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Effects of Nigerian Qua Iboe Brent crude oil on rat spleen and haematological parameters

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ABSTRACT

This study investigated the consequences of exposure to crude oil on components of the immune system of male rats. A total of 40 male albino rats were used for the experiment. Exposure to crude oil was achieved by oral administration of increasing doses (low, medium and high) of Nigerian Qua Iboe Brent crude oil to the rats every other day for 4 weeks. Haematological parameters, relative weights and histomorphological features of the spleen of rats that received the crude oil were compared to control rats. The results showed that packed cell volume values ($P<0.05$), erythrocyte counts ($P<0.05$), absolute neutrophil counts ($P<0.01$) and absolute monocyte counts ($P<0.01$) were significantly reduced in crude oil-exposed rats. The total leukocyte counts ($P<0.05$) and absolute lymphocyte counts ($P<0.01$) were increased at the low dose of crude oil, but were subsequently reduced with increase in dose of crude oil. Splenic morphology was severely altered in rats that consumed crude oil. These findings suggest that exposure to Nigerian Qua Iboe Brent crude oil adversely affected the immune system of male rats. This implies possible immunosuppression for humans and other species exposed to this environmental pollutant.

Key words: environmental pollutant, Nigerian Qua Iboe Brent crude oil, haematological parameters, splenic morphology

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Introduction

Oil spills constitute a very important environmental pollutant with its significant threat to both terrestrial and marine wildlife (LEIGHTON, 1991). Following any oil spill, a number of simultaneous processes occur: spreading, dispersion, volatilization, evaporation, photo-oxidation, emulsification, sedimentation and biodegradation, which together determine the fate of the constituent hydrocarbons (NEFF, 1990). The toxicity of a petroleum fraction is related to its hydrophobicity (FREEDMAN, 1995) because lipid solubility is an important factor in the passage of petroleum components through the plasma membrane of cells, as well as the degree of membrane disruption.

The negative consequences of petroleum hydrocarbon toxicosis have been documented in fish (TAHIR et al., 1993; ALKINDI et al., 1996), and many aquatic avian species (BRIGGS et al., 1996; NEWMAN et al., 2000). It is apparent that there are differing species' susceptibilities to petroleum toxicosis. Haematological parameters of fish exposed to crude oil have been investigated by various authors, but the reports have varied greatly (DAVIDSON et al., 1993; TAHIR et al., 1993). Similarly, the effect of crude oil on avian haematological indices has been a subject of controversy among researchers (LEIGHTON, 1985; FRY and LOWENSTINE, 1985).

Contamination with crude oil is not limited to the marine environment. Crude oil pollution at drilling sites and oil spills on lands used for agricultural purposes, as well as petroleum- or diesel-contaminated wastes poses serious exposure risks to occupational public, terrestrial wildlife mammals and livestock raised on these lands. Furthermore, some components of petroleum have the potential to bioaccummulate within susceptible aquatic organisms and thus are passed by trophic transfer to other levels in the food chain (GARDNER et al., 1991). Despite these risks, very limited information is available on the negative consequences of crude oil toxicity in mammalian species.

The present study was therefore designed to investigate the effect of Nigerian Qua Iboe Brent crude oil on splenic morphology and haematological parameters of male rats. It seeks to elucidate the consequences of exposure to crude oil on components of the immune system of the body.

Materials and methods

Experimental animals. Forty male albino rats with masses of between 135 to 150 g. were used for the study and were housed in cages with an aluminium bottom and a screen top. They were maintained on a commercially prepared diet and allowed access to drinking water *ad libitum* throughout the period of the study. The rats were randomly assigned to 4 groups (N = 10). No crude oil was given to rats of the control group. Rats of the test groups received Nigerian Qua Iboe Brent crude oil orally every other day. On

each treatment day, the low-dose group received 0.1 mL of crude oil per rat, the medium-dose group received 0.2 mL of crude oil per rat, and the high-dose group received 0.4 mL of crude oil per rat. At the end of 4 weeks of treatment with crude oil, each rat was sacrificed by cervical dislocation. Prior to sacrificing each rat, the live body mass was determined and blood was collected from the orbital sinus into EDTA-containing bijou bottles for haematological analyses.

Quantitative measurements. Following death, spleens were collected from rats in the control and test groups. These were cleared of surrounding fat and weighed. Spleen mass was assessed relative to animal live body mass (gram spleen mass per gram live body mass × 100).

Histological preparations. Spleen samples taken from control and test group rats were fixed by immersion in Bouin's fluid for 48 hours. These were then dehydrated in graded ethanol, cleared in xylene and embedded in paraffin wax. Sections 5 μm thick were cut, mounted on glass slides and stained with haematoxylin and eosin for light microscopy.

Haematological analyses. Packed cell volume (PCV) was determined by micro-haematocrit centrifugation (JAIN, 1986). Values were expressed in percentages. Erythrocyte counts were carried out according to the methods of COLES (1986). Four mL of erythrocyte diluting fluid was used to dilute 0.02 mL of the blood. A Neubauer chamber was charged with the diluted blood and examined under a light microscope. The cells were counted using a tally counter. The figure obtained was multiplied by a factor of 10,000 to give the total number of erythrocytes per microlitre of blood. The total leukocyte count was carried out by diluting 0.02 mL of blood with 0.38 mL of leukocyte diluting fluid. The Neubauer chamber was charged with the diluted blood and the leukocytes were counted under a light microscope. The figure obtained was multiplied by a factor of 50 to give the total number of leukocytes per microlitre of blood.

Thin smears stained with Leishman's stain were prepared for the differential leukocyte counts. The percentage occurrence of differential leukocytes (neutrophils, basophils, eosinophils, lymphocytes and monocytes) was determined by counting under a light microscope. The results were converted to absolute figures using the formula: occurrence per 100 cells × total leukocyte count.

Statistical analyses. Mean and standard error were calculated for each group of measurements. Data was analyzed statistically using Analysis of Variance (Analysis Toolpak, Microsoft Excel 2000).

Results

Table 1. Comparison of haematological parameters and relative mass of spleens of crude oil-exposed male rats and controls

Parameter	Control	Low dose 0.1 mL/48h	Medium dose 0.2 mL/48h	High dose 0.4 L/48h	Level of P
Packed cell volume (%)	50.5 ± 0.89^{b}	47.30 ± 1.11^{a}	48.02 ± 1.53^{ab}	46.75 ± 1.42^{a}	ab: P<0.05
Erythrocyte counts (10^6 µL)	7.89 ± 0.12^{b}	7.44 ± 0.33^{ab}	7.38 ± 0.38^{ab}	6.82 ± 0.42^{a}	ab: P<0.05
Total leukocyte counts (10^3 µL)	12.94 ± 1.27^{b}	22.48 ± 3.03^{a}	17.37 ± 3.08^{ab}	9.90 ± 0.93^{bc}	ab: P<0.05 ac: P<0.05
Absolute lymphocyte counts (10^3 µL)	5.62 ± 0.72^{c}	19.84 ± 3.08^{a}	15.59 ± 2.82^{a}	9.29 ± 0.98^{b}	ab: P<0.05 ac: P<0.01 bc: P<0.01
Absolute neutrophil counts (10^3 µL)	6.33 ± 0.60^{b}	1.82 ± 0.70^{a}	1.83 ± 0.45^{a}	0.40 ± 0.17^{a}	ab: P<0.01
Absolute monocyte counts (10^3 µL)	0.74 ± 0.16^{b}	0.44 ± 0.25^{ab}	0.17 ± 0.07^{a}	0.04 ± 0.02^{a}	ab: P<0.01
Absolute basophil counts (10^3 µL)	0.09 ± 0.03	0.05 ± 0.03	0.03 ± 0.03	0.01 ± 0.01	P>0.05
Absolute eosinophil counts (10^3 µL)	0.18 ± 0.07	0.17 ± 0.13	0.00 ± 0.00	0.04 ± 0.02	P>0.05
Relative mass of spleen (%)	0.29 ± 0.02^{b}	0.41 ± 0.03^{a}	0.37 ± 0.02^{a}	0.44 ± 0.04^{a}	ab: P<0.05

Values are presented as mean ± standard error. Different superscripts in a row indicate significant variation at the specified levels of probability.

Haematological parameters. The effect of exposure to increasing doses of Nigerian Qua Iboe Brent crude oil on haematological parameters of male rats is presented in Table 1. Both the low and high doses significantly reduced the packed cell volume relative to the control (P<0.05). The erythrocyte count was significantly reduced at the high dose relative to the control (P<0.05). The low dose significantly increased the total leukocyte count relative to the control (P<0.05). However, the total leukocyte count decreased with increase in dose of crude oil. The high-dose group had a significantly reduced total leukocyte count relative to the low-dose group (P<0.05). Absolute lymphocyte counts were significantly increased in the low-dose, medium-dose and high-dose groups relative to the control group (P<0.01). However, increase in the dose of crude oil resulted in reduction of the absolute lymphocyte counts. The high-dose group had a significantly

reduced absolute lymphocyte count relative to the low-dose and medium-dose groups ($P<0.05$). Absolute neutrophil counts were significantly reduced in the three groups of rats exposed to crude oil relative to the control group ($P<0.01$). The medium and high doses significantly reduced the absolute monocyte counts relative to the control ($P<0.01$). There were no significant differences in the absolute basophil counts or absolute eosinophil counts among the four groups of rats ($P>0.05$).

Relative mass of spleen. The mean relative mass of spleen was significantly increased in the low-dose, medium-dose and high-dose groups relative to the control group ($P<0.05$) (Table 1).

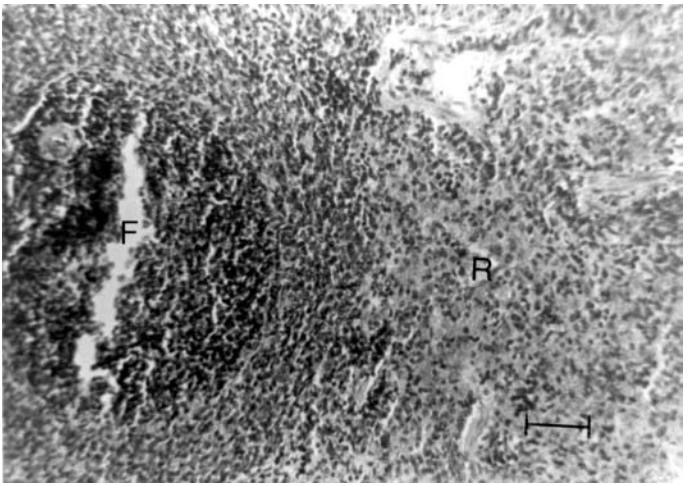

Fig. 1a. Spleen section from control rat showing fully developed splenic follicle (F) and red pulp (R). Scale bar = 40 μm.

Histopathology. Spleen sections taken from rats of the control group showed clearly discernible red pulp and splenic follicles that were not reactive (Fig. 1a). Samples of spleen from the crude oil-treated rats were moderately to severely hyperaemic, with mild deposition of haemosiderin pigments. The splenic follicles were reactive, and depopulation of these follicles was apparent (Fig. 1b). These pathological lesions increased in magnitude with increase in the dose of the crude oil. The high dose produced moderate oedematous distension of the red pulp and splenic follicles, haemosiderosis, increased lymphopoiesis and severe necrosis of mature lymphocytes (Fig. 1c).

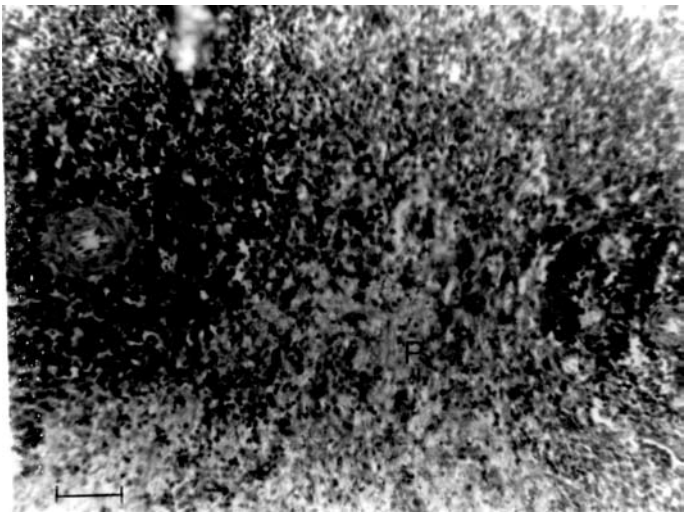

Fig. 1b. Spleen section from rat exposed to the low dose of crude oil. The splenic follicle (F) is mildly depopulated and the pulp (P) is moderately hyperaemic. Scale bar = 40 μm.

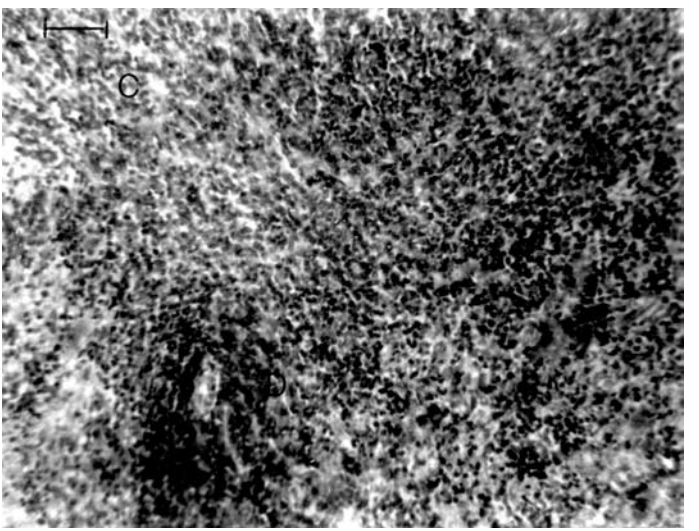

Fig. 1c. Spleen section from rat exposed to the high dose of crude oil. Note oedema/congestion (C) of the pulp and severe depopulation (D) of the spleen follicle. Scale bar = 40 μm.

Discussion

Haematological analyses, which include packed cell volume, erythrocyte counts, total leukocyte counts and differential leukocyte counts, provide information about the haematopoietic system and immunological responses. These blood tests can serve as diagnostic adjuncts in the development of a presumptive of definitive diagnosis (CAMPBELL, 1995). Significant dose-dependent reduction in the packed cell volume values of rats that consumed graded doses of crude oil was observed in this study. This suggests that treatment of rats with Nigerian Qua Iboe Brent crude oil may be associated with anaemia. Haemolytic effects of crude oil have been reported previously (LEIGHTON et al., 1985), and haemolytic anaemia was an important consideration in the survival of oiled birds (BRIGGS et al., 1996). Exposure to graded doses of crude oil in this study caused increased extravascular haemolysis in the spleen, with excessive deposition of the yellow-brown pigment, haemosiderin, in this organ. The haemolysis was probably due to sequestration of erythrocytes in the splenic sinusoids.

The present study demonstrated a significant increase in the total leukocyte count in rats that consumed the low dose of crude oil. Subsequently, the total leukocyte counts were significantly reduced in the medium and high-dose groups relative to the low-dose group. This may be an indication that an initial proliferative response by the immune cells was followed by immunosuppression. The immune system is synonymous with circulating leukocytes, all of which derive from a single precursor, the pluripotential haemopoietic stem cell (SCOTT and GORDON, 1995). It has been shown that immunodeficiency often follows contact with toxicants (ROCKE and SAMUEL, 1991). Experimentation with petroleum ingestion in mallard ducks, as well as evidence from seabird rehabilitation centres (LEIGHTON, 1986; McORIST and LENGHAUS, 1992) support the observation that exposure to crude oil gives rise to deficient immune systems.

In this study, the number of circulating lymphocytes was significantly increased in all the groups that received crude oil, relative to the control group. However, increase in the dose of crude oil significantly reduced the number of lymphocytes in the high-dose group relative to the low and medium-dose groups. The primary function of B lymphocytes is to produce antibodies. The proliferation of lymphocytes following crude oil consumption may be related to the response of the immune system to the presence of the toxicant. The splenic morphology of rats that received crude oil showed reactive splenic follicles, with increased lymphopoiesis. The apparent depopulation of these follicles may be related to the mobilization of the lymphocytes into the blood stream in response to the presence of the crude oil. Subsequent reduction in number of lymphocytes with increase in the dose of crude oil may indicate a gradual suppression of the humoral immune mechanisms by the toxicant. This suggestion is supported by the observed severe necrosis of mature lymphocytes in the spleen of rats that received the high dose of crude oil.

The number of circulating neutrophils and monocytes was significantly reduced in rats that received the crude oil. This reduction in number of neutrophils and monocytes was

found to be dose-dependent. Circulating monocytes are precursors of tissue macrophages (JUNQUEIRA et al., 1998). Macrophages and neutrophils are functionally similar cell types (GORDON et al., 1995). Both cells participate in the destruction of pathogens through phagocytic uptake, intracellular and extracellular enzymatic degradation of toxic substances. A decline in the number of these cells may depress the ability of the affected animals to phagocytize bacterial pathogens and thereby increase their susceptibility to infection (EPPLEY, 1992). It has been reported that crude oil consumption resulted in lowered resistance to *Pasturella multocida* infection among mallard ducks (ROCKE et al., 1984).

In conclusion, our study has demonstrated that exposure to Nigerian Qua Iboe Brent crude oil adversely affected components of the immune system of male rats. This may imply possible immunosuppression for humans and other species that may be exposed to this environmental pollutant.

Formal statement. The authors wish to state that the rats used in the course of this study were handled in accordance with the guidelines for the protection of animal welfare in the University of Nigeria Nsukka.

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SAŽETAK

Istraživanje je provedeno da bi se ustanovio učinak sirove nafte na imunosni sustav štakora. U pokus je bilo uzeto ukupno 40 mužjaka albino štakora. U razdoblju od četiri tjedna svi su štakori peroralno dobivali sirovu naftu Nigerian Qua Iboe Brent na način da im je svaki drugi dan povećavana doza (od najmanje preko srednje do najveće). Uspoređeni su hematološki pokazatelji, relativne mase i histomorfološka obilježja slezena pokusnih i kontrolnih štakora. Ustanovljene su smanjene vrijednosti ukupnoga obujma krvnih stanica ($P<0,05$), broja eritrocita ($P<0,05$), apsolutnoga broja neutrofila ($P<0,01$) i apsolutnoga broja monocita ($P<0,01$) u štakora izloženih sirovoj nafti. Ukupan broj leukocita ($P<0,05$) i apsolutni broj limfocita ($P<0,01$) bio je povećan tijekom razdoblja u kojem su štakori dobivali male doze sirove nafte, ali je bio smanjen s povećanjem doze. Morfologija slezene ozbiljno je bila poremećena u štakora koji su dobivali sirovu naftu. Nalazi upućuju na zaključak da izlaganje nigerijskoj Qua Iboe Brent sirovoj nafti nepovoljno utječe na imunosni sustav štakora. To ukazuje i na mogući imunosupresijski učinak na čovjeka i druge vrste izložene ovom zagađivaču okoliša.

Ključne riječi: zagađivač okoliša, nigerijska Qua Iboe Brent sirova nafta, hematološki pokazatelji, morfologija slezene