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Effects of Crude Oil Contaminated Water On Haematocrit And Histopathology of Guinea Pig: Animal Model for Investigating Crude Oil Pollution

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ABSTRACT

Twenty-four guinea pigs (*Caria porcellus*) obtained from the Animal House of College of Health Sciences, University of Port-Harcourt, Nigeria, were weighed individually and divided into six groups of four per group. They were allowed access to rat feed and tap water *ad libitum* for two weeks acclimatization. Different concentrations of crude oil (5%, 10%, 15%, 20%, 25%) were mixed with tap water and served to five experimental groups. The sixth group received 100% tap water and served as control. The study lasted for 28 days after which the following parameters were monitored in each guinea pig: appetite, body weight, packed cell volume (PCV) and the lung histopathology. Results showed loss of appetite, dull and disoriented conditions in animals with contaminated water. Microscopic examination of the lungs showed that effects of crude oil contaminated water increased with the concentration of crude oil. There was a progressive reduction in the value of Haematocrit in the experimental groups compared to the control groups. The results of the present study suggest that crude oil is toxic to the experimental animals.

Keywords: Crude oil, Haematocrit, Histopathology, Guinea pig.

Industrialization through exploitation and exploration of hydrocarbon has introduced into the ecosystem substances that are potentially toxic to life and the environment (Dede and Kagbo 2002). These toxic substances tend to disturb the delicate ecological balance. Nigeria is an oil producing country; therefore, ecological accidents related to oil exploration and exploitation readily occur. Unfortunately, the immediate and long-term effects of these accidents on the ecology have not been extensively investigated (Dede et al 2002, Dede and Kagbo 2002).

Crude oil is a complex mixture of a wide range of hydrocarbon fractions with such compounds as sulphur, oxygen, nitrogen and a range of metals and salts in smaller quantities. The hydrocarbon fractions include straight or branched alkanes, cyclo-alkane aromatic hydrocarbon and tetracyclic compounds, (Chililingerian and Yen 1987). The contents of crude oil are responsible for its toxicity. This however depends on some other factors including quality, degree of pollution, duration of exposure, the state of the crude oil i.e. whether fresh or refined products; the habitat impacted and the subject organisms involved (Dede and Kagbo 2002).

In addition, a variety of other toxic compounds are typically generated during oil exploration and exploitation. These include polycyclic aromatic hydrocarbons e.g. benzyl-pyrene aromatic hydrocarbons and related organic compounds and their derivatives. These toxic substances are in addition carcinogenic and pose serious threat to human health (WHO 1997). In humans, repeated or prolonged contact with crude oil has been reported to cause skin loss, dryness, cracking, changes in skin pigmentation, hypokeratinization, pigmented plane warts and eczematous reaction, while prolonged exposure to constituents of crude oil, such as benzopyrene and other hydrocarbons can result in dermal neoplasm (Hanbrough 1985).

Studies and interviews cited in a report by the Natural Resources Defense Council, found high rates of child malnutrition and mortality, increases in birth defects and rashes in areas impacted by oil development (Kimerling 1991).

Furthermore, Nwankwoala (2000) reported that oral administration of Nigerian crude oil on Albino Wister rats caused the rats to loose weight and appetite and become weak. Also pregnant rats aborted their fetuses after ten days of gestation. Histopathological examination of the lungs and kidney of Albino Wister rats following a single oral diesel fuel administration showed black deposits and inflammation in the pulmonary interstitial tissue and necrosis of the kidney (Dede et al 2000).

Nigeria is about the fifth largest oil-producing country in the World. Spillages of crude oil often occur during transportation of crude across the land and waterways. The current study was therefore aimed at investigating the effects of crude oil contaminated water on certain parameters of the Guinea pig, an animal model for investigating the effects crude oil contaminated water in man.

MATERIAL AND METHOD

Twenty-four Guinea pigs (*Caria porcellus*) of average body weight and of mixed sexes obtained from the Animal house of the College of Health Sciences University of Port-Harcourt were used for the current study. The animals were incubated in metabolic cages for two weeks for acclimatization. The animals were later divided into six groups of four per group. All the animals were fed *ad libitum* with animal feed (Pfizer Nigeria Ltd).

The current study was allowed to run for 28 days as follows: Group one (control) received tap water. Group two received tap water contaminated with 5% crude oil, group three received tap water contaminated with 10% crude oil, group four received tap water contaminated with 15% crude oil, group five received tap water contaminated with 20% crude oil while group six received tap water contaminated with 25% crude oil. The different concentrations of crude oil were prepared by adding appropriate quantity of crude oil to tap water.

The following parameters were then monitored body weight, packed cell volume (PCV) and the lung histopathology in the experimental guinea pigs and compared with control guinea pigs. The mode of administration of the water was orally with a syringe 8 hourly for 28 days. Results were monitored at 0,7,14, 21 and 28 days periods as indicated in the results. At the end of these days guinea pigs were sacrificed and the lungs were fixed and prepared for histological examination. The blood specimens were collected from the hearts by direct cardiac puncture for determination of packed cell volume (PCV). The packed cell volume was determined using Hawksley micro-capillary tubes centrifuged at 3000 r.p.m for 5 minutes. The different concentrations of crude oil were prepared by adding appropriate quantity of crude oil to tap water as shown in Table 1.

In group D two guinea pigs died at the end of the first week and two in the third week respectively, while in group F, three guinea pigs also died before the of the experiment: one at the end of first week, one at the third week and one at the fourth week. Their lungs were however disserted out and placed in 10% normal saline for fixation. Tissue (lung) preparation went through the normal and careful procedure of fixation, dehydration, clearing, impregnating/infiltration, embedding, cutting of sections, staining, viewing under the microscope and photographing.

RESULTS

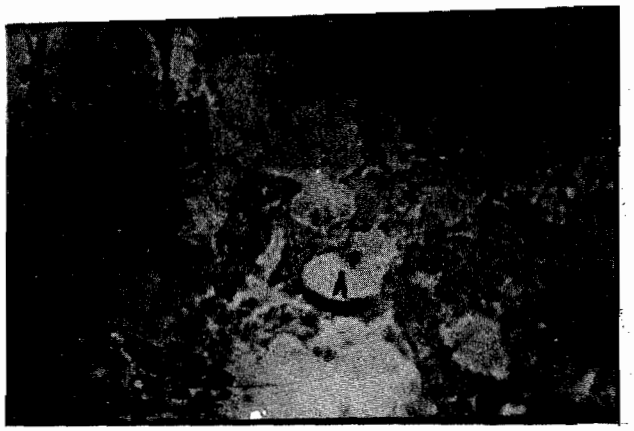
The results obtained in this study are described under four headings: General effects, gross anatomical observations, haematological effects and microscopic effects on the lungs.

TABLE 1: Effect of Crude Oil Contaminated Water On body Weight Of Guinea Pigs.

DAYS	GROUP A (CONTROL)	GROUP B 5%	GROUP C 10%	GROUP D 15%	GROUP E 20%	GROUP F 25%
0	0.525 ±0.2	0.275±0.3	0.450±0.2	0.425±0.2	0.350±0.2	0.475±0.3
7	0.53±0.2	0.250±0.3	0.325±0.3	0.400±0.1	0.325±0.2	0.450±0.2
14	0.550±0.2	0.275±0.3	0.375±0.2	0.375±0.2	0.475±0.2	0.450±0.4
21	0.550±0.2	0.250±0.2	0.325±0.3	0.350±0.3	0.450±0.2	0.400±0.2
28	0.550±0.2	0.375±0.2	0.400±0.2	-	0.425±0.2	-

Table 2: Packed Cell Volume (PVC) Of The Guinea Pigs

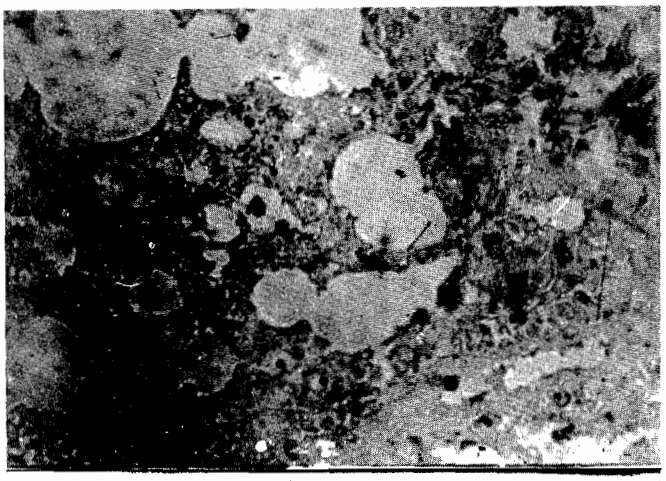
DAYS OF SACRIFICE	GROUP A (CONTROL)	GROUP B	GROUP C	GROUP D	GROUP E	GROUP F
0	25%	25%	26%	27%	28%	26%
7	15%	21%	22%	24%	21%	20%
14	21%	20%	20%	22%	20%	19%
21	17%	20%	18%	-	19%	-
28	-	-	18%	20%	18%	-



A. (control group) (X250).

B. (treated group) (x 400)

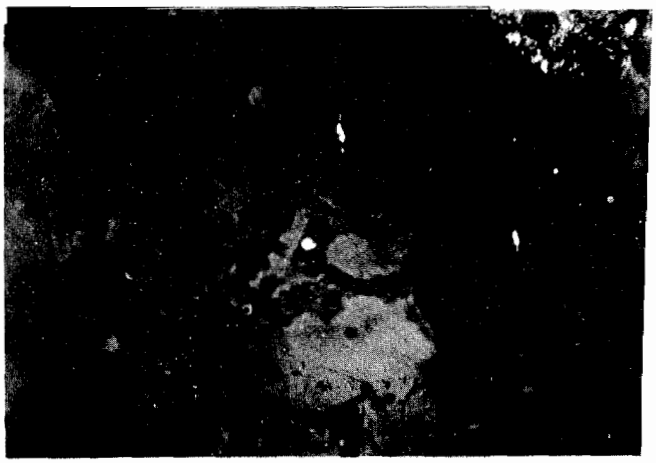
Fig. 1 Micrographs above indicates Alveolar spaces (A) and Alveolar ducts (v). These features are more prominent at a magnification of (X400), so in succeeding Micrographs (X400) Magnification will be used.



A.(control group).

B. (treated group)

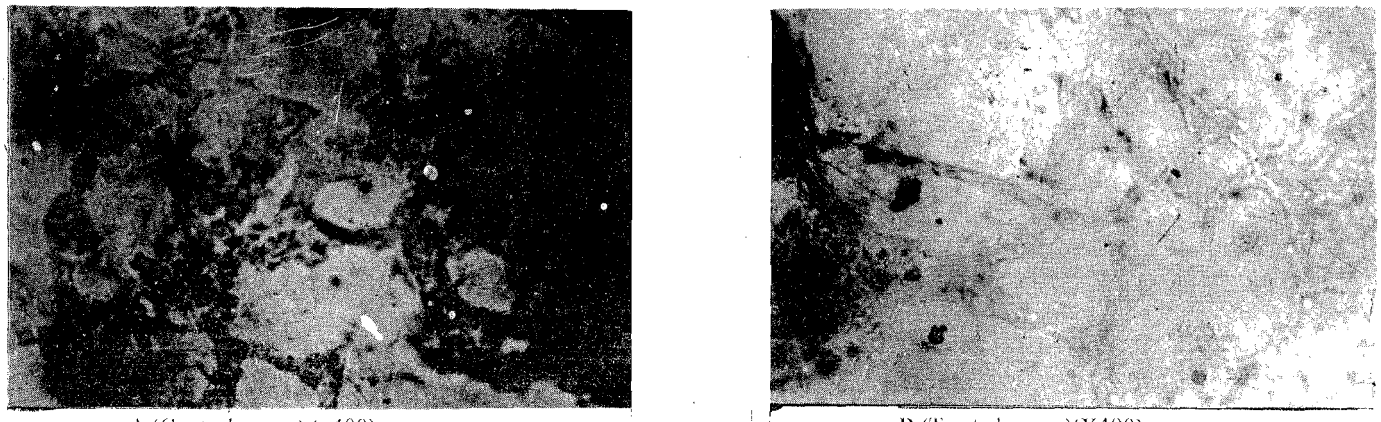
Fig. 2: Micrographs above indicates Alveolar spaces (A). There is widened Pulmonary Interstitium (I) with oedema in Group B, as compared to control.



A.(Control group)

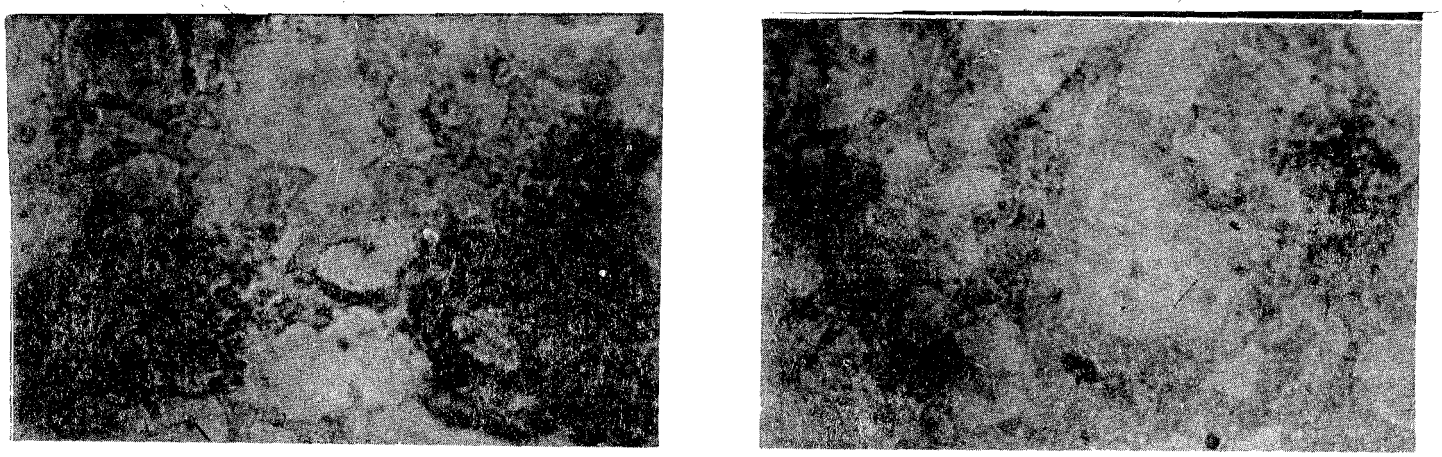
(Treated group)

Fig. 3: The Micrograph of Group C shows reduced Alveolar spaces (A) and more widened Pulmonary interstitium (I) with oedema, as compared to control.



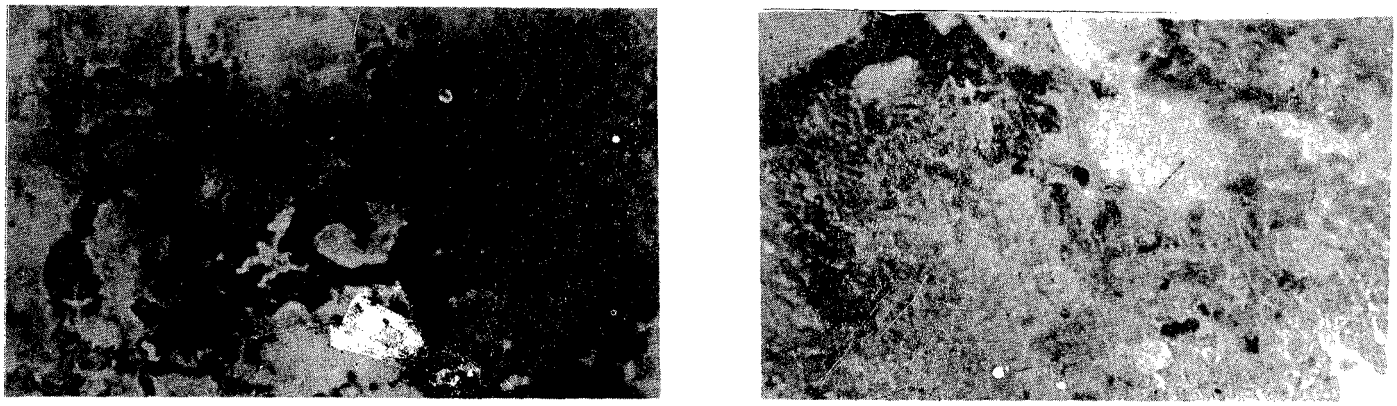
A.(Control group) (x400) B.(Treated group)(X400)

Fig.4: The Micrograph of Group D shows haemorrhage into pulmonary interstitium indicated by arrows, as compared to control.



A.(Control group) (X400) B.(Treated group) (X400)

Fig. 5: The Micrograph of Group E shows more severe haemorrhage (A) into the pulmonary interstitium, as compared to control.



A. (Control group) (X400) B.(Treated group) (X400)

Fig. 6: The Micrograph of Group F shows more haemorrhage (H) into the pulmonary interstitium, and destruction (D) of the alveolar spaces, as compared to control.

General Effects:

On the fourth day following administration of crude oil solution, the guinea pigs especially in group D to F became dull and disoriented. There was significant loss of appetite, loss of body weight (Table 2) and death of some guinea pigs. Two guinea pigs died before the end of the first week an indication that toxicity of crude oil is directly related to the concentration of the crude oil in the solution.

Cross-Anatomical Observations:

The lungs obtained from the four control guinea pigs showed no differences in their normal gross anatomical features, i.e. size, color and consistency. The lungs of the experimental group (B to F) were slightly larger in size but softer in consistency. They were dark in color with the presence of dark carbon-like deposits on them especially on 28th day. This effect was most pronounced in group F pigs.

Haematological Effects:

The blood samples were analyzed for PCV and the results obtained are shown in Table 3. It was clear from our results that PCV decreased in the experimental groups as the study progressed thus suggesting that crude oil is toxic to the haemopoetic system.

Microscopic Examination Of The Lungs:

The effect of crude oil contaminated water on the lungs also manifested at the microscopic level and the effects increased with increased concentration of crude oil contaminated solution and increased duration of feeding. The micrograph of control group (Fig. 1) shows normal lung architecture with the usual alveolar spaces, alveolar ducts and inter-alveolar spaces.

In group B (Fig. 2) there was minimal widening of the inter-alveolar spaces probably due to edema. We also observed infiltration by inflammatory cells not observed in control group.

In group C (Fig. 3) we observed few alveolar spaces due to massive collapse of these spaces as the alveolar spaces widened with increased edema. Infiltration of chronic inflammatory cells increased.

In group D (Fig.4) we noticed actual hemorrhage and widened inter-alveolar spaces.

In group F (Fig. 5) Most alveolar spaces and ducts have collapsed, more severe hemorrhage and chronic inflammatory cell infiltration have occurred.

DISCUSSION

The results obtained in the present experiment are indicative of the fact that crude oil contaminated water and by extension the environment has negative and toxic effect on the animal population. After a few days of the experiment crude oil induced a loss of appetite on the experimental animals, the animals ate less and thus lost weight. It is also possible that other factors are involved in bringing about the loss of weight in the animals; for example, crude oil in the intestines and stomach could affect absorption of substances. This effect needs possible further investigation. In 28 days only, the death of five guinea pigs was quite high and this could have resulted from the direct lethal toxicity of crude oil as observed at the microscopic level or from sub-lethal disruption of physiological and behavioural activities following the entry of hydrocarbon constituents into the food web (Akpofure 2000).

The effect of crude oil was manifested at the microscopic level of the tissue (lung) examined. The architectural alterations observed could have come about as a result of change in permeability of the cells of the lung and those of the blood vessels of both the bronchial, arterial and pulmonary systems resulting in edema, hemorrhage, inflammatory and subsequent migration of chronic inflammatory cells. The leakage of large quantities of fluid and/or blood into the inter-alveolar spaces resulted in massive collapse of alveolar spaces due to compression. Gradual reduction in packed cell volume was observed and this heightened as the concentration of crude oil and days of experiment increased. The mechanism for the production of this anemia is not yet well understood but it may be that crude oil equally affects other tissues of the haemopoetic system as it does to the lung hence decreasing haematopoeisis. Loss of appetite and/or absorption of substance from the gut further aggravate the situation.

In conclusion, we report that oral administration of various concentrations of crude oil to experimental animals (guinea pigs) caused histologic changes in the lungs with widening of the alveolar spaces, due to edema, chronic inflammatory cell, infiltration of and actual hemorrhage into the alveolar ducts. These changes are most probably due to the animals; more studies are however still needed on the effects of crude oil on other organ systems of experimental animals.

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