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Research Article

The Effect of Ethylene Glycol Monomethyl Ether on Haematological Parameters in Wistar Rats.

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Abstract

Ethylene glycol monomethyl ether is an industrial solvent with wide application including being employed as an antifreeze agent in jet fuel and hydraulic fluids. It has been found to have various deleterious effects including haematological, reproductive, developmental and neurological toxicities in in vivo models. Detailed report on the effect on blood cell indices and differential leukocyte is sparse. Thus the aim of this study is to investigate the effect of ethylene glycol monomethyl ether on the blood cell indices and differential leukocyte count. Fifty male Wistar rats were weight matched into five groups with ten animals each. Group I received distilled water only. Rats in groups II-V received daily, ethylene glycol monomethyl ether at 100, 200, 300 and 400 mg/kg respectively for fourteen consecutive days. All administrations were done orally. On day fifteen, blood was collected from the animals by ocular puncture, using heparinized capillary tubes, into di- potassium ethylene diamine tetra acetic acid (K2- EDTA) specimen bottles and haematological parameters were analyzed. The white blood cell count, platelets, red blood cell count, mean cell haemoglobin, haemoglobin concentration, mean cell haemoglobin concentration and neutrophils reduced significantly ($p < 0.05$) while lymphocytes and mean cell volume increased significantly ($p < 0.05$), especially at the 300 and/or 400 mg/kg body. Ethylene glycol monomethyl ether has toxic effect on the red and white blood cell indices.

Key Words: ethylene glycol monomethyl ether, blood cell indices, differential leukocyte count, haematological parameters

INTRODUCTION

Ethylene glycol monomethyl ether (EGME) belongs to the family of glycol ethers and is known as monomethyl glycol, 2-methoxy ethanol, monomethyl ethylene glycol ether, methyl cellosolve (commercially), methyl oxitol, monomethyl ether or methyl glycol. It is an industrial solvent with wide application including being employed as an antifreeze agent in jet fuel and hydraulic fluids (Johanson, 2000; Takei et al., 2010; Adeyemo-Salami and Farombi, 2018). It is a colourless, highly inflammable and volatile liquid at room temperature. The major metabolic pathway of EGME is oxidation via methoxyacetaldehyde to methoxy acetic acid and the metabolites are eliminated from the body via the urine (Bagchi and Waxman, 2008).

Haematopoietic, developmental and reproductive toxicities (with emphasis on testicular damage) have been reported in various species including humans, after being exposed either by inhalation, ingestion and/or dermal absorption to EGME (Bagchi and Waxman, 2008). Studies in humans have shown a relationship between occupational exposure to EGME with haematological, reproductive tissue and neurological deviations (Takei et al., 2010). Bone marrow depression, pancytopenia, leucopenia and decrease in red blood cell count are haematogical aberrations as a result of exposure to EGME (Starek et al., 2010; Bendjeddon and Khalili, 2014). Moreover, effects of EGME on the immune system include thymic atrophy, decreased cell number of the spleen and reduced thymus weight (Takei et al., 2010). However, reports on the effect on differential leukocyte count and blood constants, which are also indicators of toxicity in the blood, is scanty.

This study was therefore designed to investigate the effect of EGME on the differential leukocyte count and blood cell indices of Wistar rats.

MATERIALS AND METHODS

Reagents: EGME was a product of Loba Chemie (Mumbai, India). Dewei Mindray BC 3000 haematology reagents (China) were used with the autohaematology analyzer.

Experimental Animals and Care: Ethical approval was obtained for the study from the Animal Care and Use Research Committee of the University of Ibadan, Nigeria and the number UI-ACUREC/ APP/ 10/2016 /003 was assigned. Fifty (50) nine weeks old male Wistar rats weighing 140-190 g were obtained from the Primate Colony of the Department of Biochemistry, University of Ibadan and randomly distributed into five groups of ten animals each. They were kept in

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appropriate laboratory cages and given feed (Ladokun Feed, Nigeria) and water ad libitum. Animals were acclimatized for a week.

Experimental Design: All administrations were carried out orally daily for fourteen consecutive days. Weight of rats was monitored weekly. The protocol for administration was as follows:
Group I received distilled water only (Control)
Group II – V received EGME at 100, 200, 300 and 400 mg/kg, respectively
On day 15, blood was collected by ocular puncture into di-potassium ethylene diamine tetra acetic acid (K2-EDTA) specimen bottles using heparinized capillary tubes and the haematological parameters were determined.

Haematological analysis
The packed cell volume (PCV), white blood cell count (WBC), mean cell haemoglobin concentration (MCHC), mean cell volume (MCV), red blood cell count (RBC), mean cell haemoglobin (MCH), neutrophil, platelets count, eosinophil, lymphocytes, monocytes and haemoglobin concentration were determined using the Mindray BC 3000 Autohaematology analyzer (China).

Statistical Analysis: All data were expressed as mean ± standard error of mean (S.E.M). Statistical analyses were carried out using one-way analysis of variance (ANOVA), followed by least significant difference (LSD) post hoc test to compare treatment groups. P-values less than 5% were considered to be significant.

RESULTS

Table 1 shows that the white blood cell count decreased significantly ($p < 0.05$) in a dose-dependent manner, and haemoglobin concentration, platelet count and red blood cell count decreased significantly ($p < 0.05$), especially at 300 and 400 mg/kg doses compared to the control while the packed cell volume decreased insignificantly. Table 2 shows that the mean cell haemoglobin and mean cell haemoglobin concentration decreased significantly ($p < 0.05$), especially at 300 and 400 mg/kg doses compared to the control while mean cell volume increased significantly ($p < 0.05$) in a dose-dependent manner. The differential leukocyte count showed that the neutrophils decreased with a significant ($p < 0.05$) effect observed at the 300 mg/kg dose, while the lymphocytes increased with a significant ($p < 0.05$) effect at the same dose compared to the control (Table 3).

Table 1:
Effect of graded doses of EGME on haematological parameters

Dose (mg/kg)	**PCV (%)**	**Hb (g/ 100mL)**	**RBC (x 10^{12}/ L)**	**Total WBC (x 10^3)**	**Platelets (x 10^3)**
Control	41.80±1.62	12.77±0.49	6.68±0.25	10,857.14±939.32	509.50±59.42
100	43.80±1.69	12.89±0.52	7.16±0.26	6,800.00±371.29*	446.70±51.20
200	41.78±1.52	12.24±0.46	6.66±0.23	6,950.00±1,119.90*	352.56±35.53
300	38.25±0.88	10.70±0.27	5.99±0.17*	3,616.67±388.52*	351.67±59.21*
400	35.86±2.60	9.57±0.80*	5.27±0.41*	3,716.67±387.66*	266.50±31.88*

Note: n = 10; *- significant at $p < 0.05$; values are mean ± standard error of mean; PCV- Packed Cell Volume; Hb- Haemoglobin concentration; WBC- White Blood Cell count; RBC- Red Blood Cell count

Table 2:
Effect of graded doses of EGME on blood cell indices

Dose (mg/kg)	MCV **(fL)**	**MCH (pg)**	**MCHC (g/ 100mL)**
Control	61.50±1.45	19.10±0.41	30.40±0.34
100	61.10±1.36	18.00±0.26	29.30±0.47
200	63.22±0.95	18.11±0.56	29.22±0.72
300	63.88±1.30	18.00±0.27	28.13±0.40*
400	68.43±1.99*	16.43±1.43*	27.14±0.80*

Note: n = 10; *- significant at $p < 0.05$; values are mean ± standard error of mean; MCV- Mean Cell Volume; MCH-Mean Cell Haemoglobin; MCHC-Mean Cell Haemoglobin Concentration

Table 3:
Effect of graded doses of EGME on differential leukocyte count

Dose (mg/kg)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
Control	47.60±4.04	44.90±4.14	7.11±0.72	1.10±0.46
100	46.80±3.37	48.20±2.30	7.50±0.50	1.30±0.54
200	41.11±2.35	49.56±2.11	7.89±0.82	1.22±0.49
300	31.43±2.94*	58.63±3.83*	8.00±0.73	1.50±0.38
400	37.50±3.10	52.83±3.50	8.00±0.44	1.71±0.36

Note: n = 10; *- significant at $p < 0.05$; values are mean ± standard error of mean

DISCUSSION

This study shows that EGME elicits toxicity on the differential leukocyte count and blood cell indices. Haemoglobin concentration was significantly decreased in the treated rats, especially at the highest dose, than in the control group which may be an indication of inadequate blood formation and pigmentation (Maakaron, 2019). This implies that the oxygen carrying capacity of the blood might be impaired in rats treated with the highest dose. Packed cell volume, which is also known as the haematocrit, is a measure of the volume of the red blood cells (erythrocytes) in blood and it is used to identify anaemia (low concentration of red blood cells in the blood) and polycythaemia (high concentration of red blood cells in the blood) (Mondal and Budh, 2021). This moderate decrease in the groups treated with EGME was observed. Decrease in the production of red blood cells may be an indication of decreased bone marrow function and insufficient erythropoiesis (Zivot et al., 2018) induced by EGME. This seeks to support the observed insignificant decrease in packed cell volume. The monocytes, eosinophils, white blood cells (leucocytes), lymphocytes and neutrophils constitute the defense mechanism of the body system, and some of them are also indicators of inflammation (Adeyemo-Salami and Ewuola, 2015; Underwood, 2018; Huizen, 2020; Raymaakers, 2020). Therefore the moderate elevated levels of the lymphocytes, monocytes and eosinophils suggest that the defense mechanism is being compromised and there also may be inflammation or allergic reaction which can result in tissue damage. The significant decrease in white blood cells is an indication that the function of the bone marrow is being disrupted. Moreover, neutrophils eradicate microorganisms and clear infections via various mechanisms including granular proteins, phagocytosis, release of reactive oxygen species (ROS), production and liberation of cytokines, and chemotaxis (Mortaz et al., 2018). The decrease in neutrophils, especially at the 300 and 400 mg/kg doses is an indication of the compromise of this defense system. The mean cell haemoglobin (MCH) or mean corpuscular haemoglobin is the average mass of haemoglobin per red blood cell in a sample of blood. Significantly decreased mean cell haemoglobin may be an indication of decrease in reticulocytes (immature red blood cells) (Adeyemo-Salami and Ewuola, 2015). This may be due to decreased erythropoiesis caused by EGME, especially at 400 mg/kg dose. Mean cell haemoglobin concentration or mean corpuscular haemoglobin concentration is a measure of the concentration of haemoglobin in a given volume of packed red blood cells (Adeyemo-Salami and Ewuola, 2015). The values of mean cell haemoglobin concentration for the groups treated with 300 mg/kg and 400 mg/kg doses showed that the red blood cells were hypochromic because they were lower than that of the control. Therefore, significantly decreased Hb, WBC, platelet count, MCH, MCHC and RBC are signs of haematological abberations. These findings are consistent with the report of Shih et al. (2000; 2003) in humans exposed to EGME at the workplace. The gradual increase in MCV in the treatment groups which was significant at the 400 mg/kg dose reflects macrocytic anaemia (i.e. red blood cells that are larger than the average size) which is also a sign of haematological abberation.

CONCLUSION

The data show that Ethylene glycol monomethyl ether induces signs of toxicity in the blood cell indices and differential leukocyte count.

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