African Journal of Biotechnology Vol. 3 (6), pp. 346-348, June 2004
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2004 Academic Journals

Full Length Research Paper

Haematological changes associated with crude oil ingestion in experimental rabbits

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Accepted 30 December 2003

Blood cell profile among experimental rabbits associated with crude oil ingestion were evaluated and was significantly (P<0.05) altered. Erythrocytes decreased linearly (P<0.05) indicating an anemic condition. The decrease also affected dependable factors such as packed cell volume (PVC) and haemoglobin (Hb). Leukocyctes, the main defense cells of the animal decreased linearly (P<0.05) further indicating susceptibility of the animal to stress and infection. Granulolytic leukocytes, which include neutrophils and eosinophils increased linearly (P<0.05). This increase is a physiological response to stress. Lymphocytes, antibody forming leukocytes decreased linearly, indicating a response to stress and susceptibility of the animal to infection. Crude oil fraction present in the diet has serious consequences on hematological parameters in animals.

Keywords: Haematological changes, crude oil ingestion, experimental rabbits.

INTRODUCTION

Environmental and physiological factors are known to affect many parameters in the blood. Furthermore, many environmental factors cause stress in animals. Haematological studies are of ecological and physiological interest. Such studies help in understanding the relationship of blood characteristics to the habitat and adaptability of the species to the environment. Crude oil, have over the years led to the pollution of the aquatic and terrestrial ecosytems. Several toxic components of the crude oil have been documented (O'Clair and Rice, 1985; Delille and Vaillant, 1990). Berepubo et al. (1994) observed that a relatively short exposure to crude oil led to the inhibition of growth in weaner rabbits. Wang et al. (1994) reported similar observation in juvenile pink salmon (*Oncorhynchus gorbuscha*).

The main objective of this study was to investigate the effects of ingested crude oil on some haematological parameters in rabbits under experimental conditions. Rabbits were chosen as a model for this study because their counterparts in the wild could be exposed to crude oil pollution through farm lands, crops, water and natural pastures. The analysis in this investigation has been focused on haematological parameters owing to their relationship with energy (blood glucose level), respiration (erythrocytes, haematocrit and haemoglobin level) and defense mechanism (Leukocyte level).

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MATERIALS AND METHODS

Thirty semi-adult rabbits aged 20 to 22 weeks were acclimatized for two weeks at the Rivers State University of Science and Technology Teaching and Research Farm, Port Harcourt. They were housed in conventional hutches made of bamboo. During this period, they were administered prophylactic coccisiostat and broad-spectrum antibiotics. Their food was mainly forage prepared from grass and centro and supplemented with concentrate (growers mash). Drinking and feeding troughs were cleaned and kept under strict hygienic condition and continued throughout the experimental period.

The thirty rabbits were randomly allocated into five dietary groups. Each dietary group consisted of six animals in two blocks. The groups were:

Table 1. Total red cell counts, platelets, dependable factors and red cell indices in rabbits exposed to crude oil.

Treatment group/contamination level	Hb (g/dl) Mean ± SEM	PCV (%) Mean ± SEM	RBC ($x10^{12}$/l) Mean ± SEM	Platelets ($x10^9$/l) Mean ± SEM	MCHC (%)	MCV (cu microns)	MCH (µ/g)
Group I: 0.00%	11.35 ± 0.75^{a}	34.13 ± 2.10^{a}	4.39 ± 0.21^{a}	198.25 ± 8.18^{a}	33.26	77.75	25.85
Group II: 0.05%	10.51 ± 0.76^{ab}	32.86 ± 2.50^{ab}	4.21 ± 0.27^{ab}	185.00 ± 6.12^{ab}	32.00	78.05	24.96
Group III: 0.10%	9.78 ± 0.50^{abc}	30.63 ± 1.50^{abc}	4.04 ± 0.18^{ab}	168.75 ± 6.25^{bc}	31.92	75.92	24.20
Group IV: 0.15%	9.48 ± 0.28^{bc}	28.18 ± 0.87^{bc}	3.50 ± 0.11^{b}	163.00 ± 7.84^{bc}	33.64	80.51	27.09
Group V: 0.20%	8.50 ± 0.40^{c}	27.02 ± 1.30^{c}	3.49 ± 0.17^{b}	159.75 ± 9.92^{c}	31.46	77.42	24.36

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

Table 2. Total white cell counts and differential values in rabbits exposed to crude oil.

Treatment group/contamination level	WBC ($x10^9$/l Mean ± SEM	Neutrophils (%) Mean ± SEM	Lymphocytes (%) Mean ± SEM	Monocytes (%) Mean ± SEM	Eosinophils (%) Mean ± SEM
Group I: 0.00%	7.45 ± 0.36^{a}	46.00 ± 7.20^{b}	55.94 ± 5.01^{a}	1.04 ± 0.02^{a}	0.00 ± 0.00^{c}
Group II: 0.05%	6.93 ± 0.30^{a}	47.33 ± 3.50^{b}	54.03 ± 2.59^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}
Group III: 0.10%	5.30 ± 0.32^{b}	54.03 ± 2.59^{c}	49.78 ± 6.06^{a}	0.00 ± 0.00^{b}	0.94 ± 0.06^{b}
Group IV: 0.15%	5.27 ± 0.82^{b}	57.23 ± 3.83^{a}	41.93 ± 5.96^{b}	0.00 ± 0.00^{b}	1.04 ± 0.04^{b}
Group V: 0.20%	4.65 ± 0.30^{b}	55.87 ± 3.63^{a}	37.42 ± 2.31^{b}	0.00 ± 0.00^{b}	4.90 ± 2.88^{a}

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

I: 0.00% (No contamination) control
II: 0.05% (Crude oil contamination)
III: 0.10% (Crude oil contamination)
IV: 0.15% (Crude oil contamination)
V: 0.20% (Crude oil contamination)

Crude petroleum was obtained from the Nigerian Agip Oil Company Ltd and exposed to sunlight in shallow pans (30 cm x 30 cm x 5 cm) for 24 h at the venue of the project to allow the extremely light and volatile fractions to evaporate leaving behind the stable components. This product simulates the naturally occurring condition following spillage (Neff et al., 2000). Animals were starved for 24 h before being fed the experimental diets. The incorporation of measured amounts of crude oil into the forage/hay preparation and concentrate was by simple mixing and homogenization using a manual mixer. Water was offered *ad-libitum*.

The experimental diets were administered for a period of twelve weeks. At the end of the experimental period, blood samples were collected from two does and two bucks from each treatment group. About 5ml of blood was collected from the marginal ear vein with a sterile disposable syringe and needle. The blood was immediately transferred into sterile ethylene diamino-tetra-acetic acid (EDTA) embedded vials, properly mixed and kept in the refrigerator for hematological analysis. The total red blood cell, white blood cell and platelets were estimated by the haematocytometer method using the improved Hawkskey haematocytometer. Haemoglobin (Hb) was measured by spectrophotometry as cyanomethmoglobin using the Pye Unicon SP 6500 spectrophotometer. Packed cell volume (PCV) was determined by the use of micro haematocrit method.

All data collected were subjected to the analysis of variance (ANOVA) and where differences existed, results were further subjected to Duncan Multiple Range Test (DMRT) for mean separation according to the procedure of SAS (1999).

RESULTS

The red cell components and their indices in rabbits exposed to crude oil ingestion and those of the control group are presented in Table 1. The red blood cells (RBC), packed cell volume (PCV), platelets and haemoglobin (Hb) in the control animals were 4.39±0.21 x 10^{12}/l, 34.13±2.10, 198.25±8.18 x 10^9/l, 11.35±0.72 g/dl, respectively. There were significant differences (P<0.05) between the control and crude oil treated groups. The red cell indices were also affected. Results showed that the values of these parameters decreased with increasing concentration of crude oil in the diets.

White cell count and the differential white cell counts are presented in Table 2. The mean white blood cell count was 7.45±0.36 x 10^9/l in the control and decreased linearly with increasing dietary concentration of crude oil. The mean neutrophil and eosinophil value in the control animals was 46.00±7.20 and zero respectively. These granulocytes increased with increasing dietary concentration of crude oil. Lymphocytes and monocytes also decreased with increasing concentration of crude oil in the diets.

DISCUSSION

A major problem facing the Niger Delta environment is related to pollution by petroleum products. Jacob and Al-

Muzaini (1995) observed that petroleum pollution could range from diffused chronic exposure to considerably large single doses. These subtlethal concentrations may not necessarily lead to outright mortality but may have significant effects which can lead to physiological stress and dysfunctions in animals (Omoregie, 1998).

From this investigation, it is obvious that exposure of rabbits to crude oil ingestion caused a significant decrease in erythrocyte values. Consequently, haemoglobin (Hb) and packed cell volume (PCV) decreased with increasing concentration of crude oil (Table 1). The observed linear reduction in haemoglobin, packed cell volume and erythrocyte count demonstrate and suggest an anemic condition in the crude oil treated rabbits. Similarly, platelets were observed to decrease with increasing concentration of crude oil. Similar toxic components like chlorinated hydrocarbons exert the same effects on blood cell profile (Matsumura, 1975). In the same vein, the toxic components especially those in crude oil change blood chemistry and induce anaemia by causing bone marrow hypoplasia and interfered with platelet production in the animals, hence the reduced values (Sudakov, 1992). This assertion is supported by Snyder (1987) who demonstrated that benzene is activated in the bonemarrow. These cytotoxic effects are mediated through disturbance in DNA function. Thus bone marrow failure as in this study is characterized by inadequate production of red cell and other formed elements.

The primary function of white blood cells appears to be to defend the body against foreign bodies, which is achieved by leucocytosis and antibody production (Robbin and Angel, 1976). Total white blood cell count decreased with increasing concentration of crude oil toxicity (Table 2). The observation in this study is similar to the findings of Ngodigha et al. (1999) in which there was a reduction in total white cell count in goats as the level of crude oil concentration increased. They argued that the reduction in total white cell count in goats may be a combination of stress imposed by crude oil hydrocarbons. Neutrophils and eosinophils values increased linearly with increasing concentration of crude oil. This increase is an indication of stress imposed by crude oil fraction in the diets and confirmed Selye's (1963) finding that a stress stimulus elicits a defense response.

Lymphocytes and monocytes were found to be significantly ($P<0.05$) most depressed from group two to five. Similar observations were made by Thompson and Lippman (1974) as well as Sudakov (1992) who pointed out that ACTH and glucocorticoids cause the regression of lymphoid tissue due to stress. The observed linear reduction in lymphocytes and monocytes could be attributed to the effect of hydrocarbon in lymphoid tissues which have been found to cause antibody depression, impaired migration of phagocytic cells, lower resistance to viruses and foreign bodies (Spain, 1975).

From the results of this study, it is hereby suggested that crude oil is an environmental stressor which causes depression of total white cell and red cell count. Thus, crude oil has serious consequences on hematological parameters in rabbits.

ACKNOWLEDGMENT

We are grateful to A.J. Saibolo for technical assistance.

REFERENCES

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

Delille D, Vaillant N (1990). The influence of crude oil on the growth of sub-artartic marine bacteria. Antartic Sci. 2 (2) 123 - 127.

Jacob PG, Al-Muzaini (1995). Marine plants of the Arabian Gulf and effects of oil pollution. Mahasagar 23: 83 - 101.

Matsumura F (1975). Energy of insecticides into animal systems: In: toxicology of insecticides. Pler. Press, New York, U.S.A. pp. 165 - 179.

Neff JM, Ostazeski S, Gardner W, Steiskal I (2000). Effects of weathering on the toxicity of three offshore Australian crude oils and diesel fuel to marine animals. Environ. Toxicol. Chem. 19: 809 - 820.

Ngodigha EM, Olayimika FO, Oruwari BM, Ekweozor IKE, Wekhe SN (1999). Toxic effects of crude oil on organs and blood cells of West Africa Dwarf Goat. Nig. Vet. J. 20 (1) 82 - 91.

O'Clair CE, Rice SD (1985). Depression of feeding and growth rates of the seastar (*Evasterias troschelii*) during long term exposure to water soluble fractions of crude oil. Marine Biol. 84: 331 - 340.

Omoregie E (1998). Changes in the haematology of the Nile tilapia (*Oreochromis niloticus trewavas*) under the effects of crude oil. Acta Hydrobiol. 40: 287 - 292.

SAS (1999). Statistical Analysis System, User's Guide, SAS/STAT; Version 8 Edition. SAS Inst. Inc. Cary NC., U.S.A.

Selye H (1963). Stress and the adaptation syndrome. In: Cyclopedia of medicine, surgery and specialities Vol. X111. E.A. Davis Co. New York p. 365 - 366f.

Snyder CA (1987). Benzene in Snyde R (ed) Ethel Browning toxicity and metabolism of industrial solvents 2nd ed. Vol. 1: Hydrocarbons Armsterdam. Elsevier.

Spain DM (1975). Corticosteroids, inflammation and connetive tissue. In: Handbook of physiology. Sect. 7 Vol. 6. (eds) Blaschko H, Sayer G, Smith AD. Washington DC American Physiological Society pp. 263 - 270.

Sudakov KV (1992). Stress postulate: analysis from the position of general theory of functional systems. Pathophysiol. Exp. Ther. 4: 86 - 93.

Thompson EB, Lippma ME (1974). Mehanism of action of glucocortisoids. Metabolism 23: 159 - 202.

Wang SY, Lum JL, Carls MG, Rice SD (1994). The relationship between growth and total nucleic acids in juvenile pink salmon (*Oncorhynchus gorhuscha*) fed crude oil contaminated food. Can. J. Fish. Aquat. Sci. 50: 996 - 1001.