Toxicological and Haematological Effects of Pyrene on Whit Taker Albino Mice

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Abstract—This study explores the effect of the haematological toxicity of pyrene (toxicant) on Whit taker Albino mice. The method for haematological toxicity was oral administration of different concentrations (50, 120,250 mg of pyrene kg/b.w.) using olive oil as diluent into adult male and female Whit taker Albino mice approximately 8-12 weeks of age. The average weight ranges from 23.12- 23.30 for 35 days. After the experimental days, blood was collected from the mice through cardiovascular puncture. The result of the haematological toxicity analysed showed that pyrene caused significant decrease in white blood cells (WBC) and slight decrease in the red blood cell (RBC), haemoglobin (Hb), packed cell volume (PCV), platelets, neutrophil and significant decrease in the weight of mice. Toxicity of pyrene is directly proportional to increase in concentration and time of exposure. Precautionary measures must be taken by monitoring agencies and various petrochemical companies that discharge several PAHs into our environment. Efforts should be made to educate society on the economic and health implications of these toxicants in the environment.

Keywords — Pollution, toxicants, Polycyclic Aromatic Hydrocarbons, Haematological Toxicity, Environmental Degradation, Pyrogenic Toxicant, Petrogenic Toxicant

1 INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are pollutants in the ecosystems which are released into the environment through pyrogenic and petrogenic sources. These compounds and their derivatives are produced from incomplete combustion of organic materials or natural processes such as forest fires and volcanic eruptions. Above all, they may be produced as a result of human activities. In recent times, some important sources of PAHs pollutants include industrial processes, transportation, waste burning, evaporation and burning of plastic residues. One of the most common ways for PAHs to enter the body is by inhalation of polluted air or consuming PAH-polluted water or food. Clinical studies show that high concentration of a PAH compounds can cause a variety cancers of the skin, lung, stomach, and liver [1]. The PAH molecules exhibits biochemical persistence due to the dense cloud of π electrons on both sides of ring structures. Hence, PAHs compounds are more resistant to nucleophilic attack. PAHs compounds exhibit various noxious and hazardous properties and thus, are toxic, potential mutagens and carcinogens [2]. Petrogenic PAHs are introduced into the aquatic environment through accidental oil spills, discharge from routine tanker operations, municipal and urban runoff, etc. [3]. Pyrogenic PAHs are released in the form of exhaust and solid residues, and are largely prevalent in aquatic environments [5]. According to United States Environmental Protection Agency (USEPA) among all known PAHs Benzo[a]pyrene is recognised as the most dangerous pollutant due to its extreme carcinogenic potential. It is also a major component of smoke released from the cigarettes, coke oven emissions and roofing tar emissions [5]

Pyrene is one of the polycyclic aromatic hydrocarbons (PAHs) which have been considered priority pollutants by the United States Environmental Protection Agency emissions [5]. PAHs are ubiquitous environmental pollutants produced during incomplete combustion of fossil fuels, organic wastes and various industrial processes. Many of them have been found to exhibit cytotoxic, mutagenic and carcinogenic properties and therefore pose a serious risk to human health [5]. Pyrene is a toxic, recalcitrant, four fused ring PAH commonly found in soil. Its quinone-based metabolites are mutagenic and more toxic than the parent compound. For these reasons, pyrene is listed among the 16 USEPA priority pollutants [6]. Pyrene released to the atmosphere will likely be associated with particulate matter and may be subject to long distance transport, depending mainly on the particle size distribution and climatic conditions which will determine the rates of wet and dry deposition. If released to water, pyrene will be expected to adsorb very strongly to sediments and particulate matter. It will not hydrolyse but may undergo slight to moderate bioconcentration. If pyrene is released to soil, it will be expected to adsorb very strongly to the soil and will not be expected to leach to the groundwater. Although no information concerning biodegradation in soil was located, pyrene has been shown to be metabolized in laboratory tests by microorganisms isolated from soils and natural waters [7].

Hence, this analysis was carried out to study the haematological effects of pyrene using Whit taker albino mice as a case study. The result will further confirm pyrene as a highly toxic PAH which should strictly monitored to reduce or eliminate its effects on human health.

2 MATERIALS AND METHODS

Sample collection

Pyrene

Pyrene was purchased from Sigma Aldrich Germany (CAS:

129-00-0, C16H10, MW: 202.25 g/mol, mp: 145-148 °C (lit.). The test chemical is greater than or equal to 98% pure (HPLC).

Mice

A total of 24 adult male and female mice (8-12 weeks of age and weight ranging from 13.17g - 27.31g) were purchased from Christian Farm beside General Hospital Mgbakwu, Awka-North L.G.A. of Anambra state, Nigeria

Sample preparation

Pyrene

Different concentrations (50mg/kg, 120mg/kg, and 250mg/kg) of pyrene hydrocarbon were prepared using olive oil as diluent.

Mice

Animals were housed in groups of 6 mice/ aluminium wire cage and maintained in a controlled room temperature of about 25±2^{O}C, 12 hr light: dark cycle for one week prior to initiation of studies and throughout the experimental period of 35 days. The mice were allowed *ad libitum* access to grower feed of poultry and water.

Experimental Animal Design

An equal number of male and female animals were assigned to a control group and 3 treatment groups. The test chemical (pyrene) was dissolved in olive oil and 0.1ml was administered orally. After one week of acclimatization, the animals were weighed and randomly divided into two groups (6 mice in one group and 18 mice in group 2).Group 2 was subdivided into 3 subgroups (6 mice per subgroup ratio 1:1 for male: female) according to the dose concentration (50,120,250mg/kg).Group 1 were administered orally with 0.1ml olive oil only and served as control while group 2 were administered with similar doses (50,120,250mg/kg) daily for 35 days.

Blood Sample Collection

After the experimental period (35 days) for pyrene treated mice, blood was collected in a vacuum blood collection tube containing anticoagulant (EDTA) using agarine sterile insulin syringe (wuxiyoshon China) through cardiovascular puncture.

Blood Analysis

Haematological parameters used in this study includes white blood cell, red blood cell, haemoglobin, packed cell volume, platelets, neutrophils, lymphocytes, monocytes, eosinophils, basophils and were analysed at Comax international laboratory Anambra state.

Statistical Analysis

Statistical analyses for this study was performed and the ressults obtained from this study were expressed as mean ±SD(standard deviation). Two types of analysis were performed: one way analysis of variance (ANOVA) for comparison between groups of each dose concentrations and independent T test to compare each dose of pyrene and its corresponding control. The results were considered statistically significant if the $P \leq 0.05$.

3 RESULTS

Animal Study

Average weight of mice before and after the experiments are presented in table 1 below, minimum and maximum weight of the mice before the oral exposure ranges from 23.12to 27.11 and after the oral exposure ranges from 20.44 to 23.45.from the statistical analysis (students T test) P≤0.05 hence you accept alternate hypothesis

Table 1: Average weight of mice before and after 35 days of oral exposure to different concentrations of pyrene to mice

Concentrations	Before(g)	After(g)
50mg/kg	23.12±0.40	20.88±0.37
120mg/kg	23.30±0.35	19.43±0.04
250mg/kg	23.30±0.35	17.88±0.09

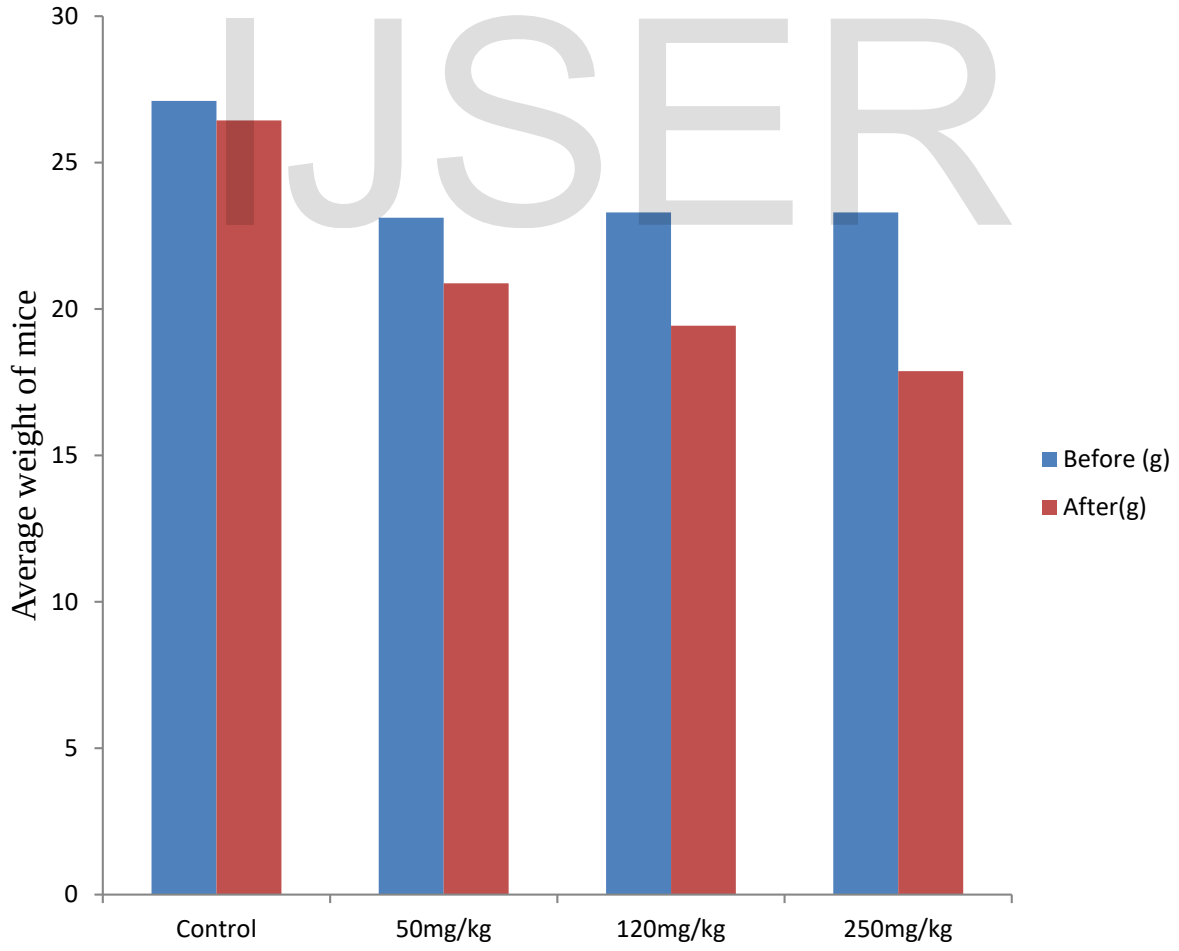

Figure 1: Average weight of mice before and after 35 days of oral exposure to different concentrations of pyrene to mice

Haematological Analysis

The haematology resultsof pyrene on mice at concentration 50mg/kg, 120mg/kg, 250mg/kg are presented in table 2. From the statistical analysis(ANOVA) P≤0.05 which shows that there is a significant difference between the different concentration of pyrene inoculated into the mice

Table 2: Effect of pyrene on haematological parameters in mice at 50mg/kg,120mg/kg and 250mg/kg

Test	50mg/kg	120mg/kg	250mg/kg	Reference range
WBC($\times 10^3$)	5.3±0.4	2.3 ±0.4	2.9 ±0.5	5.0-12.0 /L
RBC($\times 10^{12}$)	5.5±0.5	4.6±0.5	4.6±0.5	4.0-5.2 /L
Haemoglobin	16.5±0.5	13.9±0.5	13.8±0.6	11.0-16.0g/dl
PCV	51.0±0.6	42.0±0.4	42.6±0.7	35-49%
Platelets($\times 10^{10}$)	88.5±0.5	53.3±0.5	113.5±0.6	10.0-30.0$\times 10^{10}$/L
Neutrophils	30.0±0.2	40.0±0.5	30.0±0.2	40-75%
Lymphocytes	68.6±0.5	54.2±0.6	60.0±0.5	20-45%
Monocytes	1.0±0.4	3.8±0.8	5.8±0.5	2-10%
Eosinophils	0.4±0.1	2.0±0.6	3.0±0.8	1-6%
Basophils	0.0±0.0	0.0±0.0	1.0±0.4	0-1%

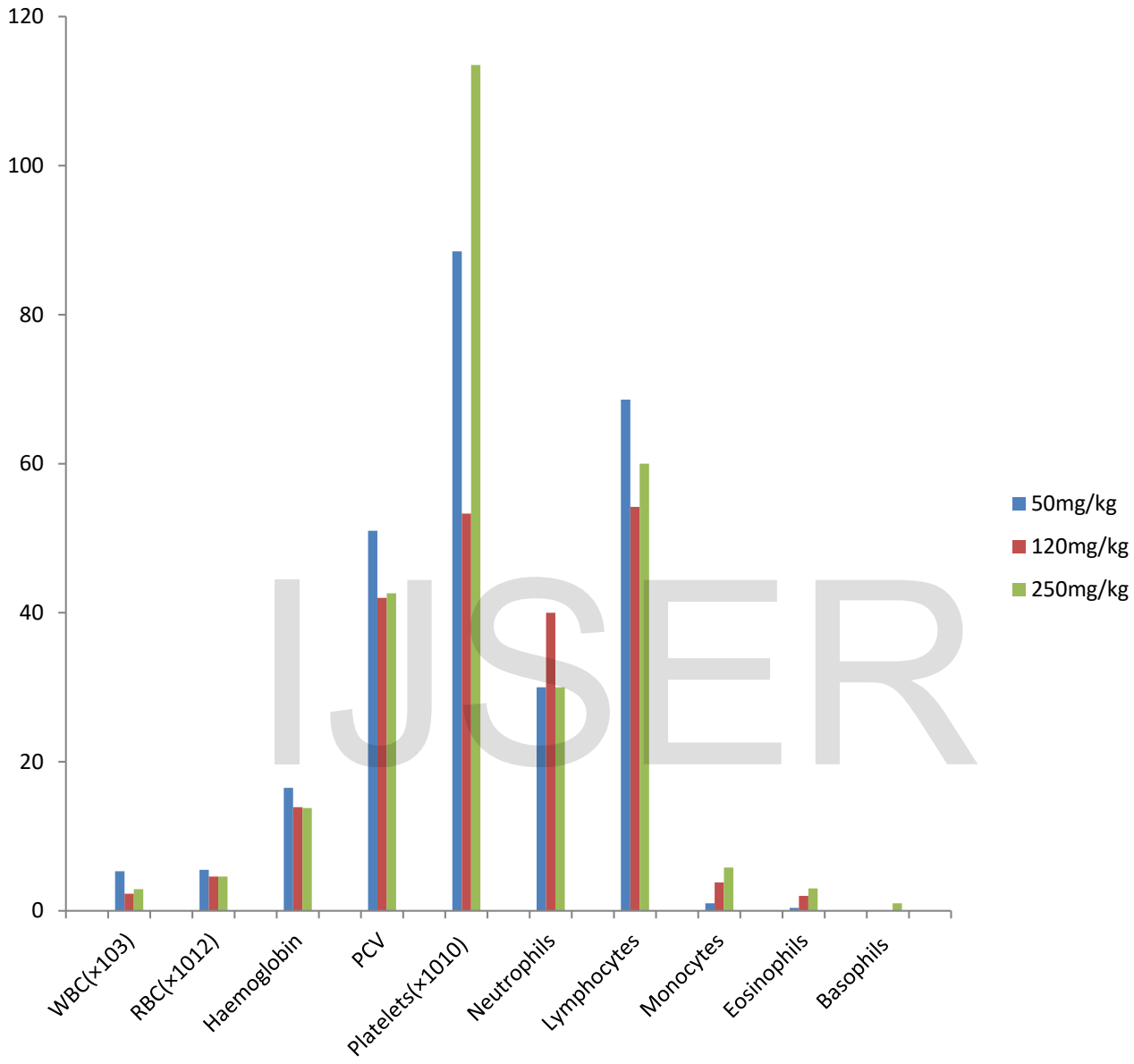

Figure 2: Effect of pyrene on haematological parameters in mice at 50mg/kg,120mg/kg and 250mg/kg

Table 3: Comparing the effect of haematological parameters at 50mg/kg of pyrene with control

Test	50mg/kg	Control	Reference range
WBC($\times 10^3$)	5.3±0.4	5.8±0.5	5.0-12.0 /L
RBC($\times 10^{12}$)	5.5± 0.5	3.3±0.6	4.0-5.2 /L
HB	16.5±0.5	9.9±0.7	11.0-16.0g/dl
PCV	51.0±0.6	30.0±0.2	35-49%
Platelets($\times 10^{10}$)	88.5± 0.5	18.5±0.3	10.0-30.0$\times 10^{10}$/L
Neutrophils	30.0±0.2	23.0±0.7	40-75%
Lymphocytes	68.6±0.5	60.0±0.5	20-45%
Monocytes	1.0±0.4	12.0±0.4	2-10%
Eosinophils	0.4±0.3	5.0±0.7	1-6%
Basophils	0.0±0.0	0.0±0.0	0-1%

Table 4: Comparing the effect of haematological parameters at 120mg/kg of pyrene with control

Test	120mg/kg	Control	Reference range
WBC($\times 10^3$)	2.3 ±0.4	5.8±0.5	5.0-12.0 /L
RBC($\times 10^{12}$)	4.6± 0.5	3.3±0.6	4.0-5.2 /L
HB	13.9±0.5	9.9±0.7	11.0-16.0g/dl
PCV	42.0±0.4	30±0.2	35-49%
Platelets($\times 10^{10}$)	53.3± 0.5	18.5±0.3	10.0-30.0$\times 10^{10}$/L
Neutrophils	40.0±0.5	23.0±0.7	40-75%
Lymphocytes	54.2±0.6	60.0±0.5	20-45%
Monocytes	3.8±0.8	12.0±0.4	2-10%
Eosinophils	2.0±0.6	5.0±0.7	1-6%
Basophils	0.0±0.2	0.0±0.2	0-1%

Table 5: Comparing the effect of haematological parameters at 250mg/kg of pyrene with control

Test	250mg/kg	Control	Reference range
WBC($\times 10^3$)	2.9 ±0.5	5.8±0.5	5.0-12.0 /L
RBC($\times 10^{12}$)	4.6± 0.5	3.3±0.6	4.0-5.2 /L
HB	13.8±0.6	9.9±0.7	11.0-16.0g/dl
PCV	42.6±0.7	30±0.2	35-49%
Platelets($\times 10^{10}$)	113.5±0.6	18.5±0.3	100-300$\times 10^{10}$/L
Neutrophils	30.0±0.2	23.0±0.7	40-75%
Lymphocytes	60.0±0.5	60.0±0.5	20-45%
Monocytes	5.8±0.5	12.0±0.4	2-10%
Eosinophils	3.0±0.8	5.0±0.7	1-6%
Basophils	1±0.4	0.0±0.2	0-1%

4 DISCUSSIONS

The experimental mice were weighed before and after the 35 days of oral administration of different concentrations of pyrene and their average weight were presented in Table 1. The result shows that there is a marked decrease in the weight of the mice. From the statistical analysis (independent T-test), P ≤05, the decrease in their weight could be as a result of toxicity potential of pyrene or other factor could be from the feed which might contain contaminant that can slow down the rate of their body metabolism. A literature shows oral administration of pyrene to infant mice for 13 weeks and the results showed slight haematological changes such as decrease in RBC, PCV, Hb [8]. After 35 days oral administration of different concentrations of pyrene, several haematological parameters obtained were presented in Table 2 with their reference ranges. The statistical analysis(ANOVA) shows that

P ≤0.05.Table 3 compares the effects of pyrene on haematological parameters on mice administered 50mg/kg b w with the control mice, there was slight decrease in WBC while the other parameters has slight increase with exception of basophil and eosinophils responsible for allergic reaction and parasitic infection. Table 4 shows comparison between the 120mg/kg treated mice and the control. The results show slight increase in in RBC, Hb, PCV, platelets and decrease in WBC count. The last table (Table 5) compares the 250mg/kg treated mice and the control which shows a significant decrease in the WBC because the bone marrow ceases to produce blood cells in the right proportion and the spleen takes over this process.

5 Conclusion

Evaluation of this study shows the effect of pyrene on haematological parameters such as WBC, RBC, Hb on Whit taker Albino mice at different concentrations. Pyrene is toxic to the environment; both plants and animals. Toxicity as a result of the emission of pyrene increases with increasing time and dose therefore extreme measures should be applied to abate environmental pollution with PAHs by the educating the society on the economic and health implications of these toxicants in the environment. Governments should enforce strict policies to ensure that the emission of PAHs is limited. Further study should be carried out on the genotoxicity, neurotoxicity, immunotoxicity and embroyonicdevelopment of PAHs

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