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Toxicological Effects of Petrol, Xylene and Thinner on *Mus musculus* (Albino Mice)

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Abstract

Background: Petrol, xylene, and thinner are volatile organic compounds (VOCs), a major class of pollutants that affect the chemistry of the atmosphere and animals including man. The principal mode of contamination is via inhalation. This study aims to elucidate the effects of these compounds on biochemical functions in the body of the mouse, *Mus musculus*

Methods: One hundred and twenty adult albino *Mus musculus* species were randomly assigned to groups (A1, A2, B1, B2, C1, C2, and D1) based on their weights (8-13g for juveniles and 15 - 21g for adults) with specific treatments. The control treatment, A1, was housed in a plastic experimental house free from the test chemical vapor while groups A2 and B1; B2 and C1; C2 and D1were exposed to petrol, thinner, and xylene respectively in wooden exposure chambers for six hours daily for sixty days. Levels of oxidative stress markers (GSH, SOD, CAT, and MDA) were determined using the spectrophotometry.

Results: A significant decrease in the values of GSH, activities of SOD and CAT, were observed when compared to the mice in the control experiment as daily exposure to the selected vapor increased. On the other hand, MDA levels increased significantly with increasing daily exposure to vapor from the test chemicals, when compared to the control rats.

Conclusion: Based on the findings from this study, the decrease in the SOD, CAT and GSH levels are indications of toxic build up in the blood implying that exposures to these VOCs inhibit natural processes. Thus, VOCs are detrimental to human health and long-term exposure to these organic compounds could result in deleterious sub-lethal effects, especially to individual exposed occupationally to them

Keywords: Oxidative stress, Environmental pollution, Volcanic Organic Compounds, Inhalation

1.0 INTRODUCTION

Crude petroleum is refined into various fractions including petrol, xylene, and thinner collectively regarded as Volatile Organic Compounds (VOCs). Among pollutants, VOCs are considered important parameters for the assessment of air quality in indoor and outdoor environments because of their ubiquitous presence, and their significant impact on the environment and human health [1,2]. Petrol contains a mixture of volatile hydrocarbons and so inhalation is the most common form of exposure [3]. In Nigeria, there is an increase in the demand for petrol and other petroleum products, which are used for various reasons at homes, in manufacturing, and petrochemical industries [4]. Exposure to petroleum products both in and outside petroleum industries have been reported to have some effects on the users, with those who are occupationally exposed being more likely to be affected than their counterparts [5,6,7]. Such effects include increased incidences of blood disorders and anemia, higher cancer risk, renal function impairment, and nephrotoxicity [8,9,5,10].

Xylene is an aromatic hydrocarbon widely used as a solvent in the printing, rubber, paint, and leather industries. It is found in small amounts in airplane fuel, petrol, and cigarette smoke. In dentistry, xylene is used in histological laboratories for tissue processing, staining, and cover slipping [11]. Exposure to xylene can occur via inhalation, ingestion, or eye or skin contact. The main effect of inhaling xylene vapor is depression of the central nervous system, with symptoms such as headache, dizziness, nausea, and vomiting. Thinner is a solvent mixture used in both household products and industrial settings. It has a highly variable composition, displaying both temporal and geographic differences [12]. The major component of thinner is toluene, a well-known neurotoxic agent [13]. Acute and chronic effects of toluene on neurons have been well documented [12]. Thinner inhalation induces oxidative stress. Activation of free radical processes underlies the effect of many toxic substances like ethanol, toluene, ionizing radiation, lead and arsenate [14].

Petrol and thinner are used on a daily basis. Electricity supply in homes Nigeria is not constant. Electric generators that are affordable and used in homes are those that are powered with petrol. In some houses in Nigeria ('Face me, I face you Houses') almost every family use it. The whole area will smell of petrol. There is a need to see the effect of long time exposure of petrol on humans using the mouse. The study was therefore carried out to determine the effect of petroleum products on the mouse, *Mus musculus.*

2.0 METHODOLOGY

2.1 Animal Model

A hundred and twenty albino *Mus musculus* species were used in this experiment (Table 1). The adult mice, weighing 15-21g, were separated from the juvenile mice of about 5 weeks old weighing 8-15g. The animals were housed under room temperature for seven days for acclimatization and fed a pellet diet (120g), obtained from the Nigerian Institute of Medical Research (NIMR), Yaba, every two days

Table 1. Distribution of *Mus musculus* into Groups

Group	No of *Mus musculus*	Treatment	Weight (g)
A1	15	Control	8 – 10g (Juvenile)
A2	15	Petrol	8 – 10g (Juvenile)
B1	15	Petrol	11 – 13 g (Juvenile)
B2	15	Thinner	11-13g (Juvenile)
C1	15	Thinner	18 – 21g (Adult)
C2	15	Xylene	15-18g (Adult)
D1	15	Xylene	18-21g (Adult)

2.2 Exposure to Test Chemical Vapor

The animal cages housing the test groups A (2), B (1and 2), C (1 and 2), and D (1) were placed in wooden exposure chambers (measuring 69cm × 38cm ×30cm) with six calibrated 1000 ml cans containing 500 ml of their respective chemical vapor placed in each chamber one hour prior to the commencement of the exposure to ensure that each chamber was saturated with test vapor. The mice were later placed in the chamber and allowed to inhale the vapor generated from the direct evaporation of liquid from the cans respectively at ambient humidity and temperature. The exposure period was done for 6 hours daily, and was adopted for 60 days [15]. The care of the animals was done in accordance with the U.S. public health service guidelines [16].

2.3 Sample Homogenization

Organs were washed in ice cold potassium chloride (KCl) solution, blotted, and weighed. Each organ, with 0.1M phosphate buffer (pH 7.2) and laboratory sand (acid

washed sand), was placed in a mortar and blended using a pestle. The resulting homogenate was centrifuge at 2500rmp speed for fifteen minutes, and the supernatant, after decantation was stored at -20˚C for further analysis [16].

2.4 Determination of Superoxide Dismutase Activity

Superoxide Dismutase (SOD) activity was determined as previously described by some resesarchers [17,18]. The reaction mixture (3ml) contained 2.95ml 0.05M sodium carbonate buffer pH 10.2, 0.02ml of liver homogenate, and 0.03ml of epinephrine in 0.005N. Hydrochloric acid (HCl) was used to initiate the reaction. The reference cuvette contained 2.95ml buffer, 0.03ml of substrate (epinephrine), and 0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480nm for 5 minutes. ∑=4020M-1cm-1

2.5 Determination of Catalase Activity

Serum catalase activity was determined using Beers and Sizer method as adjusted by by Uboh *et al.,* [15], by measuring the decrease in absorbance at 240nm resulting from the decomposition of hydrogen peroxide (H_2O_2) UV recording spectrophotometer. The reaction mixture (3ml) contained 0.1ml of serum in phosphate buffer (50mM, pH 7.0) and 2.9ml of 30mM H_2O_2 in phosphate buffer pH 7.0. An extinction coefficient at 240nm H_2O_2 of 40.0M-1cm-1 [19] was used for the calculation. The specific activity of catalase was expressed as moles of H_2O_2 reduced per minute, per mg protein.

2.6 Determination of Reduced Glutathione Activity

Tricarboxylic acid TCA (10%) was added to the homogenate and centrifuged. 1.0ml of supernatant was treated with 0.5% of Ellman's reagent, 19.8mg of 5.5-dithiobisnitrobenzoic acid (DTNB) in 100ml of 0.1% sodium nitrate and 3.0ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412nm [15,16]

∑=1.34×104M-1cm-1

2.7 Lipid Peroxidation

Malondialdehyde (MDA), an index of lipid peroxidation, was determined using 1.0ml of the supernatant was added to 2ml of thiobarbituric acid (TBA) 0.37%, 0.24N HCl and 15% tricarboxylic acid reagents in the ratio 1:1:1. The solution was boiled at 100% for15 minutes and allowed to cool. Flocculent materials were removed and absorbance read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDA-TBA complex of 1.56 ×105M-1cm-1[15]

2.8 Blood Chemistry

Ocular blood collection was down on the mice and in turn transferred into properly labeled EDTA bottles. The blood samples were used for White Blood Cell (WBC), Packed Cell Volume (PCV), Mean Corpuscular Haemoglobin Count (MCMC), and Hemoglobin Count analysis in 21,45, and 60 days following exposure respectively for the three volatile organic solvents (petrol, xylene, and thinner) [15].

3.0 RESULTS AND DISCUSSION

As the experiment proceeded, the mortality rate of the test animals within the first 21 days was 12.5%. Significant reduction in the feeding habit was observed after 45 days with those exposed to xylene eating the least. In general, the exposed test animals were restless and exhibited signs of physiological and neurotic stress. The group exposed to xylene was the most restless during the period of exposure

3.1 Biochemical Assay

3.1.1 Glutathione

Results showed significant decrease in the blood glutathione (GSH) of mice exposed to petrol, xylene, and thinner via inhalation (Figure 1 and Table 2). This goes with a previous work by Raza *et al.,* [20] that recorded decrease in brain GSH with concomitant increase in lipid peroxidation levels after gasoline exposure in plants. The decrease in glutathione levels due to inhalation of the organic chemicals is attributed to the oxidative stress induced by Fechter *et al.*,[18].

3.1.2 Superoxide Dismutase and Catalase Levels

Findings from this experiment showed significant reduction in superoxide dismutase (SOD) and catalase (CAT) concentration as the days increased, thereby inhibiting the activities of both enzymes (Figure 1 and Table 2). Also, SOD and CAT were referred to as endogenous antioxidant enzymes that act as free-radical scavengers, and hence prevent and repair damage done by reactive oxygen species [21,22].

3.2 MDA Level

The study showed significant increase in malondialdehyde level, which is an indication of lipid peroxidation of the lung and liver tissues ((Figure 1 and Table 2). This finding is in agreement with Ulakoglu *et al.,* [23], who demonstrated an increase in brain lipid peroxidation after inhalation of gasoline, and/or its additives, which include

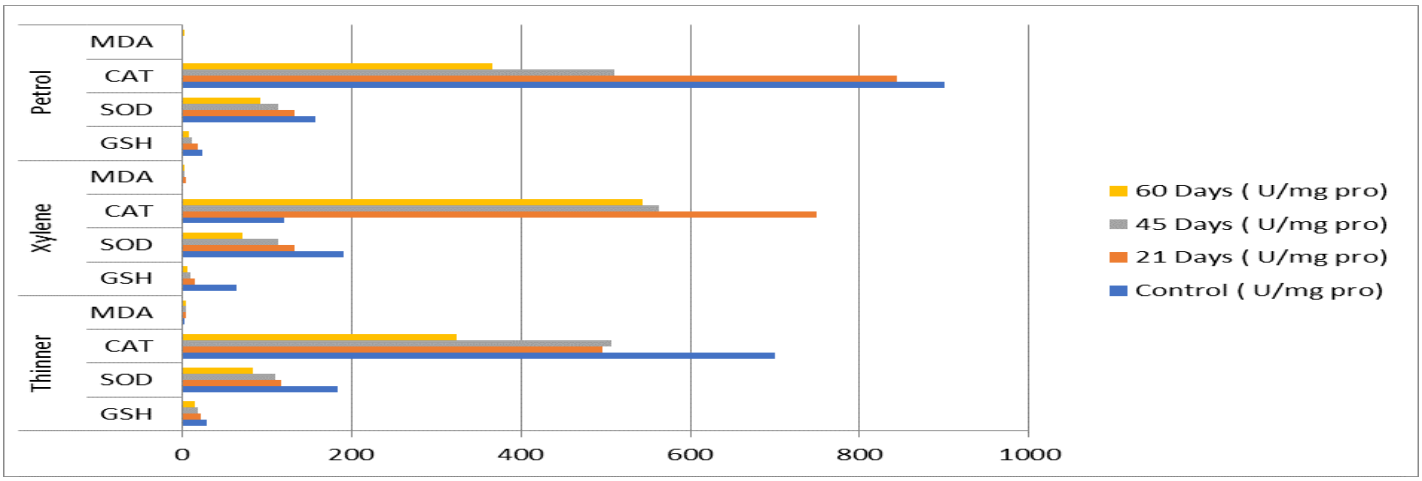

Figure 1. Concentrations of Oxidative Stress Markers of Mice in Control and in Exposure

Table 2: Concentrations of Oxidative Stress Markers of Mice in Control and in Exposure

Chemical	Parameter	Control (U/mg pro)	21 Days (U/mg pro)	45 Days (U/mg pro)	60 Days (U/mg pro)
Thinner	GSH	29.2	22.73	19.45	14.64
	SOD	183.07	116.77	109.39	84.02
	CAT	700.07	495.88	506.9	324.56
	MDA	2.81	3.97	4.38	4.9
Xylene	GSH	65.26	14.75	10.64	6.98
	SOD	191.26	133.63	114.27	71.08
	CAT	119.99	748.94	563.1	543.76
	MDA	1.85	5.08	3.39	2.28
Petrol	GSH	24.94	18.81	12.58	8.86
	SOD	157.91	132.81	114.22	92.65
	CAT	900.13	843.69	511.07	366.19
	MDA	1.41	1.67	1.97	3.06

xylene components. The toxicity of petrol, xylene, and thinner to mammalian tissue depends mainly on cytochrome P-450-mediated oxidation, as well as GSH conjugation [24]. Thus petrol, xylene, and thinner vapor produce dose dependent increases in cellular H_2O_2 resulting in lipid peroxidation [25]. Lipid peroxidation, the oxidative catabolism of polyunsaturated fatty acids, is widely accepted as a general mechanism for cellular injury and death, and has been implicated in diverse pathological conditions [26,27].

Petrol, xylene, and thinner inhalation stimulate reactive oxygen species (ROS) formation, an important pathway of these organic compounds neurotoxicity, by which it induces oxidative damage to lipids, proteins, and nucleic acids [28]. This is evident in the repeated rise in the level of catalase formation after exposure from day 21 through day 60 of this experiment.

3.3 Hematology

Exposure to the selected organic chemicals caused significant increase in WBC, PCV, and hemoglobin level, without marginal change in the MCHC count value ((Figure 1 and Table 2). Increase in white blood cells may be suggested to be due to stimulated lymphiopoiesis and/or release of lymphocytes from lymph-myeloid tissues [29]. Hence, the lymphocytes response is a stimulatory effect of the toxic substances in the lymphoid tissues.

Based on the findings from this study, the decrease in the SOD, CAT and GSH levels are indications of toxic build up in the blood of mice. This implies that exposures to these VOCs inhibit natural processes of body detoxification, impairing lipid peroxidation and anti-oxidant enzyme

activities in lipid rich organs like liver, lung, brain. and buildup of hydrogen peroxide (H_2O_2) within the issues. Thus, VOCs are detrimental to human health and long term exposure to these organic compounds could result in deleterious sub-lethal effects, especially to individual exposed occupationally to them.

Conflict of Interest

The authors declare that there is no conflict of interest.

Authors Contribution

KAK conceived and designed the study and contributed to manuscript writing; **AAI** collected data, contributed to data analysis tools and performed analysis of data; **PCA** contributed to manuscript writing. All authors approved the final version of the manuscript

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