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Haematotoxic Effects of Diets Contaminated with Petroleum Products (Kerosene and Petrol) in Wistar Albino Rats

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Abstract: Haematotoxic effects following administration of kerosene and Petrol-contaminated diets were investigated in male and female albino rats. Haematological parameters namely haemoglobin (Hb), Packed Cell Volume (PCV), White Blood Cell Counts (WBC) and differentials were used to assess the effect of kerosene and petrol-contaminated diets on rats. LD_{50} values of kerosene and petrol were obtained at levels of 212 and 128 mL kg^{-1} of feed respectively for 12 weeks. Long term exposure of the animals to kerosene and petrol-contaminated diets caused a significant decrease ($p \leq 0.05$) in PCV and Hb indicating an anaemic condition. Male and female rats fed diets contaminated with kerosene and petrol showed a significant decrease ($p \leq 0.05$) in the WBC counts. Result of the differential count showed a significant increase ($p \leq 0.05$) in neutrophils of male and female rats fed diets contaminated with kerosene and petrol while lymphocytes, antibody forming leucocytes decreased significantly ($p \leq 0.05$) among rats fed diets contaminated with kerosene and petrol indicating a response to stress and susceptibility of the animals to infection. Long term exposure of rats to kerosene and petrol-contaminated diets induced anaemia as observed in this study.

Key words: Petroleum products, haemoglobin, packed cell volume, white blood cells, anaemia

INTRODUCTION

In Nigeria today, contamination as a result of oil spillage has become one of the severest forms of environmental pollution. Generally, petroleum and its refined products reaching the terrestrial and aquatic ecosystems arrive as a consequence of spillage which may result from natural seepages, offshore exploration, leakages from oil wells or from oil tankers, accident from oil tankers, land based discharges and sabotage (Awobajo, 1981; Wardley-Smith, 1983; Jackson *et al*., 1989). The toxic effect of petroleum hydrocarbons in an organism is manifested subsequent to bioaccumulation of the offending xenobiotic.

The phenomenon of bioaccumulation is associated with direct transfer of compounds through body surface into the circulatory fluids in a process known as bioconcentration, or they are transferred through food across the gastrointestinal tract (GIT) into the circulatory fluids in a process called biomagnifications (Connell and Miller, 1980). Petrol inhalation is associated with dysfunctions that range in severity from subtle cognitive impairment to encephalopathy and death (Cairney *et al*., 2002). Individual components of kerosene are

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known to undergo dermal absorption (Singh and Singh, 2003; Mattorano *et al*., 2004) and kerosene vapour is absorbed following pulmonary exposure (Risher and Rhodes, 1995). The extent of dermal and pulmonary absorption is dose and time-dependent (Bebarta and DeWitt, 2004). Many of these substances are considered to have the potential to affect human health if consumed directly or via food (Pollock *et al*., 1991). Environmental and physiological factors are known to affect many parameters in the blood.

Haematological studies are of ecological and physiological interest. Such studies help in understanding the relationship of blood characteristics to the environment (Ovuru and Ekweozor, 2004). Several toxic components of petroleum products have been documented (O'Clair and Rice, 1985; Delille and Vaillant, 1990; Cairney *et al*., 2002). Berepubo *et al*. (1994) observed that a relatively short exposure to petroleum products led to the inhibition of growth in weaner rabbits. Wang *et al*. (1994) reported similar observation in juvenile pink salmon (Oncorhyncus gorbuscha).

Kerosene and petrol are distilled from crude petroleum. These fractions of crude petroleum contain aliphatic, aromatic and a variety of other branched saturated and unsaturated hydrocarbons (Henderson *et al*., 1993; Kato *et al*., 1993; Anderson *et al*., 1995).

Kerosene is a liquid mixture of chemicals produced from the distillation of crude oil. In the UK, kerosene is also known as paraffin and home heating oil. The word kerosene comes from the Greek word keros, meaning wax. Kerosene is a major component (>60%) of aviation (jet) fuels. It is used for central heating systems and can be used as a cleaning agent or solvent. Approximately 7½ millions tons of kerosene was used in the UK in 2005 (Chilcott, 2007). Swallowing fuel oil such as kerosene or petrol can cause vomiting, diarrhea, swelling of the stomach, cramps, coughing, drowsiness, restlessness, irritability and breathing difficulties. Drinking more than an ounce can result in coma or death. Most accidental poisonings involve children. Many of these children drink kerosene that was being kept in old soft drink bottles (WDHFS, 2000).

Much literature does not exist concerning the haematotoxic effects of kerosene and petrol in rats. However, this investigation was therefore aimed at assessing the potential haematological effects associated with the chronic exposure of rats to kerosene and petrol.

MATERIALS AND METHODS

Petroleum Samples

Kerosene and petrol used for this study was purchased from African Petroleum (AP) filling station near the University of Port Harcourt, Choba, Rivers State, Nigeria. The petroleum samples were stored in clean containers and kept in our laboratory until required for use.

Animals

Mature male and female albino rats of the Wistar strain (160-170 g) obtained from the animal house of the Department of Biochemistry, University of Port Harcourt, Port Harcourt, Nigeria were used for this study. They were housed in standard cages (Griffin and George Modular cage system) and left to acclimatize for 14 days to laboratory conditions before the commencement of the experiment. During the acclimatization period, the animals were fed with pelleted rat chow and water *ad libitum*.

Chronic Toxicity Study

Forty eight rats consisting of equal numbers of male and female rats were used for this study. The rats were separated into two sets of male and female. A set of either male or

female rats comprised of 3 groups of eight rats per group. The rats were acclimatized for two weeks. A group from each set of rats was fed with normal diets and these served as the general control. All the animals were fed *ad libitum* with their specific diets for the duration of 12 weeks.

Preparation of Feed

The LD_{50} values of the petroleum products-kerosene and petrol, as calculated were multiplied by a factor of +2.5 to make up for loss of the volatile components of these products. Calculated quantities (volumes) of the samples were then mixed with measured amounts of animal feed and the mixture was compacted with water to form a constant mass. The feed was then molded into small balls, air-dried and stored in well-labeled sacks to last for the duration of the test period.

Preparation of Samples

At the end of the 12 weeks of the test period, the rats were anesthetized in chloroform-saturated chambers. The animals were sacrificed by cervical dislocation and blood samples were collected using a 5 mL hypodermic syringe and needle through cardiac puncture. The blood samples for Hb, PCV and WBC were collected into EDTA containers without anticoagulant.

Haematological Test

The White Blood Cells (WBC) and the differentials were estimated using the improved Neubauer counting chambers as described by Dacie and Lewis (1991). The haemoglobin (Hb) concentration was determined by the Cyanmeth-haemoglobin method and the Packed Cell Volume (PCV) was determined by the micro method, also as described by Dacie and Lewis (1991).

Analysis of Data

The mean values of the control(s) and test samples were compared using the student's t-test (Zar, 1984). The significance level was set at ($p \leq 0.05$).

RESULTS

Table 1 and 2 show the result of male and female rats fed kerosene and petrol-contaminated diets respectively. Result shows a significant decrease ($p \leq 0.05$) in Hb level of male and female rats exposed to kerosene and petrol when compared to the control (Table 1, 2). The data obtained in this study also revealed that there was a significant reduction ($p<0.05$) in PCV of animals in the test groups. There was also a significant ($p \leq 0.05$) decrease in WBC of the male and female rats in the two test groups relative to the control (Table 1, 2). The differential count in male and female rats fed diets contaminated with

Table 1: Haematological parameters (Hb, WBC and differentials) in male rats

Experimental groups	Haematological parameters				
	Hb (g dL^{-1})	PCV (%)	WBC (mm^{-3})	Neutrophils (%)	Lymphocytes (%)
Control	11.50±0.49^a	4.1±0.96^a	8757±241.29^a	31.01±2.24^a	69.002±2.24^a
Kerosene- exposed	9.55±0.05^b	3.1±0.78^b	5700±413.31^b	47.00±2.98^b	53.00±3.09^b
Petrol-exposed	10.33±0.23^b	3.5±0.64^b	5900±570.09^b	37.50±2.69^c	62.50±2.69^c

Values are Means±SEM. Values with different superscripts are considered to be significantly different ($p \leq 0.05$)

Table 2: Haematological parameters (Hb, WBC and differentials) in female rats

Experimental groups	Haematological parameters				
	Hb (g dL^{-1})	PCV (%)	WBC (mm^{-3})	Neutrophils (%)	Lymphocytes (%)
Control	10.70±1.39[a]	3.7±1.78[a]	9375±302.11[a]	47.50±6.58[a]	51.25±7.53[a]
Kerosene-exposed	9.90±0.34[b]	3.0±1.54[b]	7000±141.42[b]	57.50±3.65[b]	41.00±1.80[b]
Petrol-exposed	9.45±0.34[b]	2.9±1.54[b]	7000±141.42[b]	60.00±9.50[b]	39.50±1.22[b]

Values are means±SEM. Values with different superscripts are considered to be significantly different ($p \leq 0.05$)

kerosene and petrol showed a significant increase ($p \leq 0.05$) in Neutrophils level when compared to the control. Results in this study also showed a significant ($p \leq 0.05$) decrease in lymphocyte counts in male and female rats in the two test groups relative to the control (Table 1, 2).

DISCUSSION

Haematological parameters have often been associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health (Patrick-Iwuanyanwu *et al*., 2007). This study has demonstrated that long-term exposure to petroleum samples particularly kerosene and petrol induces anaemia. The resulting anaemia is in accordance with the report of Krishan and Veena (1980), which showed the suppressive effect of petroleum samples on erythropoiesis. Leighton *et al*. (1985) characterized haemolytic anaemia as hallmark of oil toxicity in animals. In the same vein, Sudakov (1992) and Marieb (1995) have shown that the toxic components especially those in petroleum products change blood chemistry and induce anaemia by causing bone marrow hypoplasia and interfered with platelet production in the animal, hence the reduced values of Hb and PCV. Similarly, altered blood parameters have been reported in other forms of life exposed to petroleum products. Lutcavage *et al*. (1995) demonstrated the toxicity of petroleum in different species of birds, which showed significantly reduced red blood cell counts and red blood cell polychromasia with inherent regenerative anaemia. Altered blood parameters in fish exposed to pollutants have also been reported by Cowell (1971), Christensen *et al*. (1972), Anderson *et al*. (1974), Sabo and Stageman (1977), Linden (1978), Igboh (1999) and Peters (2001). Haematological changes induced by these petroleum samples demonstrated that chronic (long term) exposure of rats to petroleum products induces anaemia through the reduction of Hb and PCV probably as a result of the suppressing effect of petroleum samples on erythropoiesis (Krishan and Veena, 1980). Erythrocytopenia observed in this study in rats fed diets contaminated with kerosene and petrol could be as a result of the suppressing effect of poly aromatic hydrocarbons (PAHs) present in these petroleum products on erythropoiesis. This observation is similar to the findings of Patrick-Iwuanyanwu *et al*. (2007) in CCl_4-induced haematological anomalies in rats. Fairbarks (1967) showed that xenobiotics cause haemolytic anaemia when sulphydryl groups of the erythrocyte membrane are oxidized, which inflicts injury to the erythrocyte membrane. This is in agreement with Hb and PCV values in this study as rats fed kerosene and petrol-contaminated diets recorded lower values.

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 (Robbins and Angel, 1976; Marieb, 1995). In this study, male and female rats exposed to kerosene and petrol recorded a decrease in the total white blood cell count. This observation is similar to the findings of Ovuru and Ekweozor (2004) where WBC decreased with an increase in crude oil contamination in experimental rabbits. The result of this study is also in agreement with the findings of Ngodigha *et al*. (1999) who observed a reduction in total white blood cell in

goats as the level of crude oil contamination increased. They argued that the reduction in total white blood cell count in goat may be a combination of stress imposed by crude oil hydrocarbons. Okoro *et al*. (2006) also observed a significant reduction in WBC in humans of both sexes exposed to petroleum fumes. Benzene is reported to produce haematological changes ranging from pancytopaenia to total bone marrow aplasia, effected through its myelotoxic action (D'Azevedo *et al*., 1996). Xylene is also reported to cause leukocytopaenia (D'Azevedo *et al*., 1996). The decrease in WBC observed in this study is possibly as a result of pancytopaenia and leukocytopaenia, which may result in impaired migration of phagocytic cells, lower resistance to viruses, bacteria and foreign bodies (Marieb, 1995). The reduction in white blood cell count as observed in this study could also be a combination of stress imposed by the hydrocarbons present in kerosene and petrol.

The increase in neutrophils as observed in male and female test rats used in this study could be an indication of stress imposed by kerosene and petrol in the diets, this confirms Selye (1963) findings that a stress stimulus elicits a defense response. This is also in agreement with the findings of Ovuru and Ekweozor (2004). However, a reduction was observed in the values of lymphocytes of male and female rats exposed to kerosene and petrol. Similar observations were made by Thompson and Lippma (1974) as well as Sudakov (1992) who pointed out that Adrenocorticotropic hormone (ACTH) and glucocorticoids cause the regression of lymphoid tissues which have been found to cause antibody depression, impaired migration of phagocytic cells, lower resistance to viruses and foreign bodies (Spain, 1975). The result of this study is also similar to the observation of Ovuru and Ekweozor (2004).

In conclusion, the petroleum products namely, kerosene and petrol caused a reduction in levels of haemoglobin (Hb), packed cell volume (PCV) and white blood cells (WBC). It is hereby suggested that kerosene and petrol are environmental stressors and thus have serious consequences on haematological parameters in rats.

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