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Alterations in Antioxidant and Hematological Indices in Diabetic and Non diabetic Rats Exposed to Paint Fumes

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Abstract

Toxicities of various kinds due to inhalation of fumes from paint industries as well as housing estates in Nigeria and indeed in many Africa countries are now being realized as an occupational hazard. In this study, the effects of paint fumes on oxidative stress and hematological parameters in diabetic and normal rats were investigated. The rats were exposed to paint fumes for 7 days. The result of groups 4–6 (diabetic) showed significant increase ($P < 0.05$) in glucose levels compared to groups 1–3 (non diabetic). After exposure of groups 2, 3, 5, and 6 to paint fumes, groups 2 and 3 showed a non significant increase ($P > 0.05$) in their glucose when compared to group 1 while group 5-6 gave a significant elevation of blood glucose level when compared to group 4. Red blood cell in groups 4-6 (diabetic groups) were significantly ($P < 0.05$) reduced when compared to group 1. However, there was no significant lipid per oxidation (malondialdehyde) in groups 2, 3, 4, and 5 except in group 6 when compared to group 1 (normal control). There were non significant decreases ($P > 0.05$) in antioxidant levels across groups 1–6. The results of packed cell volume in group 6 (diabetic exposed to oil paint fumes) were significantly ($P < 0.05$) lower compared to group 1. Methemoglobin concentration showed a significant ($P < 0.05$) increase in groups 3 (normal rats exposed to oil paint fumes) and 6 (diabetic rats exposed to oil paint fumes) when compared to group 1. In conclusion, paint suppresses certain hematopoietic processes and complicates diabetic status in rats.

Keywords: Alloxan-Induced, Antioxidants, Diabetes Mellitus, Inhalation Chamber, Paint Fumes

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1. Introduction

Pancreatic β-cells and insulin receptor cells have emerged as a target of oxidative stress mediated tissue damage (Kaneto *et al.,* 1996), which can lead to diabetes mellitus. Diabetes mellitus is a metabolic disease in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced (Evans *et al.,* 2003). Most cases of diabetes mellitus fall into three broad categories: Type 1, type 2, and gestational diabetes. Other forms of diabetes mellitus include congenital diabetes, cystic fibrosis-related diabetes, steroid diabetes, and several forms of monogenic diabetes (ADA, 2012). Paint is any liquid, liquefiable, or mastic composition which after application to a substrate in a thin layer is converted to an opaque solid film (Wiggers *et al.,* 2004). Paint falls into two categories: water-based paints (acrylic emulsions) and solvent or oil-based paints (Wiggers *et al.,* 2004). Water-based paints typically emit fewer vapors and dries faster as compared to oil-based paints which usually contain toluene, xylene, and mineral spirits (Wiggers *et al.,* 2004). Hydrocarbons are the primary poisonous ingredient in oil paints. Some oil paints have heavy metals such as lead, mercury, cobalt, and barium added as a pigment. These heavy metals can cause additional poisoning if inhaled in large amounts though some of these heavy metals are no longer present in the paint. Paints have been associated

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with the release of potentially harmful chemicals such as volatile organic compounds that can also lead to free radicals generation and oxidative stress in the long run (Wax and Beuhler, 2004). This harmful effect of paint may be manifested when the fume is inhaled in a poorly ventilated room or during the act of painting (Wiggers *et al.,* 2004). Workers at various paint industries and the painters are thus prone to the deleterious effects of the paint such as a headache, nausea, increased rate of birth defects, cancers, damage to the central nervous system, and a general decrease, in general, mental faculty (Townsend and Maynard, 2002). Paint fumes may cause destruction or failure in bone marrow function, kidney disease, blood vessel injury, or leukemia, which may affect the production of Red Blood Cell (RBC) (Zuckerman, 2007). Studies have shown that prolonged exposure to paint fumes during pregnancy may increase the risk of miscarriage and fetal death (particularly during the first trimester) by crossing the placenta and combines with fetal hemoglobin, causing a more direct fetal tissue hypoxia (Omaye, 2002). This study, therefore, attempted to study the hematological and antioxidant response of alloxan-induced diabetic and non diabetic rats exposed to paint fumes.

2. Materials and Methods

2.1 Experimental Animals

Animals used for this study were male Wistar albino rats, aged between 10 and 12 weeks and were obtained from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka (UNN). The animals were housed in cages in the Animal House of the Department of Home Science, Nutrition and Dietetics, Faculty of Agriculture, UNN. The animals were handled according to the guidelines of the Ethical Committee on the Use and Care of Experimental Animals of UNN.

2.2 Composition of Paint

Paints used for this study were purchased from a production factory at Enugu, in Enugu State Nigeria with the following composition:

- Emulsion (water–based) paint: 1200 ml water, 0.2 kg polyvinyl acetate, 3.0 kg calcium carbonate, 0.2 kg titanium dioxide and 0.2 kg yellow oxide, 0.4 kg kaolin, defoamer, methylene chloride, and phenyl mercuric acetate (biocide).
- Gloss (oil-based) paint: 1.0 kg kerosene, 1.0 kg alkyd resin, 0.2 kg titanium dioxide, 0.02 kg yellow oxide, and 0.03 kg yellow paste, 0.07 kg anti skin, methylene chloride, mix drier ammonia and phenyl mercuric acetate (biocide).

3. Study Design

A total of 36 rats divided into six groups ($n = 6$). They were acclimatized for 1 week under standard environmental conditions and maintained on a regular feed and water *ad libitum.* The groups were as follows:

- Group 1: Normal rats not exposed to paint fumes (control).
- Group 2: Normal rats exposed to emulsion paint fumes.
- Group 3: Normal rats exposed to oil paint fumes.
- Group 4: Diabetic rats not exposed to paint fumes (negative control).
- Group 5: Diabetic rats exposed to emulsion paint fumes.
- Group 6: Diabetic rats exposed to oil paint fumes.

3.1 Induction of Diabetes and Blood Glucose Determination

The baseline blood glucose levels were determined before the induction of diabetes. The rats were fasted overnight prior to injection of alloxan dissolved in iced cold normal saline at a dose of 150 mg/kg body weight and the route of administration was intraperitoneal. After 72 h, rats with blood glucose levels >200 mg/dl were considered diabetic and used for the investigation (Fröde and Medeiros, 2008). Thereafter, blood glucose levels were determined following exposure to paint fumes.

3.2 Exposure to Paint Fumes

Rats in groups 2, 3, 5, and 6 were exposed to paint fumes in an inhalation chamber using a 30-s exposure (3 times daily for 7 days) as previously described (Piggott and Emmons, 1960). Briefly, the inhalation chamber consisted of a 1-L Pyrex flask with six tubular glass side arms, each at a 30° angle from the horizontal and spaced equidistantly around the flask. Prior to exposure, the bottom of the flask was filled with paint. Rats were subsequently and individually placed in each side arm (six rats per flask for each group), pushed to the bottom of the arm (so that the nose filled and extended beyond the open end

of the tube), and held in place by cotton plugs. The top of the flask was then closed with a rubber stopper, which was transverse by a glass rod, the end of which extended directly above the paint lawn. The flaxes were filled with the same quantity of the paint (300 ml); turbulence was created over the paint via the forceful expulsion of 60 cm^3 of air through the end of the glass rod, using a 60 ml syringe which was connected to the stoppered end of the glass rod by a piece of plastic tubing. This method resulted in the uniform dispersion of fumes to rats throughout the chamber. The behavioral change of the rats was equally observed.

3.3 Determination of Biochemical Parameters

The glucose concentrations were measured with Glucometer (OneTouch Ultra, UK), the malondialdehyde (MDA) concentration was determined using the method of Varshney and Kale (1990). Glutathione (GSH) was determined by the method of King and Wotton (1959). Superoxide Dismutase (SOD) was assayed using the method described by Das *et al*., (2000) and catalase (CAT) activity was assayed with the method of Aebi (1983). Packed Cell Volume (PCV), RBC count, and White Blood Cell (WBC) count were determined using the standard hematological procedure as described by Ochei and Kolhatkar (2008), Methemoglobin was determined by the method of Betrand (1982) and platelets was determined using the method described by Cheesbrough (2000).

3.4 Statistical Analysis

The data obtained from laboratory tests were subjected to one-way analyses of variance. Least significant difference multiple comparison *post-hoc* test was used to determine the level of significance between control and experimental groups. Significant differences were observed at ($P < 0.05$). The results were expressed as a mean ± standard deviation. These analyses were done using Statistical Package for Social Sciences (SPSS), version 17.

4. Results

4.1 Clinical Signs

It was observed that from the 3rd day the animals exposed to the fumes were showing some signs of weakness. From the 5th day, their feeding habit dwindled especially those exposed to oil paints. This could probably be responsible for a slight drop in weight for both water-based and the oil-based paint but demonstrated more the latter.

4.2 Glucose Concentration (mg/dl)

The results of glucose concentrations of rats are shown in Figure 1. The baseline glucose levels of test groups before induction of alloxan were not significant ($P > 0.05$) among groups 2 (77.33 ± 3.84), 3 (79.00 ± 2.65), 4 (79.50 ± 6.50), 5 (89.33 ± 3.18), and 6 (89.67 ± 10.52) when compared to that of group 1 (81.00 ± 12.34) (normal control). A similar pattern was observed when groups 5 and 6 (diabetic rats exposed to paint fumes) were compared to that of group 4 (negative control). Group 3 (normal rats exposed to oil paint fumes) and 5 (diabetic exposed to emulsion paint fumes) also recorded a non significant increase ($P > 0.05$) when compared to group 2 (normal rats exposed to emulsion paint fumes) and group 6 (diabetic rats exposed to oil paint fumes) when compared to that of group 3 (normal rats exposed to oil paint fumes) and 5 (diabetic rats exposed to emulsion paint fumes) also had a non significant increase ($P > 0.05$).

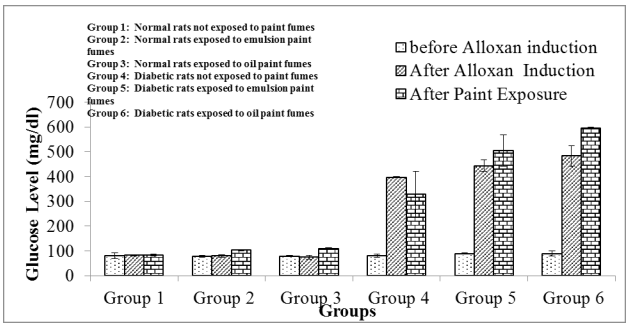


Figure 1. Effect of paint fumes on blood glucose concentrations in diabetic and non diabetic rats.

After induction of diabetes with alloxan, a significant increase ($P < 0.05$) was observed in group 4 (397.50 ± 2.50), 5 (443.33 ± 23.33), and 6 (483.33 ± 41.76) when compared to that of group 1 (82.00 ± 3.5) (normal control), 2 (79.67 ± 5.8), and 3 (74.33 ± 6.69) which were not induced. A non significant ($P > 0.05$) increase was observed when group 5 (diabetic exposed to emulsion paint fumes) was compared to group 4 (negative control), while group 6 (diabetic exposed to oil paint fumes) showed significant increase ($P < 0.05$) when compared to group 4 (negative control). In addition, a non significant increase ($P > 0.05$) was observed in group 6 when compared to group 5. The glucose level of group 3 (normal rats exposed to oil paint fumes) showed a non significant increase ($P >$

0.05) when compared to group 2 (normal rats exposed to emulsion paint), while group 5 (diabetic exposed to emulsion paint fumes) showed significant increase ($P < 0.05$) when compared to group 2 (normal rats exposed to emulsion paint fumes). Group 6 (diabetic rats exposed to oil paint fumes) also gave significant increase ($P < 0.05$) when compared to group 3 (normal rats exposed to oil paint fumes). Results of groups 4, 5, and 6 after induction of diabetes with alloxan showed a significant increase ($P < 0.05$) when compared to their results before alloxan induction.

Following exposure of groups 2, 3, 5, and 6 to paint fumes, group 2 (102.67 ± 2.19) and 3 (109.33 ± 2.85) showed a non significant increase ($P > 0.05$) in their glucose levels when compared to group 1 (83.33 ± 2.73) (normal control). Furthermore, in group 3 (normal rats + oil paint fumes), there was a non significant increase ($P > 0.05$) in glucose levels when compared to group 2 (normal rats exposed to emulsion paint fumes). The diabetic groups 4 (330.00 ± 92.00), 5 (505.00 ± 64.37), and 6 (596.67 ± 2.85) produced significant increase ($P < 0.05$) in their blood glucose levels after exposure to paint fumes when compared to group 1 (normal control). Group 4 (negative control) that was induced but not exposed to paint fumes had a significant decrease ($P < 0.05$) in blood glucose concentration when compared to groups 5 and 6 that were induced and exposed to paint fumes. Group 6 (diabetic rats exposed to oil paint fumes) gave a non significant increase ($P > 0.05$) in glucose levels when compared to group 5 (diabetic exposed to emulsion paint fumes). After exposure of groups 2 and 3 to paint fumes, a non significant increase ($P > 0.05$) was observed when compared to the result obtained before exposure to paint fumes. Group 4 that was not exposed to paint fumes had a significant decrease ($P < 0.05$) in blood glucose level when compared with those exposed to paint fumes. Groups 5 and 6 (diabetic groups exposed to paint fumes) also had significant increase ($P < 0.05$) in their blood glucose level after exposures to paint fumes when compared to their values before exposures to paint fumes.

4.3 Malondialdehyde Levels (mg/dl)

Figure 2 shows MDA values in groups 1–6. There was no significant ($P > 0.05$) increase in groups 2 (645.00 ± 19.00), 3 (748.50 ± 28.50), 4 (614.33 ± 16.59), and 5 (717.67 ± 87.51) when compared to group 1 (540.00 ± 55.16) (normal control). However, the MDA concentrations in group 6 (850.33 ± 111.82) (diabetic rats exposed to oil paint fumes) was significantly ($P < 0.05$) increased compared to that of group 1 (normal control). A non significant ($P > 0.05$) increase was also observed when group 5 (diabetic rats exposed to emulsion paint fumes) was compared to that of group 4 (negative control), while group 6 (diabetic exposed to oil paint fumes) gave a significant ($P < 0.05$) increase when compared to group 4 (negative control). The MDA values of groups 3 (normal rats exposed to oil paint) and 5 (diabetic rats exposed to emulsion paint fumes) recorded a non significant increase when compared to group 2 (normal rats exposed to emulsion paint). Similarly, non significant ($P > 0.05$) increase was observed in group 6 (diabetic rats exposed to oil paint) when compared to groups 5 (diabetic rats exposed to emulsion paint fumes) and 3 (normal rats exposed to oil paint).

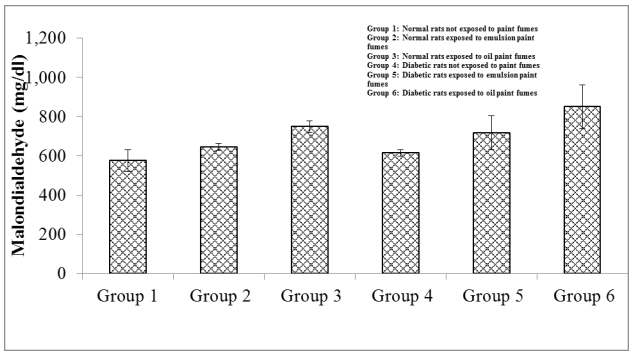


Figure 2. Effect of paint fumes on malondialdehyde values in diabetic and non diabetic rats.

4.4 Glutathione Concentrations (mg/dl)

Figure 3 represented the values of GSH, an antioxidant as obtained in the study. Groups 2 (0.85 ± 0.12), 3 (0.78 ± 0.14), 4 (0.66 ± 0.15), 5 (0.64 ± 0.07), and 6 (0.61 ±.06) recorded a non significant ($P > 0.05$) decrease when compared to group 1 (0.98 ± 0.27) (normal control). A non significant ($P > 0.05$) decrease was also observed in groups 5 (diabetic rats exposed to emulsion paint fumes) and 6 (diabetic rats exposed to oil paint) when compared to group 4 (negative control). Similarly, a non significant decrease was observed in group 5 (diabetic rats exposed to emulsion paint fumes) when compared to group 2 (normal rats exposed to emulsion paint). Similarly, a non significant decrease was observed in group 3 (normal rats exposed to oil paint) when compared to group 2 (normal rats exposed to emulsion paint). The GSH levels in group 6 (diabetic rats exposed to oil paint) showed a non significant ($P > 0.05$) decrease when compared to groups 5 and 3.

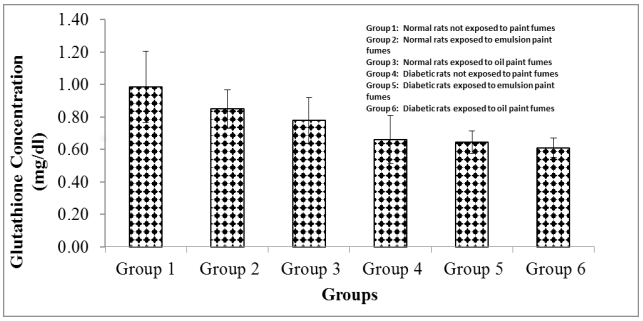


Figure 3. Effects of paint fumes on glutathione concentrations in diabetic and non diabetic rats.

4.5 Superoxide Dismutase Activity (u/l)

The SOD activity as represented in Figure 4 showed a non significant ($P > 0.05$) decrease in groups 2 (6.96 ± 0.45), 3 (6.36 ± 1.20), 4 (6.13 ± 0.68), and 5 (4.90 ± 0.45) when compared to group 1 (7.27 ± 0.91) (normal control). However, the activity of SOD in group 6 (4.09 ± 0.79) (diabetic rats exposed to oil paint fumes) was significantly ($P < 0.05$) lower compared to group 1 (normal control). A non-significant ($P > 0.05$) decrease was also observed when groups 5 (diabetic rats exposed to emulsion paint fumes) and 6 (diabetic rats exposed to oil paint) were compared to group 4 (negative control). The SOD activity in group 3 (normal rats exposed to oil paint) had a non significant reduction ($P > 0.05$) when compared to that of group 2 (normal rats exposed to emulsion paint), while group 5 (diabetic rats exposed to emulsion paint fumes) also gave a non significant decrease ($P > 0.05$) when compared to group 2 (normal rats exposed to emulsion paint). Group 6 (diabetic rats exposed to oil paint) also showed a non significant ($P > 0.05$) decrease when compared to groups 5 (diabetic rats exposed to emulsion paint fumes) and 3 (normal rats exposed to oil paint).

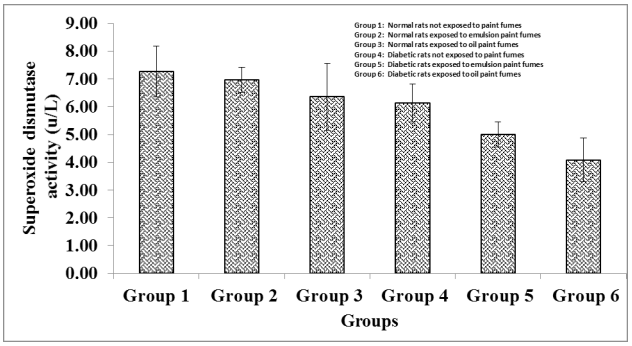


Figure 4. Effect of paint fumes on superoxide dismutase activity in diabetic and non diabetic rats.

4.6 Catalase Activity (u/l)

CAT activity results as represented in Figure 5, showed a significant ($P < 0.05$) decrease in groups 3 (0.29 ± 0.02), 4 (0.24 ± 0.09), 5 (0.20 ± 0.07), and 6 (0.18 ± 0.15) when compared to group 1 (0.59 ± 0.05) (normal control). However, the CAT activity in group 2 (0.54 ± 0.06) (normal rats exposed to emulsion paint fumes) were not significantly ($P > 0.05$) lowered compared to group 1 (0.59 ± 0.05) (normal control). A significant ($P < 0.05$) decrease was observed in group 5 (diabetic rats exposed to emulsion paint fumes) when compared to group 2 (normal rats exposed to emulsion paint fumes) and also group 3 (normal rats exposed to oil paint fumes) when compared to that of group 2 (normal rats exposed to emulsion paint fumes). A non significant ($P > 0.05$) decrease was observed when groups 5 and 6 were compared to group 4 (negative control) and non significant decrease ($P > 0.05$) also in group 6 (diabetic rats exposed to oil paint fumes) when compared to 5 (diabetic rats exposed to emulsion paint fumes).

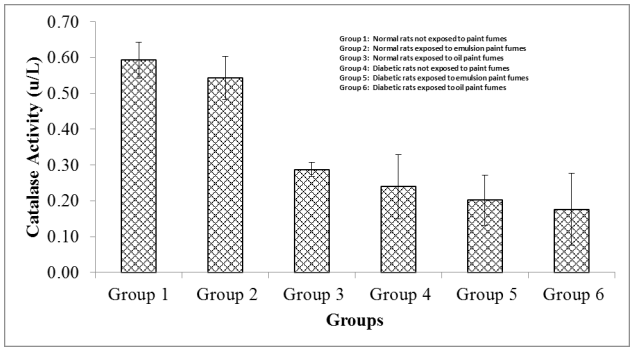


Figure 5. Effect of paint fumes on catalase activity in diabetic and non diabetic rats.

4.7 Packed Cell Volume (%)

PCV result as represented in Figure 6 showed a non significant ($P > 0.05$) decrease in groups 2 (39.67 ± 0.88), 3 (37.00 ± 2.65), 4 (39.00 ± 2.00), and 5 (36.33 ± 0.67) when compared to group 1 (41.00 ± 2.08) (normal control). However, the PCV result in group 6 (34.00 ± 0.58) (diabetic exposed to oil paint fumes) was significantly lower ($P < 0.05$) compared to group 1 (41.00 ± 2.08) (normal control). A non significant decrease ($P > 0.05$) was observed in group 5 (diabetic exposed to emulsion paint fumes) and 6 (diabetic exposed to oil paint fumes) when compared to group 4 (negative control). The PCV values of group 3 (normal rats exposed to oil paint fumes) showed a non significant ($P > 0.05$) decrease when compared to group 2

(normal rats exposed to emulsion paint fumes). Group 5 (diabetic exposed to emulsion paint fumes) was also non significantly ($P > 0.05$) reduced when compared to group 2 (normal rats exposed to emulsion paint fumes). A non significant ($P > 0.05$) decrease was observed in group 6 (diabetic exposed to oil paint fumes) when compared to group 3 (normal rats exposed to oil paint fumes) and 5 (diabetic exposed to emulsion paint fumes).

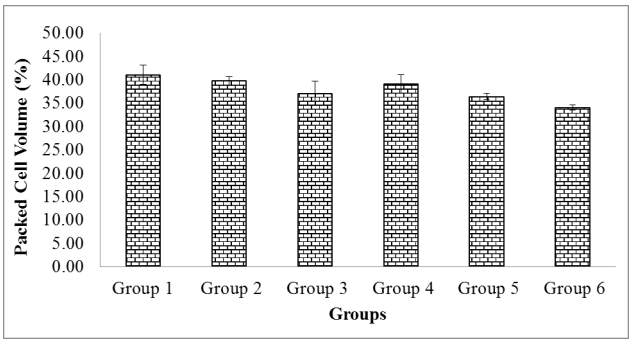


Figure 6. Effect of paint fumes on packed cell volume on diabetic and non diabetic rats.

4.8 Red Blood Cell Count (×10^9/l)

The result of RBC as represented in Figure 7 showed a non significant decrease ($P > 0.05$) in groups 2 (351.67 ± 20.48) and 3 (341.67 ± 44.75) when compared to group 1 (390.00 ± 34.64) (control group). However, RBC counts in groups 4 (298.33 ± 10.14), 5 (291.67 ± 16.92), and 6 (222.50 ± 7.50) (diabetic groups) were significantly ($P < 0.05$) reduced when compared to group 1 (390.00 ± 34.64) (normal control). A non significant decrease ($P > 0.05$) was observed in groups 5 (diabetic rats exposed to emulsion paint fumes) and 6 (diabetic rats exposed to oil paint fumes) when compared to group 4 (negative control). A similar decrease was observed in group 3 (normal rats exposed to oil paint fumes) when compared to groups 2 (normal rats exposed to emulsion paint fumes) and also when group 5 (diabetic rats exposed to emulsion paint fumes) was compared to group 2 (normal rats exposed to emulsion paint fumes). Group 6 (diabetic rats exposed to oil paint fumes) showed no significant decrease ($P > 0.05$) when compared to the RBC counts of groups 3 (normal rats exposed to oil paint fumes) and 5 (diabetic rats exposed to emulsion paint fumes).

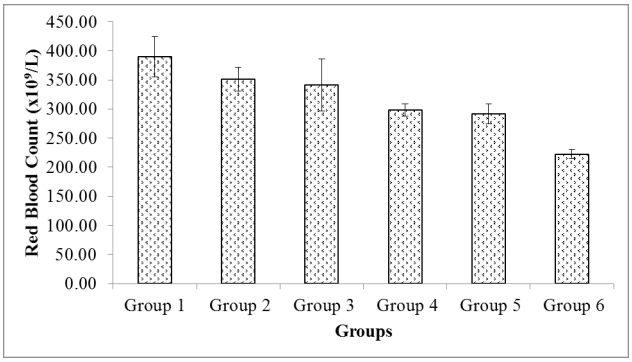


Figure 7. Effect of paint fumes on red blood cell count of diabetic and non diabetic rats.

4.9 White Blood Cell Count (/mm^3)

WBC count results as represented in Figure 8, showed a non significant increase ($P > 0.05$) in group 2 (4366.67 ± 371.18), 3 (4733.33 ± 588.11), 4 (5200.00 ± 700.00), and 5 (6766.67 ± 726.16) when compared to group 1 (4040.00 ± 1835.10) (normal control). However, WBC count in group 6 (7866.67 ± 102.76) was significantly increased ($P < 0.05$) when compared to group 1 (4040.00 ± 1835.10) (normal control). There was a non significant increase ($P > 0.05$) in groups 5 (diabetic exposed to emulsion paint fumes) and 6 (diabetic rats exposed to oil paint fumes) when compared to group 4 (negative control). A non significant increase ($P > 0.05$) was observed in group 6 (diabetic rats exposed to oil paint fumes) when compared to groups 3 (normal rats exposed to oil paint fumes) and 5 (diabetic rats exposed to emulsion paint fumes). In addition, non significant decrease ($P > 0.05$) was observed in group 2 (normal rats exposed to emulsion paint fumes) when compared to groups 3 (normal rats exposed to oil paint fumes) and 5 (diabetic rats exposed to emulsion paint fumes) and the result of platelet count showed that there was no significant increase ($P > 0.05$) in group 2 (138.33 ± 4.41) and 5 (133.33 ± 14.24) when compared to group 1 (125.00 ± 5.77) (normal control). However, significant increase ($P < 0.05$) was observed in groups 4 (212.50 ± 32.50) and 6 (201.67 ± 19.65) (diabetic groups) when compared to group 1 (normal control). A non significant decrease ($P > 0.05$) was observed in group 3 (113.33 ± 17.64) when compared to group 1 (125.00 ± 5.77). The platelet count values showed a significant decrease ($P <$

0.05) in group 5 (diabetic rats exposed to emulsion paint fumes) when compared to group 4 (negative control) and a non significant decrease in group 6 when compared to group 4 (negative control). Groups 3 (normal rats exposed to oil paint fumes) and 5 (diabetic rats exposed to emulsion paint fumes) showed no significant ($P > 0.05$) decrease in the platelet counts when compared to group 2 (normal rats exposed to emulsion paint fumes). There was significant ($P < 0.05$) increase in group 6 (diabetic rats exposed to oil paint fumes) when compared to groups 3 (normal rats exposed to oil paint fumes) and 5 (diabetic rats exposed to emulsion paint fumes).

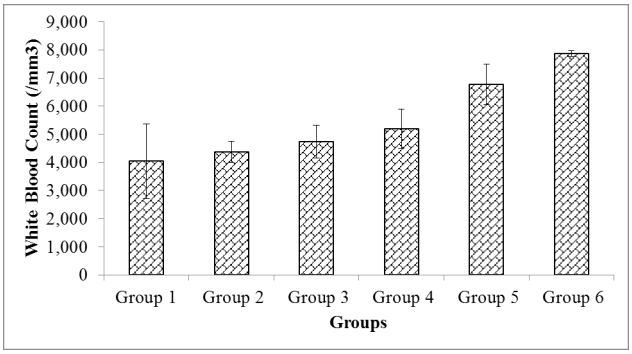


Figure 8. Effect of paint fumes on white blood cells of diabetic and non diabetic rats.

5. Discussion

An abnormally high blood glucose concentration is an index of diabetes mellitus (Anuradha and Ravikumar, 2001). The results of this study showed a normal range of baseline glucose levels in all the rats irrespective of their groups before the induction of diabetes mellitus. Abnormal high blood glucose concentration was observed (Figure 1.) after induction of diabetes with alloxan in groups 4, 5, and 6 when compared to group 1 (normal control). A high blood glucose concentration may be due to partial or complete destruction insulin-producing cells (β cells of the Islets of Langerhans) and/or resistance of the system to insulin action (Mahdi *et al.,* 2003). However, insulin concentration was not determined in this study to ascertain the degree of insufficiency. Glucose itself has been speculated to be an antioxidant at a concentration of about 4–6 mmol/l, beyond which it becomes an oxidant (Gutteridge, 1994). Thus, the high blood glucose concentration obtained in the present study could be due to alloxan- induced oxidative stress. On the other hand, diabetes may have caused a defect in the recruitment of glucose transporters from the intracellular sites to the surface of the skeletal muscles cells. High blood glucose levels in groups 2, 3, 5, and 6 (groups exposed to paint fumes) could also be attributed to the oxidative stress caused by organic solvents in paints. Wichmann *et al.*, (2005) reported that this organic solvent might result to Insulin Resistance (IR). It has also been reported that exposure to organic solvents increased cytokines such as tumor necrosis factor-alpha involved in inflammatory responses (Wichmann *et al.,* 2005; Röder-Stolinski *et al.,* 2008). Increased Reactive Oxygen Species (ROS) mediated oxidative stresses are involved in blood glucose elevation and development of IR (Röder-Stolinski *et al.,* 2008). From the result, the glucose levels in group 4 (diabetic rats not exposed to paint fumes) reduced when compared to that of groups 5 and 6 that were induced and exposed to paint fumes. This increase in groups 5 and 6 might be as a result of glycosylation observed in abnormal blood glucose level and prooxidative activity of the exposed paint fumes. A high blood glucose concentration causes more oxidative stress or damage to the cells (Gutteridge, 1994). This was evidenced by the increase in the MDA levels, an index of lipid peroxidation. Melpomeni *et al.*, (2003) reported that chronic elevation of blood glucose concentrations led to the damage of blood vessels and tissues and many other complications. The MDA levels in group 4 (diabetic rats not exposed to paint fumes) increased when compared to group 1 (normal control), suggesting that diabetes mellitus is associated with increased lipid peroxidation. The MDA values increased more in groups 2 (normal rats exposed to emulsion paint fumes) and 3 (normal rats exposed to oil paint fumes), compared with group 1, which could be attributed to increased oxidative stress caused by increased blood glucose level and organic solvents from paint fumes. A further increase in MDA values was observed when groups 5 (diabetic exposed to emulsion paint fumes) and group 6 (diabetic rats exposed to oil paint fumes) when compared with groups 1 (normal control) and 4 (diabetic rats not exposed to paint fumes). However, group 3 showed more increased values when compared to group 2 (normal rats exposed to emulsion paint fumes). A similar increase in MDA values was observed when group 6 was compared to group 5, suggesting that oil paint could illicit more damaging effect on membrane lipids compared to those exposed to emulsion paint fumes. The MDA values of group 5 (diabetic rats exposed to emulsion paint fumes) increased

more when compared to group 2 (normal rats exposed to emulsion paint), suggesting that increased lipid peroxidation could also be complicated by abnormally high blood glucose since both were exposed to emulsion paint fumes. Group 6 also showed an increased MDA values when compared to group 3 which may suggest that a shared mechanism could drive both the elevated blood sugar and lipid peroxidation. Our result which has positive correlation between blood glucose level and MDA is in line with the report of Reaven (Reaven, 1995). The MDA result showed significantly higher values in diabetic groups (4–6) than in the normal control (group 1). This finding is also consistent with the report of Szkudelski (2001) which stated that diabetic tissues produced a greater amount of free radicals with respect to healthy tissues. Oxidative stress is closely associated with diabetes and can decrease antioxidant protection (Giugliano *et al.,* 1996). Organic solvents in paint antagonize antioxidants by virtue of its affinity for free sulfhydryl groups thereby increasing the concentration of ROS. GSH is an antioxidant whose function is facilitated by the sulfhydryl group of cysteine (one of its tripeptides) (Goering et al., 2002). The decrease in the GSH level across the groups, when compared to group 1 (normal control), could be attributed to its depletion in the presence of increased free radical – mediated oxidative stress. Mercury (Hg) toxicity could affect metabolism and function of GSH by depleting Nicotinamide Adenine Dinucleotide Phosphate (NADPH) by the generation of ROS. Hg in paints could bind with selenium (Se) and by extension affect the critical role of selenium in GSH metabolism. While the mechanism for reduction is poorly understood, oil paint is entirely base and is made of organic solvent that could cause more oxidative stress. In a hyperglycemic state, the affinity of aldose reductase for glucose rises, causing much sorbitol to accumulate, and using much more NADPH, reducing NADPH meant for other processes of cellular metabolism; excessive activation of the polyol pathway increases sorbitol concentration, thus increasing concentrations of ROS and decreased concentrations GSH (Brownlee, 2001).

SOD, gave a significant decrease in groups 2, 3, 4, 5, and 6 when compared to group 1 (normal control) (Figure 4). The reduction in SOD activity suggests its anti peroxidative role. Paint fumes could generate superoxide anion $O_2^{\bullet}$ and SOD mops up these radicals and by extension diminish the concentration of SOD involved in the process. The prooxidative nature of the oil paint fumes was more pronounced when compared to the emulsion paint fumes, as the SOD activity in rats exposed to oil paint fumes (groups 3 and 6) decreased more than those exposed to emulsion paint fumes (groups 2 and 5), respectively. CAT activity also showed a decrease across the groups when compared to group 1 (normal control). Diminution of CAT could probably be due to its antioxidant role caused by increased ROS generation. The antioxidant result of GSH, CAT, and SOD showed an inverse relationship with MDA, which is in line with the report of Goering et al., (2002) who associated high lipid peroxidation with low antioxidant levels.

We also observed that the PCV as an index of anemia was lower across the groups when compared to group 1 (control group) as shown in Figure 6, however, a significant difference was observed in group 6 (diabetic rats exposed to oil paint). The significant decrease in group 6 could be associated with anemia caused by the accumulation of Hg found in the paint fumes in the blood–forming tissues (bone marrow) and may interrupt erythropoiesis. This is in line with the findings of Saljooghi and Fatemi (2010), which stated that accumulation of heavy metals in the bone marrow may lead to anemia. The decrease in PCV in the diabetic groups may have been caused by non enzymatic glycosylation of RBC membrane protein, which correlates with hyperglycemia (Oyedemi et al., 2011). This agrees with the findings of Oliveira Ribeiro et al., (2006) which stated that decrease in PCV can be as a result of anemia or a chronic disease state such as diabetes due to increased accumulation of glucose in the blood. The lower level observed in group 6 (diabetic rats exposed to oil paint fumes) when compared to group 5 (diabetic rats exposed to emulsion paint fumes) showed that oil paint fumes had more effect on PCV than emulsion paint fumes. The result also showed a decrease in group 5 when compared to group 2; this probably could mean that diabetic condition coupled with paint fume could inhibit bone marrow function as against non diabetic rats exposed to the same emulsion paint fumes. PCV may be decreased in diabetes because of higher levels of blood glucose which can potentially cause intracellular dehydration. There was a decrease in RBC count across the groups when compared to group 1, with significantly decreased values in diabetic groups (groups 4–6) when compared to group 1 (normal control). This suggests that diabetic state and paint fumes share the same mechanism that probably affects the erythropoietic process. The toxic effect of paint fumes may have caused

hypoxic environment in the renal interstitium, which could result in impaired production of erythropoietin by the peritubular fibroblasts and subsequently cause anemia (Holmes et al., 2009). Healthy kidneys produce a hormone called erythropoietin which stimulates the bone marrow to produce the proper number of RBC needed to carry oxygen to vital organs. The toxic effect on the kidney might lead to decrease erythropoietin production in response to falling PCV. This agrees with the finding of Zuckerman (2007) which stated that Hg in paints accumulates in body tissues and bone marrow and distorts the production of RBC. The decreases in PCV and RBC observed in these results agree with the findings of Oliveira Ribeiro et al. (2006) which stated that PCV provides information about the amount of RBC present in the blood. WBC count gave a non significant increase in groups 2, 3, 4, and 5 when compared to group 1. However, significant increase was seen in group 6 when compared to group 1. These increases could be a response to the toxic effect of the paint fumes on bone marrow causing abnormally high proliferation of WBC. This suggests that group 6 (diabetic rats exposed to oil paint) was most affected by the paint fumes which possibly stimulated toxic and inflammatory response when compared to other diabetic groups (4 and 5) while the non diabetic rats exposed to oil paint fumes (group 3) triggered the most inflammatory response when compared to other non diabetic groups (1 and 2). These increases may be as a result of anemia, bone marrow toxicity, stress, or tissue damage caused by the paint fumes and glycosylation. This is in agreement with the work of Petterino and Argentino-Storino (2006) which stated that the continuous rise in the WBC (a marker of inflammation) is an indication of a worse disease state. Toxic effect of paint fumes and IR (orchestrated by alloxan) could cause an inflammatory response by stimulating WBC proliferation; antigenic challenge leads to increased maturation of neutrophils (Apeh et al., 2014). The action of GSH (which plays a key role in the RBC protection) has been compromised by the binding of Hg to its sulfhydryl group; the GSH might not be able to mop up ROS within the body before they are transformed to methemoglobin (Omaye, 2002; Betteridge, 2000). When this mechanism favors the binding of Hg to the sulfhydryl group of GSH, the GSH which defends against oxidative stress within the RBC allows the hemoglobin molecule to remain in the ferric state (Fe^{3+}) and not be reduced to the ferrous state (Fe^{2+}) (Betteridge, 2000). The alterations in the redox potentials of the methemoglobin reductase and its coupling enzymes result in hypoxia (higher oxygen affinity) of diabetic rats exposed to paint fumes than normal rats. This agrees with the findings of Okonkwo et al., (2015). Platelet level was significantly increased in groups 4 and 6 (diabetic) when compared to group 1 (normal control). Reactive thrombocytosis has also been reported among diabetic patients particularly those with poor diabetic control associated with raised blood sugar level (Carobbio et al., 2008). This could be attributed to reduced membrane fluidity, which may reflect changes in the lipid composition of the membrane or glycation of membrane proteins. Similarly, the previous report has shown that raised Platelet values are commonly seen in inflammatory and infectious diseases (Wiggers, 2008). The increase in groups 2 and 3, when compared with group 1, could be due to exposure of rats to paint fumes which could damage the blood vessel probably causing increased platelet aggregability and adhesiveness. Diabetic groups showed more increase in platelet when compared with the non diabetic group. This agrees with the work of (Schmaier, 2008) which states that platelets adhere to exposed collagen in damaged vessels to initiate clotting. We observed positive correlation between raised total WBC and platelet and hyperglycemia.

This study shows that paints (water or oil) had a negative effect on both normal and diabetic rats but showed a more adverse effect in diabetic state when compared to the control group. The inference was drawn from increased blood glucose, MDA and depletion of antioxidant shown in this study. Since it has been established that paint fumes, be it emulsion (water) or oil paint fumes are hazardous to the health of both normal and diabetic rats, it is, therefore, necessary to allow new and renovated houses for proper evaporation of the fumes. Further studies are needed to evaluate the liver and kidney function as well as the histopathological effect of paint fumes.

6. References

1. Kaneto H, Fujii J, Myint T, Miyazawa N, Islam KN, Kawasaki Y, et al. Reducing sugars trigger oxidative modification and apoptosis in pancreatic beta-cells by provoking oxidative stress through the glycation reaction. Biochem J. 1996; 320(Pt 3):855–63.
2. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and {beta}-cell dysfunction? Diabetes. 2003; 52:1–8.

3. American Diabetes Association (ADA). Standards of medical care in diabetes. Diabetes Care. 2012; 35:11–3.
4. Wiggers GA, Peçanha FM, Briones AM, Pérez-Girón JV, Miguel M, Vassallo DV, et al. Low mercury concentrations cause oxidative stress and endothelial dysfunction in conductance and resistance arteries. Am J Physiol Heart Circ Physiol. 2008; 295:H1033–43.
5. Wax PM, Beuhler MB. Hydrocarbons and volatile substances. In: Tintinalli JE, Kelen GD, Stapczynski JS, Ma OJ, Cline DM, editors. Emergency Medicine: A Comprehensive Study Guide. 6th ed. New York: McGraw-Hill; 2004. p. 1330–400.
6. Townsend CL, Maynard RL. Effects on health of prolonged exposure to low concentrations of carbon monoxide. Occup Environ Med. 2002; 59:708–11.
7. Zuckerman K. Approach to the anemias. In: Goldman L, Ausiello D, editors. Cecil Medicine. 23rd ed. Philadelphia, PA: Saunders Elsevier; 2007. p. 1103.
8. Omaye ST. Metabolic modulation of carbon monoxide toxicity. Toxicology. 2002; 180:139–50.
9. Fröde TS, Medeiros YS. Animal models to test drugs with potential antidiabetic activity. J Ethnopharmacol 2008; 115:173–83.
10. Piggott WR, Emmons CW. Device for inhalation exposure of animals to spores. Proc Soc Exp Biol Med. 1960; 103:805–6.
11. Varshney R, Kale RK. Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. Int J Radiat Biol. 1990; 58:733–43.
12. King EJ, Wotton ID. Microanalyses in medical biochemistry. New York: McGraw-Hill; 1959. p. 14.
13. Das K, Samanta L, Chainy GB. A modified spectrophotometric assay of superoxide dismutase using nitrite formation by superoxide radicals. Indian J Biochem Biophys. 2000; 37:201–4.
14. Aebi HE. Catalase. In: Bergmeyer HU, editor. Methods of Enzymatic Analyses. 3rd ed. Deerfield Beach, Florida: Weinheim; 1983. p. 273–85.
15. Ochei J, Kolhatkar A. Medical laboratory science: Theory and oractice. New Delhi: Tata McGraw-Hill Publishing Company; 2008. p. 281–3.
16. Betrand E. Determination of serum methaemoglobin by second derivative spectroscopy. Clin Chim Acta. 1982; 123:121–6.
17. Cheesbrough M. District laboratory practice in tropical countries. Part 2. United Kingdom: Cambridge University Press; 2000. p. 284–90.
18. Anuradha CV, Ravikumar P. Restoration on tissue antioxidants by fenugreek seeds (Trigonella foenum graecum) in alloxan-diabetic rats. Indian J Physiol Pharmacol. 2001; 45:408–20.
19. Mahdi AA, Chandra A, Singh RK, Shukla S, Mishra LC, Ahmad S. Effect of herbal hypoglycemic agents on oxidative stress and antioxidant status in diabetic rats. Indian J Clin Biochem. 2003; 18:8–15.
20. Gutteridge JM. Antioxidants, nutritional supplements and life-threatening diseases. Br J Biomed Sci. 1994; 51:288–95.
21. Wichmann G, Mühlenberg J, Fischäder G, Kulla C, Rehwagen M, Herbarth O, et al. An experimental model for the determination of immunomodulating effects by volatile compounds. Toxicol In Vitro. 2005; 19:685–93.
22. Röder-Stolinski C, Fischäder G, Oostingh GJ, Feltens R, Kohse F, von Bergen M, et al. Styrene induces an inflammatory response in human lung epithelial cells via oxidative stress and NF-kappaB activation. Toxicol Appl Pharmacol. 2008; 231:241–7.
23. Melpomeni P, Jaime U, Helen V. Glucose, advanced glycation end products, and diabetes complications: What is new and what works. Clin Diabetes. 2003; 21:186–7.
24. Reaven P. Dietary and pharmacologic regimens to reduce lipid peroxidation in non-insulin-dependent diabetes mellitus. Am J Clin Nutr. 1995; 62(6S):1483S–9S.
25. Szkudelski T. The mechanism of alloxan and streptozotocin action in β-cells of the rat pancreas. Physiol Res. 2001; 50:537–46.
26. Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. Diabetes Care. 1996; 19:257–67.
27. Goering PL, Morgan DL, Ali SF. Effects of mercury vapor inhalation on reactive oxygen species and antioxidant enzymes in rat brain and kidney are minimal. J Appl Toxicol. 2002; 22:167–72.
28. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001; 414:813–20.
29. Saljooghi AS, Fatemi SJ. Clinical evaluation of deferasirox for removal of cadmium ions in rat. Biometals. 2010; 23:707–12.
30. Oyedemi SO, Yakubu MT, Afolayan AJ. Antidiabetic activities of aqueous leaves extract of Leonotis leonurus in streptozotocin induced diabetic rats. J Med Plant Res. 2011; 5:119–25.
31. Ribeiro CAO, Filipak Neto F, Mela M, Silva PH, Randi MA, Rabitto IS, et al. Hematological findings in neotropical fish Hoplias malabaricus exposed to subchronic and dietary doses of methylmercury, inorganic lead, and tributyltin chloride. Environ Res. 2006; 101:74–80.
32. Holmes P, James KA, Levy LS. Is low-level environmental mercury exposure of concern to human health? Sci Total Environ. 2009; 408:171–82.
33. Petterino C, Argentino-Storino A. Clinical chemistry and haematology historical data in control Sprague-Dawley rats from pre-clinical toxicity studies. Exp Toxicol Pathol. 2006; 57:213–9.
34. Apeh VO, Agu CV, Ogugua VN, Uzoegwu PN, Anaduaka EG, Rex TE, Agbalu IS. Effect of cooking on proximate, phytochemical constituents and hematological parameters of tetracarpidium conophorum in male albino rats. Eur J Med Plants. 2014; 4(12):1388–99.
35. Betteridge DJ. What is oxidative stress? Metabolism. 2000; 49(2S1):3–8.

36. Okonkwo CC, Agu CV, Njoku OU, Abonyi U, Apeh VO, Anaduaka EG, Iloabuchi KV, Odo CE. Hypoglycaemic and haematinic properties of ethanol leaf extract of artocarpus Heterophyllus in alloxan induced diabetic rats. Afr J Tradit Compl Altern Med. 2015; 12(2):133–48.
37. Carobbio A, Antonioli E, Guglielmelli P, Vannucchi AM, Delaini F, Guerini V, et al. Leukocytosis and risk stratification assessment in essential thrombocythemia. J Clin Oncol. 2008; 26:2732–6.
38. Schmaier AH. Laboratory evaluation of hemostatic and thrombotic disorders. In: Hoffman Hematology: Basic Principles and Practice. 5th ed. Philadelphia, PA: Churchill Livingstone Elsevier; 2008. p. 1122.