Protective influence of antioxidant vitamins on haematological indices of rabbits fed crude-oil contaminated diet

Achuba FI[1,*], Awhin PE[2]

[1]*Department of Biochemistry, Delta State University, P.M.B 1, Abraka, Nigeria;* [2]*Department of Medical Biochemistry, Delta State University, P.M.B 1, Abraka, Nigeria*

Abstract

The effects of chronic exposure to petroleum on hematological parameters as well as the possible protective role of vitamins E and C were studied. The hematological parameters assessed were red blood cell counts, hematocrit value, haemoglobin concentration and white blood cell counts. The red blood cell counts and haemoglobin concentration were significantly ($P < 0.05$) decreased in the blood of petroleum-fed rabbits. However, there was a significant increase ($P < 0.05$) in white blood cell count in rabbit exposed to crude oil contaminated feed. Pretreatment with the antioxidant vitamins E and C restored these parameters to normal. [Life Science Journal. 2008; 5(1): 55 – 58] (ISSN: 1097 – 8135).

1 Introduction

The Niger delta region of Nigeria is rich in petroleum resource. One drastic effect associated with its exploration, exploitation, processing, transportation and uses is the contamination of the immediate environment with petroleum hydrocarbons (Adeyemi, 2004). Most of the land in oil producing area in the Niger delta region of Nigeria is used for farming because the major occupation of the people in the area is farming and fishing (Egborge, 1991). This may culminates in the contamination agricultural produce (Onyesom and Okoh, 2006). The petroleum hydrocarbon may eventually gets into man and animals through the ingestion of contaminated food or bioconcentration through the food chain (Jessup and Leighton 1996).

Crude oil is a potent toxicant that is released into the environment during prospecting, production, processing and transportation (Egborge, 1991). The toxicity of crude oil and its refined product cannot be over emphasized. It causes oxidative stress (Onwurah, 1999; Achuba and Osakwe 2003), proliferation of white blood cells and changes in blood chemistry (Kori-Siakpere, 1998; Bartimeous

et al, 2002). The deleterious effect of crude oil has been linked to its ability to induce lipid peroxidation in organs and tissue (Val and Ameida-Val, 1999). Volatile components of oil can burn eyes, burn skin, and irritate or damage sensitive membranes in the nose, eye and mouth. Hydrocarbon can trigger pneumonia if it enters lungs. Benzene, toluene and other light hydrocarbons of oil and fuels if inhaled are transferred rapidly to the blood stream from the lungs and can damage red blood cells, suppress immune systems, strain the liver, spleen, and kidneys and can even interfere with the reproductive system of animals and humans (Etkin, 1997). The use of antioxidants in ameliorating the involvement of free radicals in diseases process has been the subject of recent investigations (Farris, 1991; Verma and Nair, 2001; Ognjanovic et al 2003). Most previous reports have centred more on the toxicity of crude oil and its refined product. There is paucity of data on the use of antioxidants to reduce the effects of crude oil on exposed subject. The aim of the present investigation is to determine whether the antioxidant vitamins E and C can ameliorate the toxic effects of crude oil.

2 Materials and Methods

2.1 Animals and feeding

25 male English rabbits (initial mean weight 1.45 kg,

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*Corresponding author. Email: fiachubabch@yahoo.com

aged about 16 weeks) were purchased from a local animal dealer in Benin. The animals were housed individually in clean metal hutches and acclimatized on growers mash (product of Bendel feed and flour mills (BFFM) Ltd, EWU; Nigeria) for 3 months prior to the commencement of the experiments. The animals were divided into five groups, each containing five rabbits. Members of each group were housed singly in clean metal hutches, and the feeding experiment was conducted at room temperature of 28 ºC. Rabbits in the control group were fed with growers mash only, while four other groups received growers mash with 2.5% W/W of crude oil per kg of food. One of the four groups fed crude-oil-contaminated food received no vitamin, one received 500 mg of vitamin E per kg of food, another received 500 mg of vitamin C per kg of food, and the last received 500 mg each of vitamins E and C per kg of food. Preliminary investigation has established that this level of crude oil was tolerable to the rabbits on a prolonged basis without any drastic effect. The crude oil-mash feed was prepared fresh everyday. Before feeding, the feeds were mixed with water so as to achieve a texture acceptable to the animals. Clean drinking water was liberally provided and uneaten food was regularly discarded. The animals were exposed to the contaminated food for six months. Haematocrit, WBC count, haemoglobin concentration, and RBC count were determined by conventional procedures as described in Baker *et al* (1998).

2.2 Determination of heamatocrit

Blood was allowed to flow into the capillary tube by capillary action. The capillary tube was then sealed at one end and spurred into the centrifuge and spind for 5 minutes to separate the blood cells from the plasma. The capillary was taken from the centrifuge and read with the hematocrit reader.

2.3 Determination of WBC

Dilution factor of 1 : 20 ml was made by pipeting 0.38 ml of WBC reagent (Turks fluid) and adding 20 μl of the blood sample into it. The improved number counting chamber was covered with a slip and charged with the mixture of blood and Turk's fluid before viewing under the microscope for counting with the chamber. A working formula for WBC is used for the calculation.

WBC (per litre) = $(N \times D_f \times 10^6) / (A \times D)$

Where N: Number of cell counted;

D_f: Diluting factor;

A: Area;

D: Depth of the counting chamber.

2.4 Determination of haemoglobin concentration

Blood is diluted in a buffered solution of potassium ferricyanide and potassium cyanide to field the stable haemoglobin derivative of cyanmethaemoglobin. The absorbance of this solution is read at 540 nm.

20 μl of blood was pipeted into 5.0 M of Drabkins reagent. This was allowed to stand for 5min and the absorbance read at 540 nm. Cyanmethaemoglobin standard was measured the same time as test. The final haemoglobin concentration in the test was calculated using a working formula as follows:

Hb concentration (g/100 ml) = $(T \times C \times D) / (A \times 1000)$

T: Test absorbance at 540 nm;

A: Standard absorbance at 540 nm;

C: the concentration of cyanmethaemoglobin standard (mg/dl);

D: Dilution factor, 1000 converts from mg/dl to g/dl.

2.5 Determination of RBC

The most common diluents used for visuals red cell counts is a solution of formol-citrate prepared by mixing 10 ml of formalin (40% formaldehyde) with 1 litre of 31.3 g/L trisodium citrate solution. This fluid was filtered and stored in a clean glass container until required. The blood sample is diluted by washing 1 μl of blood taken into a positive displacement pipette into 4.0 ml of diluents to give a final dilution of 1 in 20 L. The diluted sample was then mixed and loaded into the haemocytometer. When the cells have settled of suspension, the number was counted. For the final result to be expressed as the number of cells per litre, the following working formula is used for evaluating RBC.

Red blood cell count (per litre) = $(N \times D_f \times 10^6) / (A \times D)$

Where N: Number of cell counted;

D_f: Diluting factor;

A: Area;

D: Depth of the counting chamber.

2.6 Statistical analysis

The result of the study was subjected to analysis of variance (ANOVA). This was to ascertain if the effect of the feed treatment with vitamins on the haematological parameters was significantly different from that of the control.

3 Results

Incorporation of crude oil in the rabbits' feed decreased RBC count, haemoglobin concentration, but increased WBC count (Table 1). The addition of vitamins C and

Table 1. Red blood cells counts, heamatocrit value, white blood cell counts and heamoglobin concentration of control rabbit, fed with petroleum, contaminated feed and petroleum contaminated feed, vitamin C and vitamin E

Parameter	Control (n = 5)	2.5% W/W crude oil/kg feed	2.5% W/W crude oil/kg + Vitamin E	2.5% W/W crude oil/kg feed + Vitamin C	2.5% W/W crude oil/kg feed + Vitamins C and E
Haematocrit (%)	33.0 ± 1.08	25 ± 1.96	28 ± 0.40	27.6 ± 0.36	34 ± 1.41
Erythrocyte count (10^{-12}/L)	8.8 ± 0.34	4.8 ± 0.34*	7.2 ± 0.34	6.6 ± 0.36	7.2 ± 0.31
HB (mml/L)	9.6 ± 0.61	6.6 ± 0.46*	8.8 ± 034	8.2 ± 0.34	9 ± 0.45
WBC /mm^3	2900 ± 31.6	21032 ± 413.7*	5960 ± 45.61	7950 ± 194.4*	3080 ± 100.6

Values are expressed as means ± SEM of five animals in each group. *significantly different from control, $P < 0.05$.

E, either separately or together, restored these values to normal . Table 1 also shows that WBC was significantly ($P < 0.05$) increased in rabbits fed with crude oil contaminated diet while inclusion of antioxidant vitamins tended to reverse the change to control value. The highest reversal was found in rabbits fed with vitamins E and C simultaneously.

4 Discussion

Crude oil and its refined products are potentially toxic to plants and animals (Albers, 1995; Achuba, 2003; Etkin, 1997). Previous reports showed that ingestion of crude oil caused reduction in haemoglobin concentration and induced anaemia (Ben-David, 2000) while chromic injection of crude oil increased white blood cell counts in rats (Dede *et al*, 2002).The results obtained in this study show that ingestion of petroleum contaminated feed induced anaemia and increased the number of white blood cells in rabbits (Table 1). The decreased in haematological indices, especially haematocrit value, in exposed animals indicate destruction of erythrocytes (Kori-Siakpere, 1998; Dede *et al*, 2002). Conversely the increase in white blood cell counts suggests induction of the immune system defensive mechanism during disease processes (Baker *et al*, 1998). Fariss (1991) had earlier reported that antioxidants are useful in protecting against chemical toxicity. The supplementation of the food with vitamins E and C decrease the toxic effect of petroleum on the haematological values and has a protective role in anaemia and illness (Table 1). This is because administration of the antioxidant vitamins tends to restore haematological parameters to control values. The protective role of these vitamins is more effective with vitamins E and C administered simultaneously compare to vitamin E and C used separately, however, vitamin E is more efficient than vitamin C. Vitamin E has been reported to be the first line of defense against chemically induced oxidative stress (Ibrahim *et*

al, 2000) whereas vitamin C has an important role in the regeneration of reduced form of vitamin E (Tanaka *et al*, 1997). Hence, antioxidants such as vitamin E and vitamin C can act synergistically to prevent cell destruction (Beyer, 1994; Chen and Tappel, 1995; Lass and Sohal, 2000).

5 Conclusion

It's pertinent to extrapolate that ingestion of petroleum contaminated food could predispose humans to anaemia and illness. Therefore, the inhabitants of the Niger delta region of Nigeria and other parts of the world where petroleum is produced be enlightened on the need supplement their diet with antioxidant vitamins.

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