Original Research Article

HAEMATOLOGICAL EFFECT OF TOLUENE IN WISTAR RATS

Abstract

Toxicity of toluene arising from solvent abuse, occupation hazards and environmental pollution has generated a lot of concern in recent times. Young people are getting more involved in the abuse of toluene by deliberate inhalation of toluene-containing substances which may result in high level of exposure to toluene. This abuse may have adverse effect on their health. This study was therefore designed to investigate the effect of oral exposure to toluene on haematological parameters using male albino rats as model. Twenty animals were randomly assigned to 4 groups of 5 rats each. Group A (Control) received 0.5 ml of olive oil (vehicle) while groups B, C and D received 31.8, 63.6 and 127.2 mg/kg respectively of toluene for 21 days by oral gavage. At the end of the treatments, the animals were anaesthetized and blood samples were collected for haematological investigations. No significant ($p > 0.05$) variation occurred in the mean values of PCV, haemoglobin concentration, RBC and platelet counts in comparison with the control. There was a significant ($p < 0.05$) increase in total WBC and lymphocyte counts with a higher increase ($p<0.01$) in total neutrophil count. No significant ($p > 0.05$) change in the total monocyte and eosinophil counts relative to the control. Oral administration of toluene as used in this study may be toxic to health depending on the dose and duration of exposure.

Keywords: blood, solvent abuse, toluene, toxicity

Introduction

In recent times, toluene has emerged as the most commonly abused solvent [1,2] and which, with increased dose and duration of exposure, can lead to toxicity. Toluene is an organic hydrocarbon used in the manufacturing of dyes, nail, shoe polish, inks and paint thinners. It is widely used in cosmetic industry and is also a component of nylon and plastic bottles [2]. Toxicity of toluene can occur from accidental or deliberate inhalation of fumes, ingestion or transdermal absorption, toluene abuse or "glue sniffing" which has become rampant, especially among young people, since it is readily available and affordable [2]. Among children and adolescents, toluene is frequently abused by dousing cloth with paint, inks and the like, and placing it over the nose and mouth for inhalation in a bid to get intoxicated and a sensation of euphoria. Toluene, when inhaled, is known to be readily absorbed into the bloodstream.

Blood, as integral component of the body system, is used to detect any disorder or anomaly arising from exposure to all forms of injuries that can adversely affect health. Haematological study is important in assessing the toxicity of drugs and pollutants in the body since blood is the major transport system of the

body [3]. According to Oke *et al.* [4], anything that affects the blood will to a great extent affect the entire body either adversely or moderately in terms of health, growth, maintenance and reproduction. It is against this background therefore that this study was designed to investigate the effect of toluene toxicity on blood parameters using male albino rats as model[14]. This study has become necessary considering the increasing rate at which young people engage in the abuse of organic solvents in order to get to a point of ecstasy.

Materials and Method

Chemicals and Reagents

Toluene, with CAS No: 108-88-3, was purchased from Bernaco Enterprises Nigeria as clear colourless liquid with pleasant aromatic petroleum odour. The desired doses were prepared in Goya® olive oil which was purchased from the supermarket.

Animals and Treatment

Twenty (20) mature male albino rats weighing an average of 200g, purchased from the Animal House of Department of Pharmacology, College of Health Sciences, University of Port Harcourt were used for this study. The rats were acclimatized for two (2) weeks before the study was commenced. They were fed *ad libitum* with commercially sourced feed (Top Feeds Nigeria Limited) and supplied with clean drinking water all through the study[13]. After acclimatization, the animals were randomly assigned to four (4) groups – A, B, C and D. Group A served as the control and was given 0.5ml of olive oil (vehicle) while the treatment groups B, C and D received 31.8 mg/kg, 63.6mg/kg and 127.2 mg/kg, respectively of toluene which corresponded to 1/20, 1/10 and 1/5 of the LD50 which is 636mg/kg according to Doro-on [5]. Treatments were by oral gavage daily for 21 days. At the end of the treatments, the animals were anaesthetized and blood samples were collected by cardiac puncture into EDTA bottles. The collected blood samples were used for the estimation of haematological parameters such as packed cell volume (PCV), haemoglobin concentration (HB), red blood cell count (RBC), white blood cell count (WBC), platelets count, lymphocyte and neutrophil levels according to Cheesebrough [6].

Statistical Analysis

Statistical analysis was done using SPSS 21. All values were expressed as mean ± SEM and data were assessed by one-way ANOVA followed by the Tukey post-test. The significance level was set at $p<0.05$.

Results

The effect of different doses of toluene on various haematological parameters are summarized in figures 1 - 5 and Table 1. Treatment of rats for 21days with 31.8, 63.6 and 127.2 mg/kg doses of toluene had no significant ($p>0.05$) effect on PCV, haemoglobin concentration, RBC and platelet counts relative to the control as shown in figures 1 - 4.

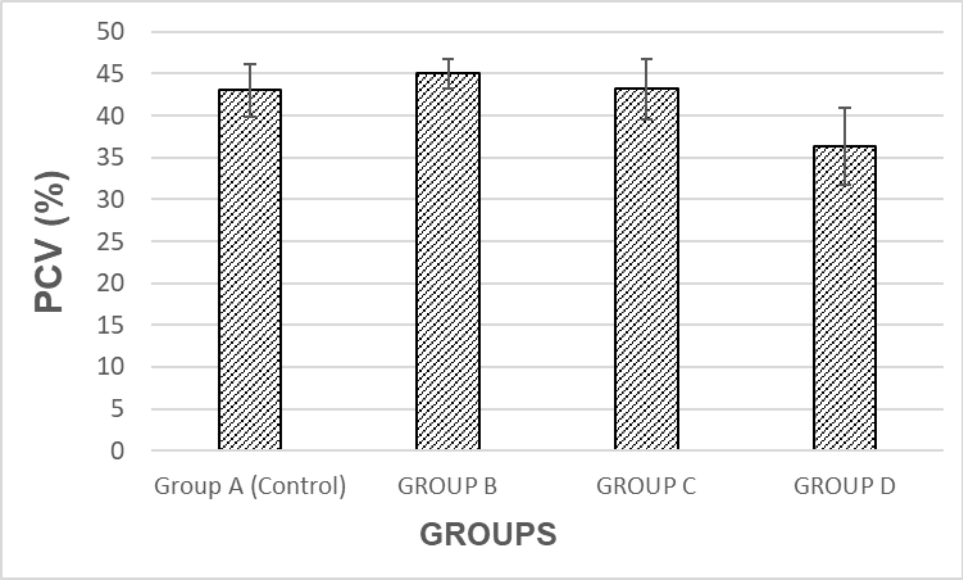

Fig. 1. Effect of Toluene on Packed cell volume (PCV) of rats treated for 21 days. Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with group A (control). No significant difference at a 95% confidence interval ($p >0.05$). Groups A, B, C and D represent the control (given 0.5 ml olive oil), 31.8 mg/kg treated rats, 63.6mg/kg treated rats and 127.2 mg/kg treated rats, respectively.

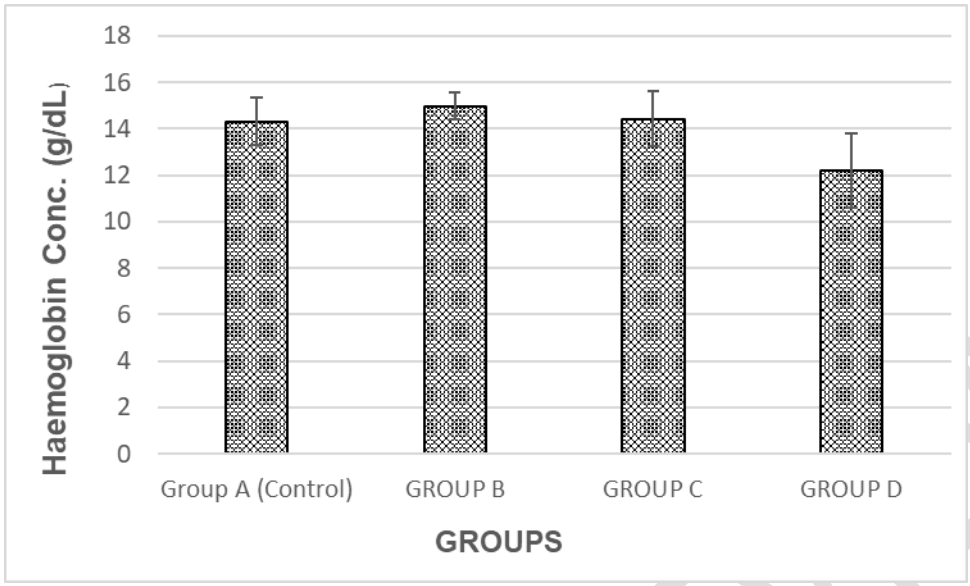

Fig. 2. Effect of Toluene on Haemoglobin Concentration of rats treated for 21 days. Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with group A (control). No significant difference at a 95% confidence interval ($p > 0.05$). Groups A, B, C and D represent the control (given 0.5 ml olive oil), 31.8 mg/kg treated rats, 63.6mg/kg treated rats and 127.2 mg/kg treated rats, respectively.

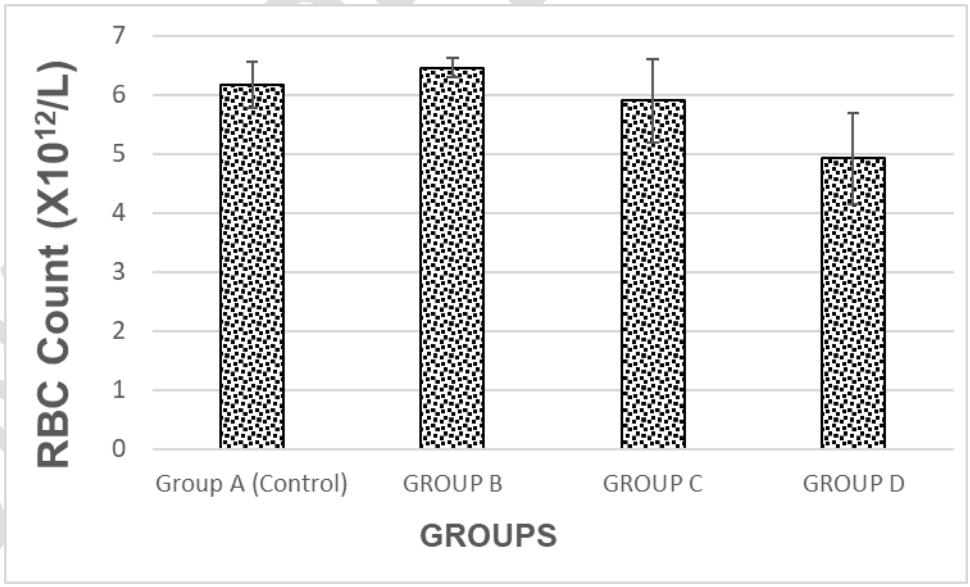

Fig. 3. Effect of Toluene on Red Blood Cell (RBC) Count of rats treated for 21 days. Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with group A (control). No significant difference at a 95% confidence interval ($p > 0.05$). Groups A, B, C and D represent the control

(given 0.5 ml olive oil), 31.8 mg/kg treated rats, 63.6mg/kg treated rats and 127.2 mg/kg treated rats, respectively.

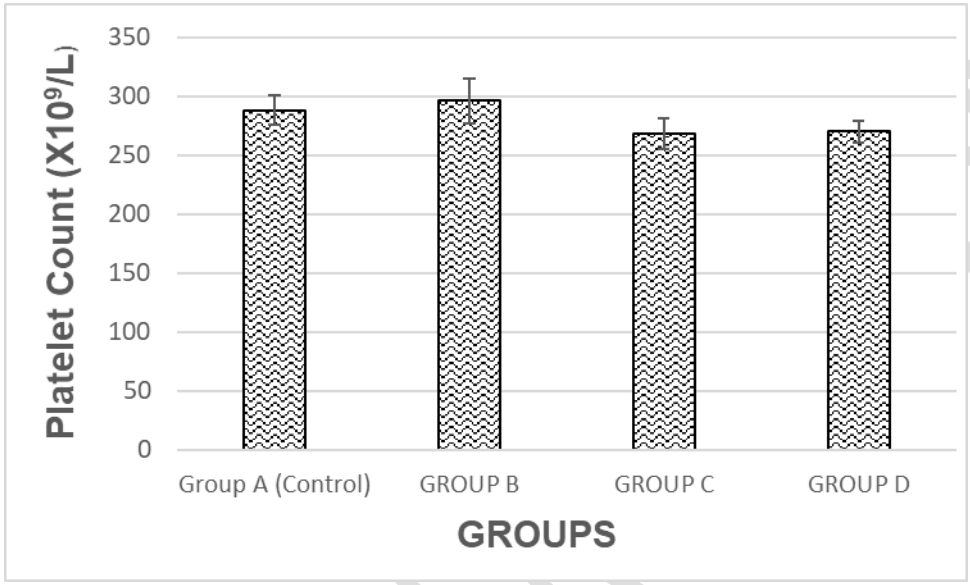

Fig. 4. Effect of Toluene on Platelet Count of rats treated for 21 days. Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with group A (control). No significant difference at a 95% confidence interval ($p > 0.05$). Groups A, B, C and D represent the control (given 0.5 ml olive oil), 31.8 mg/kg treated rats, 63.6mg/kg treated rats and 127.2 mg/kg treated rats, respectively.

Figure 5 shows that treatment of rats for 21days with 31.8 and 63.6 mg/kg doses of toluene produced no significant ($p>0.05$) change on the WBC count in relation to the control. However, only toluene treated group D (127.2 mg/kg) showed significant increase ($p<0.05$) in the WBC count in comparison with the control.

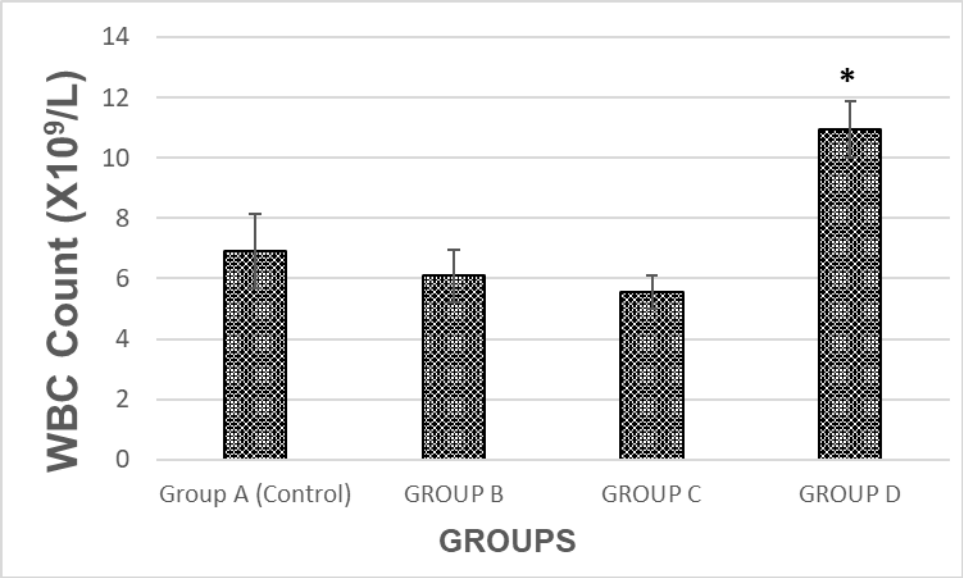

Fig. 5. Effect of Toluene on White Blood Cell (WBC) count of rats treated for 21 days. Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with group A (control). * indicates a significant difference at $p < 0.05$. Groups A, B, C and D represent the control (given 0.5 ml olive oil), 31.8 mg/kg treated rats, 63.6mg/kg treated rats and 127.2 mg/kg treated rats, respectively.

Treatment of rats for 21 days with 31.8, 63.6 and 127.2 mg/kg doses of toluene caused no significant ($p>0.05$) change on the total eosinophil and monocyte counts in relation to the control (Table 1). Although the total neutrophil and lymphocyte counts were not significantly varied ($p>0.05$) in rats treated with 31.8 and 63.6 mg/kg doses of toluene, the toluene treated group D (127.2 mg/kg) showed an increase ($p<0.05$) in the total lymphocytes count which was highly significant ($p<0.01$) in the total neutrophil count relative to the control (Table 1).

Table 1: Effect of Toluene on Differential Leucocyte Count of rats exposed for 21 days

PARAMETERS / GROUPS	**Total Neutrophil Count** ($X10^9/L$)	**Total Lymphocyte Count** ($X10^9/L$)	**Total Eosinophil Count** ($X10^9/L$)	**Total Monocyte Count** ($X10^9/L$)
A	2.07±0.39	4.68±0.89	0.04±0.02	0.12±0.06
B	2.25±0.43	3.81±0.58	0.00 ± 0.00	0.05±0.03
C	1.46±0.16	3.91±0.41	0.06±0.02	0.08±0.04
D	3.88±0.17**	7.56±0.42*	0.07±0.07	0.22±0.13

Results are given as mean ± SEM for 5 rats in each group. Experimental groups are compared with group A (control). *p<0.05, **p<0.01 vs. Control. P: statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test. Groups A, B, C and D represent the control (given 0.5 ml olive oil), 31.8 mg/kg treated rats, 63.6mg/kg treated rats and 127.2 mg/kg treated rats, respectively.

Discussion

Haematological parameters are health markers which aids diagnoses and the evaluation of physiological and pathological status of animals. According to Olafedehan *et al.* [7], blood acts as a pathological reflector of the status of exposed animals to toxicant and other conditions. From this study, oral ingestion of toluene by albino rats produced no significant effect on their PCV, RBC count, haemoglobin concentration and platelet count. There was a significant increase in the total WBC, neutrophil and lymphocyte counts at the highest dose of 127.2mg/kg. This increase in mean WBC count could be attributed to the presence of toluene in the body.

Higher WBC count is usually associated with prevalence of stress, inflammation, infection, allergy or certain diseases [8]. Similarly, animals with high WBC counts produce antibodies during the process of combating the causative agents through phagocytosis [9]. Generally, the elevated total WBC count triggered by the presence of causative agent ranging from biological, physical, chemical or thermal agents, in turn, leads to immunity to diseases, infection or allergy as a result of the generated antibodies in the body. Neutrophils are known to increase naturally in response to infections, injuries, and other types of stress[13]. The highly significant increase in the total neutrophil count indicates that the animals were exposed to injuries / oxidative stress due to the prolonged consumption of toluene. Long term exposure to air pollutants and organic solvents causes environmental pollution as well as oxidative stress in a biological system. Similarly, the high lymphocyte blood level recorded in this study could be an indication that the body is dealing with an infection or other inflammatory conditions. Since lymphocytes are associated with the immune system [10], this finding suggests that the immune responses of the body to infection may have been compromised as the lymphocytes' functions are mainly immunologic. Furthermore, toluene administered at high dose of 127.2mg/kg for a duration of 21 days, may have caused chronic inflammatory reactions in the animal as a result of the prolonged oxidative stress since lymphocytes are known to be present in very small numbers until the inflammatory reaction has become chronic.

Inflammation is a natural defense mechanism against pathogens and is usually linked with various pathogenic diseases such as microbial and viral infections, exposure to allergens, radiation and toxic chemicals while Oxidative stress refers to the overproduction of reactive oxygen species (ROS) in the cells and tissues in a way that the antioxidant system is unable to neutralize them [11]. Inflammation and oxidative stress are related in that oxidative stress is regarded as the difference between the production of reactive oxygen species (ROS) and their elimination by protective mechanisms, which can lead to chronic inflammation. The inflammation caused by oxidative stress is the cause of many chronic diseases [11].

This result of this study is in line with the work done by Ita and Udofia [12], who reported that the increase in WBC count of rats that orally ingested gasoline for 21 days could be a defensive mechanism developed by the body against toxicity of the gasoline constituents, which include toluene.

Conclusion

The present study, which provided insight into the toxicity of subacute exposure to toluene, concludes that oral administration of toluene as used in this study may be toxic to blood in particular and the entire body system in general, depending on the dose and duration of exposure. Further study on the effect of toluene in experimental animal for a longer duration of exposure is recommended.

Competing Interests

Authors have declared that no competing interests exist.

Ethical Approval

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee

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