Although our laboratory had previously shown, for the first time, the involvement of free radicals in the BLCO-induced pathogenesis in the testes, there is still much to understand about the mechanism of its toxicity. The ability of the adult liver to restore its function and mass after injury or extended resection is unique.[10] Considering the defence capacity of the body, the question then arises as to whether hepatic damage resulting from exposure to BLCO is transient or permanent.

*Correspondence to: Ebenezer O. Farombi, Drug Metabolism and Toxicology Research Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria.
E-mail: olatunde_farombi@yahoo.com; eo.farombi@mail.ui.edu.ng

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In taking up the present study as a result of our continued interest in BLCO toxicity, we attempt to determine the role played by hepatic antioxidant defence mechanism following withdrawal of BLCO-treatment from experimental rats. It seemed rational to investigate using lower doses of BLCO and longer duration than previously reported.

MATERIAL AND METHODS

Bonny light crude oil

Bonny light crude oil was obtained from the Nigerian National Petroleum Corporation, Ekpan, Warri, Delta State, Nigeria.

Chemicals

Glucose-6-phosphate, adenosine monophosphate, L-γ-glutamyl-3-carboxyl-4-nitronilide, glycylglycine, epinephrine, glutathione (GSH), 5, 5′-dithio-bis-2-nitrobenzoic acid, hydrogen peroxide (H_2O_2), thiobarbituric acid and 1-chloro-2, 4-dinitrobenzene were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade and obtained from the British Drug Houses (Poole, Dorset, UK). Kits for serum biochemistry were purchased from Randox Laboratory Limited, United Kingdom.

Experimental protocol. Forty-eight healthy adult male Wistar rats weighing approximately 175 ± 15 g obtained from the Department of Biochemistry, University of Ibadan, Nigeria, were randomly assigned to control, 50, 100 and 200 mg kg^{-1} groups of 12 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-h 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.[11]

Bonny light crude oil was dissolved in corn oil and administered orally at doses of 50, 100 and 200 mg kg^{-1} body weight per day for 21 days. Corresponding group of animals were administered with corn oil alone and served as control. One-half of the rats from each group were sacrificed on day 22, and the remaining half stayed for an additional 21 days without treatment before they were sacrificed. In each case, the livers were quickly removed, weighed and placed on an ice bath. The body weights of rats were taken before exposure to various treatments and prior to sacrifice.

Histopathology. Biopsies from each liver were fixed in 10% formalin and processed accordingly for histology. All slides were coded before examination with light microscope by investigators who were blinded to control and treatment groups.

Serum biochemistry. Serum activities of aspartate and alanine aminotransferases (AST and ALT) were determined by the method of Reitmann and Frankel.[12] The level of serum bilirubin was determined according to Jendrassik and Grof.[13]

Biochemical assay. Remaining portions of liver were homogenized in 50 mM Tris–HCl buffer (pH 7.4) containing 1.15% potassium chloride, and the homogenate was centrifuged at 10 000 g for 15 min at 4 °C. The supernatant was collected for the estimation of catalase (CAT) activity by using H_2O_2 as substrate according to the method of Clairborne.[14] Superoxide dismutase (SOD) was assayed by the method described by Misra and Fridovich.[15] Gamma-glutamyl transferase (GGT) was assayed using L-γ-glutamyl-3-carboxyl-4-nitronilide and glycylglycine as substrates according to the method of Szasz,[16] and protein concentration was determined by the method of Lowry *et al.*[17] Reduced GSH was determined at 412 nm by using the method described by Jollow *et al.*[18] H_2O_2 generation was assessed by the method of Wolff.[19] Glutathione-S-transferase (GST) was assayed by the method of Habig *et al.*[20] Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method described by Farombi *et al.*[21] and expressed as micromoles of MDA g^{-1} tissue.

Statistical analysis. All values are expressed as the mean ± standard deviation. Levels of statistical significance were analysed by ANOVA, followed by Student's *t*-test to compare the means between BLCO-treated groups and controls. Significance was set at $p < 0.05$ using the Student's *t*-test.

RESULTS

Body and organ weights and serum biochemical indices

Data on the body and organ weights and biochemical indices are presented in Table 1. There were neither abnormal

Table 1. Body and organ weights (g) of male rats sacrificed immediately after BLCO treatment and after the recovery period

	21-day treatment with BLCO				21-day withdrawal period			
Parameters	Control	50 mg kg^{-1}	100 mg kg^{-1}	200 mg kg^{-1}	Control	50 mg kg^{-1}	100 mg kg^{-1}	200 mg kg^{-1}
Final weight	194 ± 27	195 ± 24	192 ± 31	187 ± 30	232 ± 48	225 ± 21	220 ± 24	211 ± 25
Initial weight	174 ± 32	175 ± 41	176 ± 33	175 ± 31	194 ± 27	195 ± 24	192 ± 31	187 ± 30
Weight gain	20 ± 5	20 ± 7	16 ± 4	11 ± 2	38 ± 21	30 ± 4	28 ± 7	24 ± 6
Liver weight	3.57 ± 0.23	3.73 ± 0.44	4.25 ± 0.13	4.37 ± 0.16	4.64 ± 2.14	4.71 ± 1.48	4.88 ± 1.34	4.92 ± 1.68

BLCO, bonny light crude oil.

clinical signs nor changes in the behaviour of animals following 21 consecutive days of BLCO treatment. The table revealed a non-significant dose-dependent decrease in the final body weight as well as body weight gain in animals sacrificed immediately after BLCO treatment and those sacrificed after the recovery period at all dose levels when compared with respective control rats. Serum activities of AST and ALT were dose-dependently increased following exposure of rats to BLCO (Table 2). Whereas AST activity was significantly ($p < 0.05$) increased by 25%, 45% and 39%, ALT activity was significantly increased by 9%, 90% and 88% at 50, 100 and 200 mg kg^{-1}, respectively, when compared with the control. Serum bilirubin level was unaffected in all treated BLCO animals compared with the control values. In animals left to recover, whereas ALT activity was significantly ($p < 0.05$) increased, AST activity was unaffected in all treated animals when compared with the control animals. However, a significant increase in both total and conjugated bilirubin levels was observed following 21 days withdrawal of BLCO treatment, whereas the unconjugated bilirubin level was unaffected.

Hepatic antioxidant status. Table 3 shows the effect of BLCO on hepatic antioxidant status in rats. Following 21 days of BLCO exposure to animals, a significant ($p < 0.05$) dose-dependent increase in the activities of antioxidant enzymes SOD, CAT and GST were observed in the livers in all treated groups. Whereas the hepatic CAT activity increased by 11.2%, 15.5% and 17.8, SOD activity increased by 22.2%, 25.8% and 36.6%. Whereas we noted 7.4%, 20.9% and 19.8% increase in GST activity at doses of 50, 100 and 200 mg kg^{-1}, respectively, when compared with the control animals. In addition, the levels of GSH, H_2O_2 generation and MDA (an index of lipid peroxidation) were significantly ($p < 0.05$) increased in a dose-dependent manner following BLCO administration.

When compared with the control values, the percentage elevation in the levels of H_2O_2 generated were 12.7%, 38.4% and 40.6%; GSH by 100.7%, 123.8% and 132.8% and MDA by 26.8%, 29.8% and 35.2% above the control values at 200, 400 and 800 mg kg^{-1} doses, respectively. However, the activity of GGT decreased dose-dependently by 0.15%, 22.5% and 24.8% at 50, 100 and 200 mg kg^{-1} doses, respectively, when compared with control animals. In animals left to recover, activities of GGT and MDA were consistent with those of the animals sacrificed after 21 days of treatment (Figures 1 and 2). However, whereas a non-significant increase in H_2O_2 level and CAT activity were observed, activities of SOD and GST were significantly decreased following the 21-day withdrawal of BLCO treatment (Table 3).

Histopathology. Histological examination of the livers of animals sacrificed immediately after 21 days of BLCO intoxication revealed that BLCO at 50 mg kg^{-1} dose caused periportal necrosis and fibroplasias, 100 mg kg^{-1} dose resulted in central venous congestion with few numbers of neutrophils and 200 mg kg^{-1} dose caused necrosis, portal

Table 2. Bilirubin level in male rats sacrificed immediately after BLCO treatment and after the recovery period

	21-day treatment with BLCO				21-day withdrawal period			
Parameters	Control	50 mg kg^{-1}	100 mg kg^{-1}	200 mg kg^{-1}	Control	50 mg kg^{-1}	100 mg kg^{-1}	200 mg kg^{-1}
Total bilirubin	6.2 ± 1.8	9.3 ± 1.6	8.4 ± 1.8	8.7 ± 1.2	4.8 ±0.6	7.7 ± 0.4*	6.7 ± 0.6*	6.9 ± 0.5*
Conjugated bilirubin	4.7 ±1.7	8.0 ± 1.8	7.0 ± 3.5	7.3 ± 0.5	3.8 ± 0.5	6.9 ± 1.2*	6.0 ± 0.6*	6.2 ± 0.4*
Unconjugated bilirubin	1.5 ± 0.4	1.3 ± 1.2	1.4 ± 0.7	1.4 ± 0.9	1.0 ± 0.2	0.8 ± 0.1	0.7 ± 0.2	0.7 ± 0.1
ALT	51.7 ± 0.29	64.5 ± 3.54*	75.0 ± 5.6*	72.0 ± 2.51*	50.8 ± 2.5	55.5 ± 4.0*	57.3 ± 3.5*	57.3 ± 3.5*
AST	25.5 ± 4.27	27.67 ± 1.6	48.5 ± 4.9*	48.0 ± 2.0*	20.8 ± 2.9	20.8 ± 0.5	21.8 ± 2.2	22.0 ± 1.2

BLCO, bonny light crude oil; ALT, alanine aminotransferase; AST, aspartate aminotransferase.
The data are expressed as mean ± standard deviation of 12 animals.
*Values differ significantly from control ($p < 0.05$).

Table 3. Hepatic antioxidant status in male rats sacrificed immediately after BLCO treatment and after the recovery period

	21-day treatment with BLCO				21-day withdrawal period			
Parameters	Control	50 mg kg^{-1}	100 mg kg^{-1}	200 mg kg^{-1}	Control	50 mg kg^{-1}	100 mg kg^{-1}	200 mg/ kg^{-1}
SOD	1.52 ± 0.6	1.84 ± 0.2*	1.91 ± 0.1*	2.08 ± 0.2*	1.54 ± 0.6	1.25 ± 0.4*	1.19 ± 0.6*	1.17 ± 0.5*
CAT	10.1 ± 0.2	11.9 ± 0.3*	13.8 ± 0.5*	14.1 ± 0.5*	10.2 ± 0.5	10.3 ± 0.7	10.8 ± 0.6	10.7 ± 0.4
GST	1.55 ± 0.3	1.72 ± 0.1*	1.87 ± 0.6*	1.82 ± 0.2*	1.60 ± 0.9	1.32 ± 0.5*	1.15 ± 0.2*	1.10 ± 0.32*
GSH	10.3 ± 1.2	28.5 ± 2.4*	32.2 ± 2.1*	34.0 ± 2.5*	10.1 ± 2.5	12.5 ± 1.0*	15.3 ± 3.5*	16.6 ± 1.5*
H_2O_2	26.1 ± 3.4	32.3 ± 3.1	37.4 ± 2.7*	38.2 ± 4.9*	25.8 ± 2.2	26.3 ± 2.1	26.7 ± 3.2	27.1 ± 3.1

BLCO, bonny light crude oil. Superoxide dismutase (SOD) activity (units mg^{-1} protein), catalase (CAT) activity (μmoleH_2O_2 consumed min mg^{-1} protein), glutathione-S-transferase (GST) activity (μmole1-chloro-2,4-dinitrobenzene—GSH complex formed min mg^{-1} protein), glutathione (GSH) level (μmol g^{-1} tissue), hydrogen peroxide (H_2O_2) level (μmolH_2O_2 generated mg^{-1} protein). The data are expressed as mean ± standard deviation of 12 animals.
*Values differ significantly from control ($p < 0.05$).

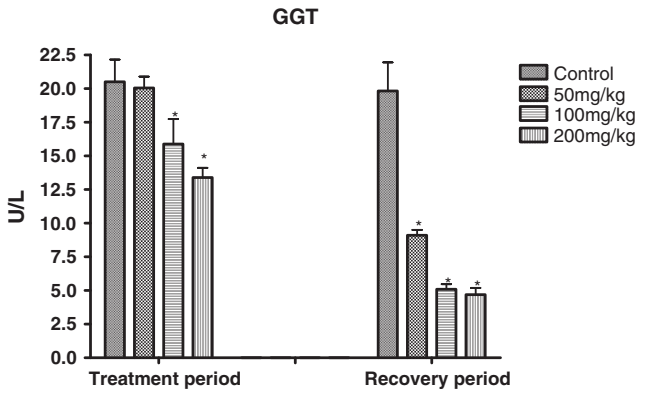

Figure 1. Effect of bonny light crude oil on the activity of gamma-glutamyltransferase (GGT) in the livers of treated and recovering rats

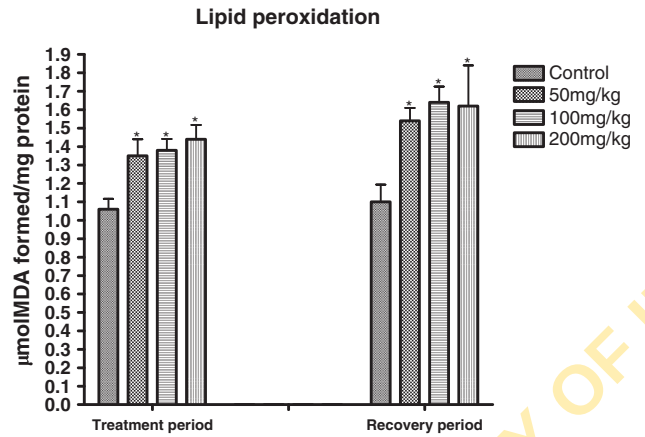

Figure 2. Effect of bonny light crude oil on the lipid peroxidation in the livers of treated and recovering rats

congestion and cellular infiltration by macrophages and neutrophils (Figure 3). The result from the recovery study was consistent with the result following the 21-day intoxication (Figure 4).

DISCUSSION

The severity of oxidative damage depends on the extent of disturbances in normal redox state within the cells. Although a cell can regain its original functional state after overcoming small perturbations, more severe oxidative stress can cause cell death and necrosis. To counteract the damaging effect of reactive oxygen species (ROS), aerobic cells are provided with extensive antioxidant defence mechanisms.[22] Endogenous antioxidant enzymes such as SOD, CAT and GST as well as non-enzymatic antioxidant GSH can limit the effects of ROS but quickly become overwhelmed by large quantities of ROS.

The present study demonstrated that administration of BLCO for 21 consecutive days resulted in a dose-dependent increase in the activities of SOD, CAT and GST. 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.[23] A simultaneous increase in CAT activity is essential for overall beneficial effect of increase in SOD activity.[24] GST is directly responsible for the elimination of electrophilic oxidants at the expense of GSH.[20] The dose-dependent induction of GST by ROS may represent an adaptive response to detoxify peroxide-containing metabolites, such as lipid and nucleic acid hydroperoxides and alkenals, generated during oxidative stress[25,26] and might be partly related to the increased availability of its substrate, GSH, observed in the present study. In addition, GSH plays an important role in protecting cells against oxidants that are produced during normal metabolism.[27] Elevated level of intracellular hepatic GSH concentration observed in the BLCO-treated rats indicates an adaptive response to reduce damage and promote better survival under the conditions of oxidative stress induced by BLCO treatment. BLCO has been shown to mediate the induction of GSH in testes and spermatozoa.[8]

Lipid peroxidation is a degenerative pathway of membrane components mediated through free radicals produced in the cell.[28] This reaction leads to the formation of MDA which is cytotoxic and mutagenic. Because a large proportion of crude oil components is lipophilic in nature, biological membranes may be the target sites where the adverse effect occur.[3,4] 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.[29] The sequential elevation in the H_2O_2 level along with increased MDA level observed in this study clearly indicates a state of stress in the liver possibly induced by BLCO or its metabolites.

Gamma-glutamyltransferase (GGT) is involved in the catabolism of GSH, the main thiol antioxidant in mammalian cells. It is the most sensitive enzymatic indicator of hepatobilliary diseases.[30] The decreased GGT activity in the livers of BLCO-treated rats observed in the present study might indicate its inactivation by ROS which may be responsible for the elevated GSH level. The decrease in GGT activity is in contrast with that of 7-day exposure.[8] However, the present result is consistent with Aslan *et al.*[31] who reported similar reduction in the activity of GGT in rat liver and attributed it to free radicals generated from the unsaturated chemical components of crude oil ingested through contaminated diet. The differences in these data may be due to the differences in treatment time. The increase in GGT activity reported after acute BLCO treatment may possibly indicate an initial adaptive response of the liver to the BLCO exposure but become overwhelmed by large quantities of ROS because of a longer period of exposure as observed in this study.

It is evident from the results of the present study that the applied dosages of BLCO did not affect both the body and

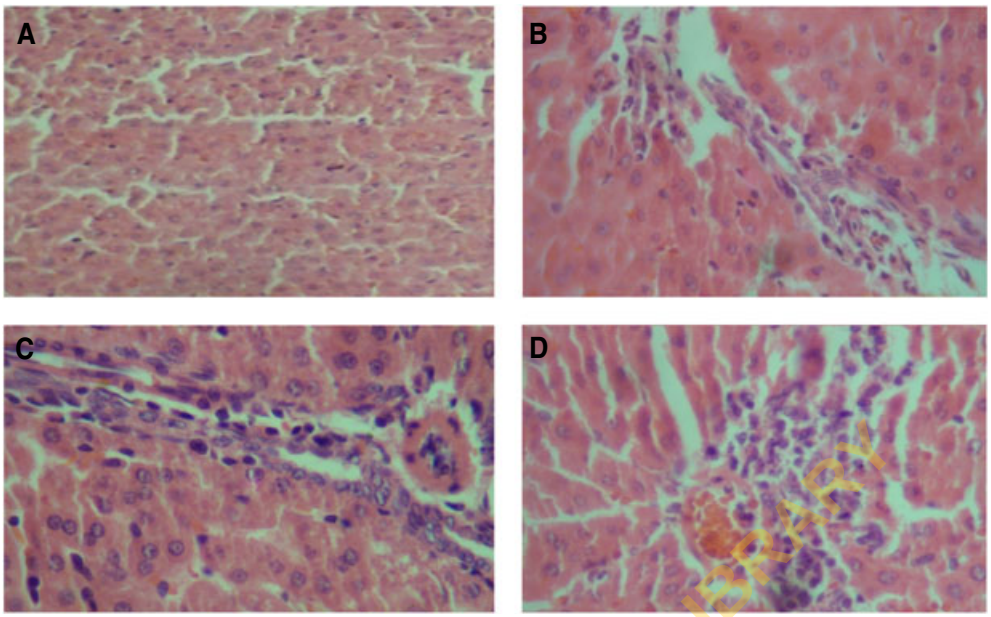

Figure 3. Histopathological alterations identified in the livers of rats treated with bonny light crude oil for 21 consecutive days. (A) Control liver with normal architecture. (B) 50 mg kg^{-1} treated liver showing periportal necrosis and fibroplasia. (C) Marked central venous congestion with a few number of neutrophils in livers treated with 100 mg kg^{-1} dose. (D) 200 mg kg^{-1} treated liver showing severe foci necrosis, portal congestion cellular infiltration by macrophages and neutrophils

liver weights. The liver damage was determined by measuring the levels of serum transaminases and bilirubin along with microscopic examination of the organ. Elevated levels of AST and ALT in circulation were indicative of a dose-dependent hepatic injury after 21 days of BLCO exposure which is well supported by the histopathological report. However, the levels of unconjugated, conjugated and total bilirubin levels remain unaffected, suggesting that erythrocyte degradation rate and liver conjugating function were not affected, although there was hepatic injury after 21 days of BLCO treatment.

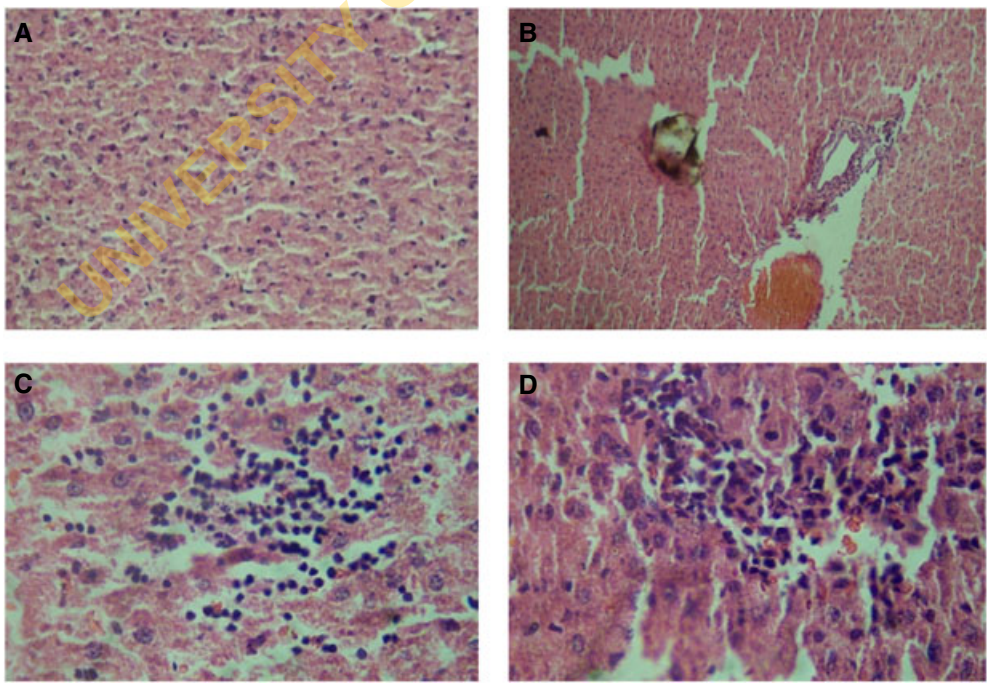

Figure 4. Histopathological alterations identified in the livers of rats sacrificed after the recovery period following bonny light crude oil treatment. (A) Control liver with normal architecture. (B) 50 mg kg^{-1} treated liver showing vacuolar degeneration and haemorrhage. (C) Cortical congestion and infiltration in liver treated with 100 mg kg^{-1} dose. (D) 200 mg kg^{-1} treated liver showing cortical congestion, cellular infiltration by macrophages and neutrophils with mild haemorrhage

It is interesting to discover that most of the above-mentioned parameters were consistent in animals from withdrawal experiment. Although we observed that whereas CAT activity and H_2O_2 level were completely reversed, activities of SOD and GST were significantly decreased after 21 days of BLCO treatment withdrawal. The low level of H_2O_2 might be due to decreased SOD activity which could have informed the normal CAT activity observed in the recovering animals. The decreased GST activity may lead to decreased protection against oxidants,[32] whereas the decrease in SOD activity in BLCO-treated animals suggests increased production of superoxide radical and enzyme inactivation.[8] Hepatic degeneration was accompanied with elevation in serum ALT activity, whereas serum AST activity was normal in recovering animals. In addition, the observed elevation in total and conjugated bilirubin levels without affecting the unconjugated bilirubin level suggests BLCO-induced post-hepatic toxicity possibly caused by an interruption to the drainage of bile in the biliary system after treatment withdrawal.

Taken together, the results suggest that induction of oxidative stress may be the initial mechanism of hepatotoxicity effect of BLCO, and this was not reversible upon withdrawal of treatment within the time course of investigation in male rats. To the best of our knowledge, the data presented are novel and have significant implications for human health problems especially if extrapolated to individuals using BLCO as folklore medicine.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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