Effect of Oral Exposure to Nitrocellulose Thinner on Haematological Profiles of Male Albino Wistar Rats

Friday E. Uboh, Itoro F. Usoh, Promise Nwankpa and Godwin O. Obochi

1Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria
2Department of Biochemistry, Faculty of Health Sciences, University of Uyo, Uyo, Nigeria
3Department of Medical Biochemistry, Faculty of Medicine, Imo State University, Owerri, Nigeria
4Department of Biochemistry, Cross River University of Technology, Calabar, Nigeria

Corresponding Author: Friday E. Uboh, Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria

ABSTRACT

Haematological profiles provide important information about the internal environment of a given organism. In this present study, varying concentrations (10, 15, 20 and 25 mg kg⁻¹) of nitrocellulose thinner were orally administered to male albino rats, as single daily dosages for 30 days, to assess the haematological changes associated with oral exposure to solvent. The results showed a significant (p<0.05) dose-dependent decrease in Red Blood Cells (RBC) count, haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), neutrophils and increase in total White Blood Cells (WBC), platelets and lymphocytes, compared to the control. However, there was no significant (p>0.05) difference in the changes in the level of these parameters in rats exposed to 10 mg kg⁻¹ when compared to the rats exposed to 15 mg kg⁻¹ and 20 mg kg⁻¹ when compared to the rats exposed to 25 mg kg⁻¹ of the solvent. The results recorded in this present study suggested that exposure to nitrocellulose thinner’s constituents, or their metabolites, may be a risk factor for haematotoxicity in mammals. Hence, the haematotoxic properties of nitrocellulose thinner are reported in the study.

Key words: Nitrocellulose thinner, haematotoxicity, blood cells, rats

INTRODUCTION

Nitrocellulose thinner is an industrial solvent containing different organic chemical substances, such as ethylbenzene or toluene and butyl acetate. These chemical substances are known to constitute chemical pollutants in the environments where they are used. Particularly, they have been detected in household and workplace air (WHO, 1996, 2005). Nitrocellulose thinner is generally used in mixtures with other solvents in domestic or industrial products (e.g., varnishes). Particularly, the solvent is used in furniture, paint and automobile spray painting industries. Hence, furniture, paints and automobile spray painting workers may be considered to be among those that are frequently exposed to this solvent, occupationally. Typically, occupational exposure to mixtures of toluene, ethylbenzene and butyl acetate have been reported in workplaces involving painting or lacquering (Vincent et al., 1994; Muttray et al., 1995; Seeber et al., 1996; Jovanovic et al., 2004). According to ATSDR (2000), toluene is readily absorbed from the respiratory and gastrointestinal tracts and to some degree through the skin. While 40-60% of
inhaled toluene is reported to be absorbed (IPCS, 1985), oral absorption is reported to be complete (ATSDR, 2000). Exposure to different organic solvents as gasoline, benzene, toluene, hexane, carbon disulphide, insecticides and pesticides such as malathion, methyl parathion, phosphonid, monocrotophos and fenvalerate, among others, has been reported to cause adverse effects on the haematological profiles in animal and humans (Synder and Hedli, 1996; Dhembare and Pandhe, 2000; Uboh et al., 2005, 2007-2010; Savithri et al., 2010; Ita and Udofia, 2011).

The adverse effects reported to be associated with exposure to different chemical substances are likely to results from the interaction of the metabolites of the constituents of these solvents with blood components, or blood forming tissues. Blood is one of the specialized body fluids, responsible for the transportation of such chemical substances as nutrients, oxygen, hormones and other metabolites to the body's cells and metabolic waste products away from those cells to sites of elimination. It is known to be the most important body fluid that regulates various vital functions of the body, including respiration, circulation, excretion, osmotic and temperature balance, as well as the transport of several metabolic substances. Circulation of blood within the mammalian system transports such specific chemical substances as gases, nutrients, minerals, metabolic products and hormones between different tissues and organs (Baynes and Dominiczak, 2005).

Blood or haematological parameters are probably the more rapid and detectable variations under stress and are fuel in assessing different health conditions (Hymavathi and Rao, 2000). Hence, the significance of haematological parameters in clinical and experimental studies in life sciences cannot be overemphasized. Particularly, literature reports have proved that the alterations in the haematological parameters, from normal state/levels, may be used as valuable indicators of disease, or stress in different animal species (Solanki and Singh, 2000; Das and Mukherjee, 2003; Jee et al., 2005; Rahman and Siddiqui, 2006; Yakubu et al., 2002; Uboh et al., 2005, 2007-2010). Literature reports indicated that haematological profile of different species of animals may be influenced adversely by phenylhydrazine (Sanni et al., 2005), some antiretroviral drugs (Kayode et al., 2011), diabetic condition (Edet et al., 2011) and aqueous Ocimum gratissimum leaf extract (Obianime et al., 2011). On the other hand, different extracts of some plants' parts have been reported to express a positive impact on the haematological profile of several animal species (Sanni et al., 2005; Edet et al., 2011; Ilkeme et al., 2011; Kolawole et al., 2011, Prasad and Priyanka, 2011). Assessment of hematological parameters can therefore be used to determine the extent of deleterious effect of foreign substances on the blood constituents of an animal. The present investigation was therefore aimed at assessing the effect of nitrocellulose thinner on the haematological parameters in albino rats.

MATERIALS AND METHODS

Animal handling and experimental design: Twenty five mature albino Wistar rats, weighing between 120 to 150 g were obtained from Biochemistry Department Experimental Research Animal House of the University of Calabar, Calabar, Nigeria. They were fed with a standard laboratory diet and tap water. Illumination was 12 h light/dark cycle and room temperature was 25±2°C. The animals were divided into five groups, i.e., one control (I) and four experimental groups (II, III, IV and V) which consisted of five apparently normal albino Wistar rats per group. The experimental groups II, III, IV and V were exposed daily to 10, 15, 20 and 25 mg kg⁻¹ body weight, respectively, of nitrocellulose thinner by oral administration for 30 days, while the control group was given normal saline. In this study, all the animal experimentations were carried out following the guidelines for the care and use of laboratory animals obtained from the Institutional Animal Ethics Committee.
Collection and preparation of blood specimen for analyses: Blood samples for bioassays were obtained from rats by cardiac puncture, under chloroform vapour anaesthesia, after 48 h of termination of nitrocellulose thinner administration into EDTA treated screw-cap sample bottles. The anticoagulated blood samples were used for haematological analyses. All haematological analyses were carried out within 24 h of sample collection.

Haematological analyses: Full blood counts including Red Blood Cells (RBC), Packed Cell Volume (PCV), haemoglobin (Hb), Total White Blood Cells (TWBC), platelet count, differential WBC (including lymphocytes, neutrophils, monocytes and eosinophils) and red cell indices, including Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC), were estimated using the Sysmex® Automated Haematology Analyzer KX-21N, Sysmex Corporation, Kobe-Japan.

Statistical analysis: The results obtained in this study were presented as Mean±SEM. Statistical analysis was carried out using SPSS window statistical software programme. One-way analysis of variance (ANOVA) was used for comparison of the mean differences among and within the respective groups. This was followed by paired-wise post hoc test using Student t-test. The mean differences were considered significant at p-value less than 0.05 (p<0.05).

RESULTS

The results of this study, on the effect of oral exposure to nitrocellulose thinner on the haematological parameters in rats, are presented in Table 1 and 2. The results showed that the Red Blood Cells (RBC) count, haemoglobin (Hb), Packed Cell Volume (PCV), mean corpuscular volume (MCV), Mean Corpuscular Haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and percentage neutrophils levels obtained for rats exposed to nitrocellulose thinner were significantly (p<0.05) lower in a dose-dependent pattern, compared to the control (Tables 1 and 2). On the contrary, the total White Blood Cells (WBC), platelets and lymphocytes levels obtained for rats exposed to nitrocellulose thinner, following the same pattern, were significantly (p<0.05) higher, compared to the control (Table 2). However, the results of this present study also indicated that there was no significant (p>0.05) difference observed in the changes in the level of these parameters in rats exposed to 10 mg kg⁻¹ when compared to the rats exposed to 15 and 20 mg kg⁻¹ when compared to the rats exposed to 25 mg kg⁻¹ of the solvent.

Table 1: Effect of nitrocellulose thinner on red blood cells, packed cell volume, haemoglobin and red blood indices in rats after 30 days of oral exposure

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>RBC (×10⁶ μL⁻¹)</th>
<th>PCV (%)</th>
<th>Hb (g dL⁻¹)</th>
<th>MCV (fL)</th>
<th>MCH (pg)</th>
<th>MCHC (g dL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control (Normal saline)</td>
<td>8.72±2.12</td>
<td>47.64±2.11</td>
<td>13.85±1.86</td>
<td>62.32±2.00</td>
<td>18.86±1.00</td>
<td>29.46±1.02</td>
</tr>
<tr>
<td>II</td>
<td>10 mg kg⁻¹ of NeT</td>
<td>6.80±1.85*</td>
<td>43.20±1.45*</td>
<td>11.78±1.00*</td>
<td>60.62±2.06*</td>
<td>16.52±1.22*</td>
<td>27.42±1.30**</td>
</tr>
<tr>
<td>III</td>
<td>15 mg kg⁻¹ of NeT</td>
<td>6.52±1.28**</td>
<td>41.88±2.61***</td>
<td>10.46±1.28***</td>
<td>60.06±1.86***</td>
<td>16.00±1.33***</td>
<td>27.08±1.52***</td>
</tr>
<tr>
<td>IV</td>
<td>20 mg kg⁻¹ of NeT</td>
<td>5.00±1.46*</td>
<td>38.24±1.08*</td>
<td>8.68±1.56*</td>
<td>57.48±2.00*</td>
<td>13.44±1.50*</td>
<td>24.22±1.40**</td>
</tr>
<tr>
<td>V</td>
<td>25 mg kg⁻¹ of NeT</td>
<td>4.96±2.00*</td>
<td>37.69±2.20*</td>
<td>8.00±1.62*</td>
<td>56.86±1.78*</td>
<td>13.40±1.78*</td>
<td>23.06±1.44*</td>
</tr>
</tbody>
</table>

Data are presented as Mean±SEM, n = 5, *p<0.05 compared with I, **p<0.05 compared with II, ***p<0.05 compared with IV, ****p<0.05 compared with II, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean cell haemoglobin Concentration, NeT: Nitrocellulose thinner.
Table 2: Effect of nitrocellulose thinner on the platelets, total white blood cells and differential cell counts in rats after 30 days of oral exposure

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Platelets (×10^6 µL^-1)</th>
<th>TWBC (×10^9 µL^-1)</th>
<th>Lymphocytes (%)</th>
<th>Monocytes (%)</th>
<th>Neutrophils (%)</th>
<th>Eosinophils (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control (Normal saline)</td>
<td>806.95±2.55</td>
<td>15.06±1.32</td>
<td>72.08±2.00</td>
<td>2.62±0.65</td>
<td>23.42±2.56</td>
<td>2.88±0.05</td>
</tr>
<tr>
<td>II</td>
<td>10 mg kg^-1 of NoT</td>
<td>870.39±2.00</td>
<td>18.22±2.99</td>
<td>77.80±1.86</td>
<td>2.68±0.78</td>
<td>20.04±1.88</td>
<td>2.90±0.46</td>
</tr>
<tr>
<td>III</td>
<td>15 mg kg^-1 of NoT</td>
<td>873.31±1.68^b</td>
<td>18.63±1.87^b</td>
<td>78.92±1.57^b</td>
<td>2.87±0.36^b</td>
<td>19.42±1.05^b</td>
<td>2.94±0.30^b</td>
</tr>
<tr>
<td>IV</td>
<td>20 mg kg^-1 of NoT</td>
<td>880.02±2.02^a</td>
<td>23.56±1.58^a</td>
<td>82.33±1.82^a</td>
<td>2.90±0.69</td>
<td>15.30±1.22^a</td>
<td>3.08±0.66</td>
</tr>
<tr>
<td>V</td>
<td>25 mg kg^-1 of NoT</td>
<td>884.19±1.88^a^d</td>
<td>24.02±1.69^a^d</td>
<td>84.56±1.70^a^d</td>
<td>2.92±0.72^d</td>
<td>14.88±1.20^a^d</td>
<td>3.16±0.58^c</td>
</tr>
</tbody>
</table>

Data are presented as Means±SEM, n = 5. ^a,b^p<0.05 compared with I, ^c^p<0.05 compared with II, ^d^p<0.05 compared with IV, ^e^p<0.05 compared with II, NoT: Nitrocellulose thinner

DISCUSSION

Haematological profiles are known to provide important information about the internal environment of a given organism. Several factors, including nutritional and environmental, have been reported to pose a variety of adverse effects on the haematological profiles of most organisms. Nutritional factors include folic acid or Vitamin B12 deficiency (Jee et al., 2005; Murray et al., 2007), while the environmental factors include exposure to solvents generating environmental pollutants, such as gasoline, benzene, toluene, hexane, carbon disulphide, insecticides and pesticides such as malathion, methyl parathion, phosphomidon, monocrotophos and fenvalerate, among others (Synder and Hedli, 1996; Dhembare and Pandhe, 2000; Uboh et al., 2005, 2007-2010; Savithri et al., 2010; Ila and Udofia, 2011). The results of this present investigation showed that oral exposure to nitrocellulose thinner caused a significant decrease in RBC, Hb and PCV, MCV, MCH, MCHC, neutrophils; and increase in WBC and lymphocytes. The observations made from the results of this study correlate with that of our earlier work on gasoline vapour (Uboh et al., 2005, 2007-2010). Similar effects on haematological parameters have been reported for such insecticides and pesticides as chlorpyrifos (Savithri et al., 2010), thiodan 55 E.C (Solank and Singh 2000), chlorpham (Fujitani et al., 2001), endosulfan (Choudhary and Joshi, 2002) and lindane and endosulfan (Baig, 2007) and deltamethrin (Yeeken et al., 2007). The haematoxic condition may results from different mechanisms, including decrease in the rate of blood cells synthesis and/or increase in the rate of blood cells destruction.

Although the specific mechanism(s) responsible for haematoxic effects reported to be associated with exposure to nitrocellulose thinner was not considered in this study, it is possible that the solvent's constituents or their metabolites might have interacted with the blood-forming tissues/organs. Such interaction(s) may cause inhibit the rate at which some specific or generalised haemopoietic committed stem cells are synthesized by these tissues. For instance, it has been reported that benzene, carbon disulphide and hexane induced haematoxic effect is associated with the interaction of their metabolites with the haematopoietic tissues and cause depression of their haematopoietic actices (Amarnath et al., 1991; Valentine et al., 1993; Synder and Hedli, 1996). The reports also indicated that some of the metabolites of these solvents can interact with the red blood cell membrane proteins to increase the rate of red blood cells destruction. The observed decrease in RBC count, Hb and PCV may therefore be assumed to be associated with retarded haemopoiesis, destruction and shrinkage of RBC. While the decrease in MCV, MCH and MCHC recorded in this present investigation may likely be due to destruction of RBC and decrease in Hb synthesis and hemoglobin content. The haematoxic condition reported in this study, according to the report of Rahman et al. (1990) and Savithri et al. (2010) suggests that exposure to
nitrocellulose thinner may induce anaemic condition rats. The anaemic condition observed in this study may then be due to the inhibition of erythropoiesis and haemosynthesis in the haemopoietic organs and/or increase in the rate of erythrocytes destruction by the nitrocellulose thinner's constituents, or their metabolites. Based on the report of Patel et al. (2006) on induction of DNA damage in hematopoietic system, including, spleen, bone marrow and lymphocytes in mouse, the results of this study also indicated that nitrocellulose thinner might have induced chromosomal berrations and micronucleus formation in rat bone marrow and other haematopoietic tissues.

Increase in total white blood cells and lymphocyte, as well as decrease in neutrophils, is also reported in this study. These results are consistent with the reports of some researchers on the effect of such insecticides and pesticides as aldrin, dione, endosulfan, fenvalerate, lindane, malathion, methyl parathion, monocrotophos, novel phosphorothionate and phosphonidon, among others, on the total white blood cells and the differential counts in experimental animals (Massod et al., 1991; Kumar et al., 1996; Synder and Hedli, 1996; Dhembare and Pandhe, 2000; Rahman and Siddiqui, 2006; Baig, 2007; Savithri et al., 2010). The increase in total white blood cells and lymphocyte observed in this work, may be suggested to be due to stimulated lymphopoiesis and/or enhanced release of lymphocytes from lymph myeloid tissue (Das and Mukherjee, 2003). This lymphocyte response might be a direct stimulatory effect of toxic substances on lymphoid tissues. Alternatively, this response may be assumed to be associated with the pollutant induced tissue damage and disturbance of the non-specific immune system leading to increased production of leukocytes. Also, a decrease in the percentage of neutrophils in peripheral blood reported in this study agrees with the findings of Savithri et al. (2010) on the effect of different doses of chlorpyrifos on the neutrophils count in rats. Moreover, studies on human beings involving in the production of liquid pesticides (Klucinski et al., 1996) and mice intoxicated with higher deltamethrin dose (Harstym, 2002), reported a significant decrease in the number of neutrophils. Generally, neutrophils are known to be involved in the phagocytosis of foreign chemical substances in the body, during which some of them are ruptured. This may therefore explain why the neutrophils count consistently decreased following administration of different doses of nitrocellulose thinner in rats.

CONCLUSION

In conclusion, significant adverse changes in haematological parameters are reported to be associated with exposure to nitrocellulose thinner, in this present study. This therefore suggest that exposure to nitrocellulose thinner may be considered to be among the risk factors for the development of anaemic condition in mammals. Hence, exposure to this solvent, in the course of occupational involvements, should be with some cautious measures to avoid the risk of suffering from anaemia.

REFERENCES


