Haematological Response of African Catfish (Clarias gariepinus) and Rat to Crude Oil Exposure

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Abstract

The effect of Bonny light crude oil on some haematological parameters was studied in African catfish (Clarias gariepinus) and rats. A total of 120 catfish were grouped into 6 of 20 catfish each and held for 30 hours in 5 different mixtures of crude oil polluted water (0.1%, 0.25%, 0.5%, 0.75% and 1% v/v). Catfish in the control group were held in borehole water. At the expiration of 30 hours, the catfish were harvested and used to formulate diet. Albino rats (n = 60) were grouped into 6 of 10 rats each and fed on the formulated diet for a period of 30 days. The control rats were fed on diet containing catfish cultured in borehole water while those in groups 2 to 6 were fed on diets containing catfish exposed to the various mixtures of crude oil. The red blood cell (RBC) count reduced significantly ($p<0.05$) as the concentration of crude oil increased in both the catfish and rat indicating an anaemic condition. The decrease also affected dependable factors such as packed cell volume (PVC), haemoglobin (HGB), platelet (PLT), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Furthermore, white blood cells (WBC), the main defense cells of the animals decreased significantly ($p<0.05$) further indicating susceptibility to stress and infection. Overall, the results indicate that exposure to crude oil has serious consequences on haematological parameters of catfish and rat which may be attributed to the toxic components of crude oil.

INTRODUCTION

Crude oil is a complex mixture of hydrocarbons from which various petroleum products such as gasoline, kerosene, fuel oil, lubricating oil, wax and asphalt are derived [1, 2]. Several toxic components of crude oil such as polycyclic aromatic hydrocarbons (PAHs) and water soluble fractions (WSF) have been documented [3, 4]. Crude oil and its refined petroleum products contains several organic and inorganic substances including atoms of sulphur, nitrogen, oxygen, oxygen as well as metals such as iron, vanadium, nickel and chromium [5].

Crude oil spillage has over the years, led to the pollution of the aquatic and terrestrial ecosystems. Many blood parameters are known to be affected by environmental and physiological factors. Therefore, haematological studies are of ecological and physiological interest such as helping to understand the relationship of blood characteristics to the habitat and adaptability of the species to the environment [6].

The effects of oil spill on aquatic lives are caused by either the physical nature of the oil (physical contamination and smothering) or by its chemical components (toxic effects and accumulation leading to tainting). Aquatic lives may also be affected by clean up operations or indirectly through physical damage to the habitats in which plants and animals live [7]. The main threat posed to living resources by the persistent residues of spilled oils and water-in-oil emulsions (“mousse”) is one of physical smothering. The animals and plants most at risk are those that could come into contact with a contaminated sea surface. These include aquatic mammals and reptiles; birds that feed by diving or form flocks on the sea as well as aquatic lives on shorelines [8].

Previous studies have shown that crude oil can have both lethal and sub-lethal effects on a wide range of organisms. These include the observation that a relatively short exposure to crude oil led to the inhibition of growth in weaner rabbits [9]. A similar observation was also reported in juvenile pink salmon (Oncorhyncus gorbuscha) [10]; while drastic changes in liver enzyme activities of catfish (Clarias gariepinus) were reported following exposure to crude oil [11]. The main objective of the present study is to investigate the effect of crude exposure on some haematological parameters of catfish and rat under experimental conditions. Haematological parameters serve as the focus of this study because of their relationship with energy (blood glucose), respiration (RBC, PCV and HGB levels) and defense

mechanism (WBC level).

MATERIALS AND METHODS

A. COLLECTION OF CRUDE OIL AND PREPARATION OF VARIOUS MIXTURES

Bonny light crude oil was obtained from the Department of Petroleum Resources (DPR), Nigerian National Petroleum Corporation (NNPC), Port Harcourt, Nigeria and diluted with borehole water to obtain mixtures of 0.1%, 0.25%, 0.5%, 0.75% and 1% by volume (Table 1). These concentrations are representative with a view to mimicking the effect of natural dilution as the crude oil moves along with water in the event of oil spillage.

Figure 1

Table 1: Preparation of Various Mixtures of Crude Oil

Mixture (%v/v)	Crude oil (cm^3)	Borehole (cm^3)	Total Vol.(cm^3)
0.10	0.10	99.90	100
0.25	0.25	99.75	100
0.50	0.50	99.50	100
0.75	0.75	99.25	100
1.00	1.00	99.00	100

B. EXPERIMENTAL FISH AND TREATMENTS

One hundred and twenty apparently healthy juvenile catfish (Clarias gariepinus) with a mean weight of 75.33±3.00g were obtained from a commercial fish pond at Unity Road in Ilorin, Kwara State, Nigeria and acclimatized for ten days prior to the commencement of the experiment. The catfish were grouped into six of twenty catfish and were kept in 30L plastic aquaria. Group 1 served as control and the catfish here were cultured in borehole water while those in Groups 2 to and 6 were exposed to the different mixtures (0.1%, 0.25%, 0.5%, 0.75% and 1% v/v) of crude oil. The catfish were fed ad libitum with commercial fish meal for 30 hours during which the experiment lasted. After harvesting from the aquaria, the catfish were allowed to stay in a dissecting tray for about ten minutes to reduce the slime on their bodies. They were thereafter dissected and blood sample was collected with a disposable syringe and needle and immediately transferred into sterile ethylene diamine tetra-acetic acid (EDTA) embedded vials for haematological analysis.

C. FORMULATION OF DIET

At the end of the 30 hours experimental period, the catfish were harvested, oven dried at 40oC and used as a source of protein to formulate diet for albino rats. The diet for each group was formulated by mixing known quantities of sources of each food class (Table 2). The food items were mixed together and manually made into pellets to feed albino rats.

Figure 2

Table 2: Composition of the Formulated Diet

Feed Components	Percentage composition (%)
*Contaminated catfish	25
Corn starch	52
Oil	4
Cellulose (maize cob)	4
Sucrose	10
**Mineral/vitamin mixture	5
Total	100%

* Catfish cultured in different concentrations of crude oil polluted water (0.1%, 0.25%, 0.5%, 0.75% and 1.0%). Control catfish were cultured in borehole water

** Vit A 15,000,000i.u, Vit. D, 32,000i.u, Vit E, 12,000i.u, Vit K, 2i.u, thiamine 1.5g, riboflavin 25g, pyridoxine 5g, folic acid 0.5g. For the mineral mixture, manganese 75g, zinc 45g, iron 20g, copper 5g, iodine 1g and selenium 100mg

D. EXPERIMENTAL RATS AND TREATMENTS

Sixty albino rats (Rattus norvegicus) with an average weight of 50.20±4.24g were obtained from the Small Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were grouped into six with each group containing ten rats. The rats in Group 1 served as the control and they were fed on the control diet, which was formulated with catfish cultured in borehole water. Animals in Groups 2 to 6 were fed on diet formulated with catfish exposed to the different mixtures of crude oil (0.1%, 0.25%, 0.5%, 0.75% and 1.0% v/v respectively). The feeding lasted for a period of thirty (30) days after an acclimatization period of ten days. The rats were anaesthetized by placing them in a jar containing cotton wool soaked with chloroform followed by jugular puncture with a sharp sterile blade. Blood sample was collected with a disposable syringe and needle and immediately transferred into sterile ethylene diamine tetra-acetic acid (EDTA) embedded vials for haematological analysis.

E. DETERMINATION OF HAEMATOLOGICAL PARAMETERS

The Automated Haematologic Analyzer (Sysmex KX – 21) was used to analyze the haematological parameters like PCV, WBC, RBC, MCH, MCHC, HGB and PLT. The analyses were carried out based on standard methods [12, 13].

F. STATISTICAL ANALYSIS

All data were analysed statistically using Analysis of Variance (ANOVA) test [14]. Significant difference between the treatment means was determined at 5% confidence limit using Duncan's Multiple Range Test [15].

RESULTS

The haematological parameters of catfish and rat exposed to various levels of crude oil polluted water and diet are as presented in Tables 3 and 4 respectively. The data obtained in the study revealed that there was a significant reduction ($p<0.05$) in the concentration of all the haematological parameters analyzed in both the catfish (Table 3) and rat (Table 4) as the level of exposure to crude oil increased. Indeed, the least concentration of all the blood parameters analyzed was observed in the catfish and rat exposed to 1% concentrated polluted water and diet respectively.

Figure 3

Table 3: Some haematological parameters of catfish exposed to various concentrations of crude oil for 30h

Conc. of Crude oil	PCV (%)	WBC (x10⁹/L)	RBC (x10¹²/L)	MCH (pg)	MCHC (g/dl)	HGB (g/dl)	PLT (x10⁹/L)
Control	37.0±2.18^a	298.5±3.50^a	2.12±0.05^a	46.0±1.60^a	27.0±1.05^a	9.8±1.05^a	132.0±1.00^a
0.1%	35.0±1.98^a	290.0±2.15^b	2.11±0.05^a	45.0±1.25^a	25.0±1.05^a	9.8±1.00^a	124.0±1.06^b
0.25%	31.0±1.50^b	266.3±1.98^c	2.11±0.05^a	39.0±1.05^b	25±1.00^a	9.7±1.06^a	118.0±1.05^c
0.50%	27.0±1.93^c		230.5±3.50^d	1.77±0.03^b	32.0±1.00^a	18±1.01^b	5.9±0.95^b	111.0±0.85^d
0.75%	19.0±1.50^d	191.9±2.75^e	1.24±0.02^a	28.0±1.01^d	12.0±1.06^a	3.9±0.50^a	96.0±0.50^e
1.00%	7.0±0.50^e	166.5±1.85^f	0.43±0.04^b	23.0±1.85^a	9.0±0.50^d	2.6±0.46^b	78.0±0.50^f

Values are means ± SEM for 20 catfish

^{a,b,c,d,e,f} Column values with different superscripts are significantly different ($p<0.05$)

Figure 4

Table 4: Some haematological parameters of rats fed on crude oil contaminated catfish over a period of 30 days

Conc. of Crude oil	PCV (%)	WBC (x10⁹/L)	RBC (x10¹²/L)	(pg)	MCH (g/dl)	MCHC (g/dl)	HGB (x10⁹/L)	PLT
Control	39.0±1.00^a	299.5±2.50^a	2.51±0.05^a	49.0±1.10^a	30.0±0.95^a	9.9±0.85^a	153.0±1.50^a	
0.10%	36.0±0.90^b	291.0±2.05^b	2.11±0.05^b	44.0±1.05^b	26.0±1.00^b	9.6±0.50^b	134.0±1.65^b	
0.25%	32.0±1.10^c	270.3±1.85^c	2.00±0.05^c	38.0±1.05^c	25.0±1.00^c	9.2±0.26^c	121.0±1.05^c	
0.50%	28.0±1.15^d	235.5±2.50^d	1.75±0.03^d	33.0±1.00^d	20.0±0.55^d	6.9±0.50^d	113.0±1.16^d	
0.75%	22.0±1.05^e	196.9±2.15^e	1.25±0.02^e	27.0±0.90^e	15.0±1.01^e	4.5±0.30^e	98.0±1.55^e	
1.00%	9.0±0.55^f	169.5±1.55^f	0.65±0.04^f	21.0±0.85^f	10.0±0.50^f	2.9±0.36^f	75.0±1.50^f	

Values are means ± SEM for 10 rats

^{a,b,c,d,e,f} Column values with different superscripts are significantly different ($p<0.05$)

DISCUSSION

One of the major problems of the inhabitants of the Niger Delta region of Nigeria is contamination of water and aquatic lives by crude oil. This contamination may not necessarily lead to outright mortality but may have significant effects which can lead to physiological stress and dysfunction in animals [16]. The severity or degree of the problems in the inhabitants of the area is dependent upon the point of contact with the polluted water. Hence, justifying the need for the preparation of different crude oil concentrations.

In the present study, it is obvious that exposure of catfish and rat to crude oil caused a significant reduction ($p<0.05$) in RBC count. Consequently, HGB and PCV reduced significantly ($p<0.05$) as the concentration of crude oil increased. The observed reduction in the concentrations RBC, HGB and PCV suggest an anaemic condition in the crude oil treated catfish and rat. The significant reduction ($p<0.05$) in RBC count may be attributed to cytotoxic effect and suppression of erythropoiesis caused by constituents of the crude oil. This is in line with previous studies which showed that the erythroid colony-forming unit (CFU-e) was very susceptible to the cytotoxic effect of the crude oil derivative benzene [17].

The RBC count dropped significantly ($p<0.05$) as the concentration of crude oil increased. This would imply reduction in the level of oxygen that would be carried to the tissues and the level of carbon dioxide returned to the lungs would also be reduced. The values obtained for MCH and MCHC serve to indicate variations in erythrocyte shape, size and haemoglobin content. Reduced MCH and MCHC as observed in the present study, which is an indicator of anaemia, was also reported in previous studies [18].

Similarly, PLT concentration was observed to reduce significantly ($p<0.05$) as the concentration of crude oil increased (Tables 3 and 4). In a related study, toxic components especially those in crude oil changed blood chemistry and induce anaemia by causing bone marrow hypoplasia and interfered with platelet production in the animals, hence the reduced values [19].

The major functions of WBC are to fight infection, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. A significant reduction ($p<0.05$) in WBC count with increase in crude oil concentration (Tables 3 and 4) suggests that the catfish and rat are exposed to high risk of infection. The observation in this study is similar to the findings of previous studies in which there was a reduction in total WBC count in goats as

the level of crude oil concentration increased [$_{20}$]. It was argued that the reduction in WBC count in goats may be as a result of stress imposed by crude oil hydrocarbons.

The result generated from this study is suggestive of the fact that crude oil is an environmental stressor which causes depression of RBC and WBC counts. Thus, it can be concluded that crude oil has serious consequences on haematological parameters in catfish and rat.

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