Bull. Environ. Contam. Toxicol. (2004) 73:132–138

DOI: 10.1007/s00128-004-0404-2

Bulletin of Environmental Contamination and Toxicology

Morphological Alterations in Liver Tissues from Rabbits Exposed to Crude Oil Contaminated Diets

S. S. Ovuru,[1] D. N. Ezeasor[2]

[1] Department of Animal Science, Rivers State University of Science and Technology, PMB 5080, Port Harcourt, Nigeria
[2] Department of Veterinary Anatomy, University of Nigeria, Nsukka, Nigeria

Received: 20 January 2002/Accepted: 21 April 2004

Crude oil is a complex mixture of both organic and inorganic components. Polycyclic aromatic hydrocarbons, weathered crude oil and even the water soluble fractions are groups of toxic and mutagenic contaminants which are highly ubiquitous, hydrophobic and may bioaccumulate in lipoprotein membranes of both aquatic and terrestrial animals (Carls et al., 1999). Exposure of terrestrial animals to crude oil may result in sublethal effects, which include biochemical alterations, tetratogenic effects and reproductive impairment. Habitat requirement including contact with and ingestion of contaminated forage is likely the most important route by which terrestrial animals come in contact with crude oil.

In many developing petroleum-exporting countries, frequent spillages occur and are contained using cheap labour. Labour hands are exploited for abatement of spillages without protective devices (masks, gloves) and they may be exposed to crude oil by various routes. Hence, workers could be exposed via dermal contact and oral intake is possible should their contaminated hands come in contact with their mouths. Many studies have been done on the effects of crude oil on marine and terrestrial animals (Berepubo et al., 1994, Marty et al., 1999, Ngodigha et al., 1999). In all these works none considered biochemical serum and liver histopathological changes. In the present work, animals are exposed to crude oil through the oral route and clinicobiochemical/histological methods are employed to assess and aid in the diagnosis of crude oil exposure in terrestrial animals.

MATERIALS AND METHODS

Thirty-two New Zealand white breed rabbits weighing 1. 10kg - 1.42kg made up of 50:50 male/female ratio were procured. They were subjected to pre-experimental acclimatization for a period of two weeks. The animals were housed in hutches made of local materials (bamboo) at the Rivers State University of Science and Technology Teaching and Research Farm, Port Harcourt. The preconditioning also included medication against coccidiosis and also broad-spectrum antibiotics (tetracycline). They were fed forage prepared from

Correspondence to: S. S. Ovuru

grass and centro and supplemented with commercial feed (grower's mash) from Pfizer (Nigeria) Plc. Their drinking and feeding troughs were cleaned and continued hygienically throughout the preconditioning and study period. Crude oil was obtained from the Agip Terminal of the Nigerian Agip Oil Company Ltd. The oil was allowed to weather after being exposed for 24hrs in shallow pans to sunlight in order to allow the volatile fractions vaporise. The weathered crude oil was incorporated into the forage/hay mix and poultry feed by simple mixing and homogenization with a manual mixer.

At the start of experimentation, thirty rabbits of both sexes were randomly allocated in batches of six animals to five dietary groups of varying dose levels in two blocks of crude oil contamination as follows: Group A (control, 0.00% - no contamination); Group B (0.05% - crude oil contamination), Group C (0.10% crude oil contamination), Group D (0. 1 5% - crude oil contamination) and Group E (0.20% - crude oil contamination).

Animals were starved for 24 hours before being introduced to the experimental diets. Rabbits were exposed to the crude oil contaminated diet for 84days. Water was served ad-libitum. At the end of the experimental period, blood samples were obtained from two bucks and two does from each treatment group through the marginal car vein with a sterile disposable syringe and needle. Blood was transferred into plain tubes and centrifuged within lhour after collection. Serum was harvested and transferred to plastic tubes. The tubes were stored at -2O°C until ready for analysis. Analyses were completed within 14days after collection. AST, ALT and ALP activities were determined using standard methods. Total and conjugated bilirubins were measured using the Jendrassic and Grof method. The animals whose blood samples were taken were sacrificed and autopsied immediately. Liver tissues were obtained and fixed in 10% neutral buffered formalin and embedded in paraffin wax. The fixed tissues were sectioned to about 6µm thin layers and stained with haematoxylin and eosin (H&E), examined and photographed with a Zeiss photomicroscope III.

RESULTS AND DISCUSSION

Serial determination of biochemical parameters revealed a progressive increase in metabolites and indicator enzymes with increasing concentration of crude oil in the diets (Table 1). The mean concentration of total and conjugated bilirubin was zero in the controls and increased to 5.18 ± 0.28 µmol/l and 0.50 ± 0.29, µmol/l, respectively in the crude oil treated animals. The maximum contribution to this rise was unconjugated bilirubin. Gupta et al. (1988), working on the effects of nuvacron and furadan in mice observed a rise in total bilirubin. They pointed out that this rise indicated obstruction jaundice in the treated animals. However, in this study, elevated bilirubin levels could be due to hemolysis, which may have been

Table 1. Biochemical responses of rabbits exposed to crude oil contaminated diets

Trt group/contam. level	Total bilirubin *(μmol/L)* Mean ± SEM	Conjugated bilirubin *(μmol/L)* Mean ± SEM	Asparate transaminase *(μmol/L)* Mean ± SEM	Alanine transminase *(μmol/L)* Mean ± SEM
A: 0.00%	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	24.50 ± 4.17^{c}	19.25 ± 1.80^{e}
B: 0.05%	3.98 ± 0.49^{c}	0.00 ± 0.00^{c}	56.75 ± 5.27^{b}	39.00 ± 2.80^{d}
C: 0.10%	4.08 ± 2.35^{b}	0.00 ± 0.00^{c}	60.50 ± 9.61^{b}	47.00 ± 2.86^{c}
D: 0.15%	4.40 ± 0.87^{b}	0.43 ± 0.25^{b}	70.75 ± 19.28^{a}	73.75 ± 3.95^{a}
E: 0.20%	5.18 ± 0.28^{a}	0.50 ± 0.29^{a}	61.00 ± 3.58^{b}	56.00 ± 3.08^{b}

Within column, Mean ± SEM with different superscript(s) differ significantly ($P<0.05$)

caused by excessive rapid destruction of erythrocytes. This was evident by low RBC levels seen in the exposed animals (results no shown) and the absence of hemosiderin in the kupffer cells. Another reason for hyperbilirubinemia could also be defects in the transport of bilirubin from sinusoids into hepatocytes or defects in microsomal enzyme like glucoronyl transferase required in the conjugation of bilirubin. The inability of the liver to function properly may also be as a result of injury to the liver parenchyma (Henry, 1979). The activities of aspartate transaminase, alkaline transaminase and alkaline phosphatase also showed significant ($P<0.05$) elevation in treated as compared to control animals. Elevated ALT appear to reflect hepatic changes more specifically than ALT. In any case, elevated levels of ALT, AST and ALP together, may reflect some necro-inflamatory damage of liver (Henry, 1979). Besides, biochemical assay of ALT and AST provide a method for screening populations for potential liver necrosis caused by environmental toxins (Redlich et al., 1990). The observed rise in ALP activity could also be due to fatty degenerative changes in livers similar to that observed in chemically induced liver injury (Cullen and Ruebner, 1991; De Levee, 1994). With the observed elevation in AST, ALT and ALP activities, it is expected that there could be necrosis, inflammation and fatty degeneratiive changes in the exposed animals.

The biochemical changes observed were confirmed by histological studies. Liver tissues from the control animals showed normal arrangement of hepatocytes clear and visible sinusoids together with central vein as well as lymph vessel (Portal triad). All nuclei in the hepatocytes showed normal vesicular structure (Fig. 1a, Trt. 1). Fatty degenerative changes were observed in many of the crude oil treated animals (Fig. lb, Trt. 2, Fig. 2d, Trt. 4 and 5). The hepatocytes showed swellings with hypergranularity of the cytoplasm together with the formation of vacuoles in some cells. This posture is suggestive of cloudy and hydropic swelling in the

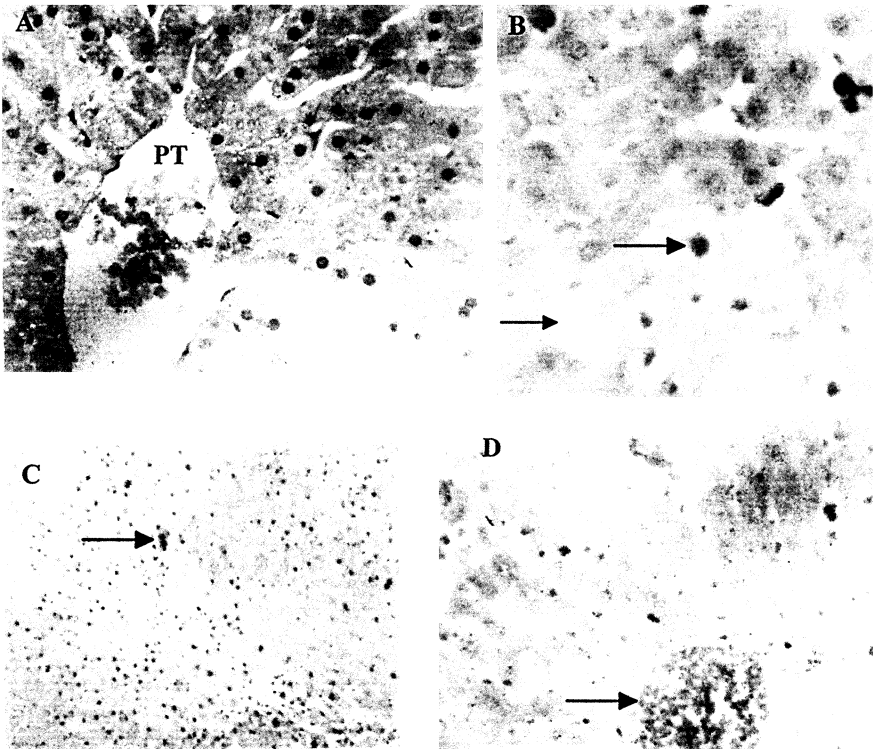

Figure 1. Effects of crude oil contaminated diets on liver tissues of rabbits.

(A) Control: Central vein, sinusoids and normal vesicular structure of nuclei (PT=Portal triad)
(B) Less severe fatty degeneration with some inflammatory cells
(C) Kupffer cells with dark pigmentation due to hemosiderosis
(D) Centrolobular necrosis and infiltration of inflammatory cells

hepatocytes. It further suggests excessive accumulation of tryglycerides within the hepatocytes, which may result from disturbances in the sequence from fatty acid entry to lipoprotein exit (Cortran et al, 1989).

These changes were rather a common observation in all crude oil treated animals. In some instances, the loss of high-energy phosphate could bring about a change in the permeability of plasma membrane of the cells. Accordingly, this situation may cause an influx of Ca++ into cells, which may result in changes in Na+ and K+ concentrations (Inns et al., 1990; Bright et al., 1991). The changes in electrolyte balance could cause swellings of the hepatocytes and inhibit RNA and protensyntheis (Hassan et al., 1991). These changes would eventually lead to marked elevated levels of liver tryglycerides, culminating in cell death.

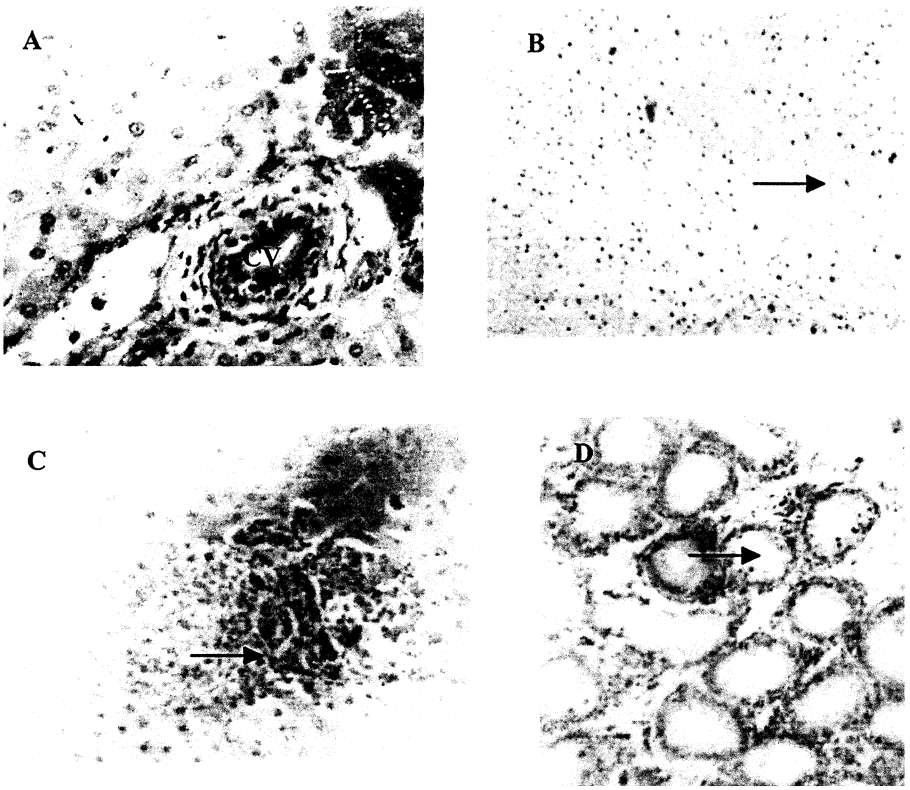

Figure 2. Effects of crude oil contaminated diets on liver tissues of rabbits.

(A) Congested central vein, and accumulation of inflammatory cells (CV=Central vein)
(B) Focal necrosis with infiltration of a variety of inflammatory cells
(C) Focal necrosis and infiltration of a variety of inflammatory cells
(D) Severe fatty degenerative change

Hemosiderosis of kupffer cells was noted in groups fed crude oil contaminated diets (Fig. lc, Trt. 3). Usually, hemosiderosis can be seen in physiological erythrocyte breakdown where hemosiderin could be found in mononuclear phagocytes of liver (Contran et al, 1989). Other conditions for hemosiderosis could be impaired utilization of dietary iron and in fasting and cachectic states. In any case, cachexia was observed in some of the animals with reduced feed intake (data not presented). Hemosiderosis in kupffer cells may be related to adverse hepatocyte function and/or malnutrition with the subsequent insufficient production of iron carrier protein (Transferin). Earlier reports concerning liver structure pigmentation were associated with hepatotoxins where besides liver morphological changes, a brownish pigmentation of kupffer cells representing at

least in part hemosiderin (Kimborough et al., 1972). Centrolobular necrosis (Fig. Id, Trt. 3), focal necrosis (Fig. 2c, Trt. 4, 5) infiltration of a variety of inflammatory and mononuclear cells (Fig. 2b Trt. 3) and congested central vein (Fig. 2a, Trt. 3) were observed in many animals in the crude oil treated group.

One important consequence of the observed sublethal effect of crude oil is hepatotoxicy. Although focal necrotic changes appear to be the dominant type of necrosis, centrolobular necrotic changes were common in most experimental animals. Many toxins cause centrolobular necrosis and crude oil seems to be in this category too. The findings in this study agree with the observations of Popp (1991) who reported that the distribution of the metabolising system in the liver results in a higher concentration of the toxicant in the centrolobular region which accounts for the frequency and occurrence of centrolubular toxicity resulting in centrolobular necrosis.

Around the necrotic foci, there was increased number of cells, which were considered local hepatic inflamationary reactions. Leukocytosis was also observed following treatment with crude oil. Similar observations were made in minks after treatment with PCB (Gilltte et al., 1987). These changes may affect health and well being of individuals in contact with crude oil and petroleum related products and hence may cause a decline in efficiency and loss of man-hour at work.

REFERENCES

Berepubo NA, Johnson NC, Bese BT (1994) Growth potential and organ weights of weaner rabbits exposed to crude oil contaminated feed. Int J Anim Sci 9: 73- 76.

Bright JE, Inns RH, Tuckwell NJ, Graffiths GD, Mars TC (1991) A histochemical study of changes observed in the mouse diaphragm after organophosphate poisoning. Human Exp Toxicol 10: 9-14.

Carls MG, Rice SD, Hose JE (1999) Sensitivity of fish embryos to weathered crude oil: Part 1. Low level exposure during incubation causes malformations, genetic damage and mortality in larval pacific herring (*Clupea pailasi*). Environ Toxicol Chern 1 8: 481- 493.

Cotran R, Kumar V, Robbins SL (1989) Cellular injury and adaptation. In: Robbins Pathologic Basis of Diseases. 4th ed. W. B. Saunders Co. Philadelphia p 1-3 8.

Culler JM, Ruebner BH (1991) A histopathologic classification of chemically induced injury of the liver. In: Meeks, RG, Harrison, SD, Bull, RJ (eds) Hepatotoxicology. Boca Raton, FL. CRC Press p. 67-92.

De Levee LD (1994) Dacarbazine toxicity in murine liver cells: A model of hepatic endothelial injury and glutathione defence. J Pharmacol Exp Therap 268: 1261-1270.

Gillette DM, Corey RD, Lowenstine LJ, Shull LR (1987) Comparative Toxicology of tetrachlorobiphenyls in minks and rats 11. Pathology Fund. Appl Toxicol 8:15-22.

Gupta M, Bagchi G, Sumitra D, Gupta RC, Mukhedee S, Roy A, Roy D (1988). Hepatorenal toxicity of nuvacron and furadan in mice. Indian J Exp Biol 26: 237-240.

Hassan MQ, Numan, II, AI-Nasiri N, Stoh SJ (1991) Endrine induced histophathological changes and lipid peroxidation in livers and kidneys of rats, mice, guinea pigs and hamsters. Toxicol Pathol 19: 108-114.

Henry JB (1979) Clinical Diagnosis and Management by Laboratory Methods. Todd, Sanford and Davidson (eds). p311-338. W.B. Saunders, Philadelphia.

Inns RM, Tuckwell NJ, Bright JE, Mars TC (1990) Histochemical demonstration of calcium accumulation in muscle fibres after experimental organophosphate poisoning. Human Exp Toxicol 9: 245-250.

Kimborough RD, Linder RE, Gaines TB (1972) Morphological changes in liver of rats fed polychlorinated biphenyl. Light microscopy and ultrastructure. Arch Environ Health 25: 354-364.

Marty GD, Okihiro, MS, Brown EO, Hanes D, Hinton DE (1999) Histopathology of adult pacific herring in Prince William Sound, Alaska, after the Exxon Valdez Oil Spill. Canadian J Fish Aqua Sci 56: 419-426.

Ngodigha EM, Olayimika FO, Oruwari BM, Ekweozor IKE, Wekhe SN (1999) Toxic effects of crude oil on organs and blood cells of West African Dwarf goat. Nigerian Vet J 20: 82-91.

Popp JA (1991) Hepatobiliary system In: Handbook of toxicologic pathology. Haschek, VM, Rousseaux, CG (eds). Academic Press Inc. New York. p. 279-314.

Redlich CA, West AB, Fleming L. (1990) Clinical and pathological characteristics of hepatotoxicity associated with occupational exposure to dimethylformamide. Gastroenterology 99: 748-757.