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Assessment of Post Exposure of Benzene on Some Hematology Parameters and DNA Lesions on Adult Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author OPK designed the study and wrote the first draft of the manuscript. Author AAO performed the statistical analysis. Author AAL wrote the protocol, author ARAF managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study sought to investigate the effect of post-exposure of benzene on some haematology parameters and DNA lesions on adult wistar rats.

Methods: A total of twenty-eight rats were grouped into 4 groups, with group 1 serving as a control. The remaining 3 groups were interperitoneally administered 0.2 ml of benzene 48 hourly for 4 weeks and were left of 0, 3 and 6 weeks respectively before sacrifice.

Results: The result obtained showed that White Blood Cell (WBC), Red Blood Cell (RBC) and platelet counts were significantly reduced in benzene post exposed groups ($p<0.05$). 8-hydroxydeoxyguanosine generation in liver and bone marrow were significantly higher in benzene post exposed groups. The photomicrograph of blood film of benzene exposed group showed promyelocyte and myeloblast.

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Conclusion: It is concluded that benzene and its metabolite are highly toxic and are potentially damaging agents to the hematopoietic system. Evidence from our study suggested that elevated levels of 8-OHdG in liver and bone marrow compare to control would be a sign of increased oxidative stress, impaired antioxidant defence or inadequate repair of oxidative damaged DNA.

Keywords: Interperitoneally; hematology; 8-hydroxydeoxyguanosine; promyelocyte; myeloblast; benzene.

1. INTRODUCTION

Benzene is an aromatic hydrocarbon and a component of crude oil and gasoline. It is a widely used chemical formed from both natural processes and human activities and a ubiquitous contaminant in the environment [1]. Occupational exposure to benzene occurs through solvent exposure in the chemical industry, in petroleum refineries, oil pipelines, on ships and tankers, bus garages and auto repair shops [2]. Human exposure to benzene occurs in diverse ways such as through dermal absorption, inhalation, ingestion of contaminated food and water, and active and passive tobacco smoking [3].

Benzene exposure causes many adverse effects, such as skin irritation, inflammation of the nasal airways and throat, central nervous system depression, immunotoxicity and hematoxicity [4]. The cells of the hematopoietic system and bone marrow are the most sensitive target organs of benzene toxicity. Repeated occupational and non-occupational exposure to benzene over long periods may affect several hematopoietic parameters such as red blood cell, white blood cell and platelet counts [5] and eventually induce toxicity of the blood and blood forming organs. Benzene exposure is associated with increased risk of acute myeloid leukemia [6], multiple myeloma and non-Hodgkin's lymphoma. Studies indicate that exposure to benzene produce toxicity and an increased risk of malignancies of the blood and blood-forming organs [7]. Benzene toxicity is linked to bone marrow depression, aplastic anemia, acute myeloblastic leukaemia and acute non-lymphocytic leukemia [8] and even at low doses can affect white blood cell and platelet counts [9]. This research was designed to study the immunotoxicity and hematotoxicity of post exposure to benzene in adult rats of Wistar strain.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Citric acid, sodium citrate, sodium chloride and tris-hydrochloric acid were products of British Drug House (Pool, England). Benzene was obtained from Sigma, St. Louis MO while 8-hydroxydeoxyguanosine assay kit was obtained from Cloud-Clone Corp, USA. The total protein kit was obtained from *Fortress Diagnostics Limited* (Muckamore, United Kingdom). All the chemicals and reagents were of analytical grade and were used and stored according to the manufacture's instructions.

2.2 Preparation of Reagents

2.2.1 Citrate buffer

21.01 g of citric acid and 29.41 g of sodium citrate were dissolved in 1 liter of distilled water each. 9.5 ml of citric acid solution was added to 41.5 ml of sodium citrate solution and was made up to 100 ml and the pH was adjusted to 6.0 and stored at 4°C.

2.2.2 Homogenizing buffers

7.86 g of 50 mM Tris-HCl and 11.2 g of potassium chloride was dissolved in 900mls of distilled water and the pH was adjusted to 7.4. The solution was made up to 1 L and stored at 4°C.

2.3 Experimental Design

A total of 28 Albino rats of Wistar strain, with an average weight of 190 g were obtained from the Animal house of Physiology Department, Ladoke Akintola University of Technology, Nigeria. The animals were divided into 4 groups and housed in separate cages in the same environment. The animals were allowed to acclimatize in the laboratory for two weeks before the commencement of the experiments.

Benzene was administered to Wistar rats by interperitoneally injection of 0.2 ml of benzene solution, given every 48 hours for 4 weeks.

The animals were randomly selected into 4 groups, each containing 7 rats.

Group I: Rats in this group served as the negative control. Group II: Rats in this group

were administered benzene for 4 weeks and were sacrificed immediately (0 week) after administration. Group III: Rats in this group were administered benzene for 4 weeks and were left for 3 weeks before sacrifice. Group IV: Rats in this group were administered benzene for 4 weeks and were left for 6 weeks before sacrifice.

2.4 Collection of Blood and Organs

The rats were fasted overnight and sacrificed according to the experimental protocol. Blood was collected by cardiac puncture. Part of the blood was collected inside plain sample bottles (without anticoagulant), allowed to coagulate and then centrifuged at 3000 rpm for 15 minutes to obtain serum. The serum was kept under refrigeration at 4°C for biochemical parameters. The other part of the blood was collected into vacuum EDTA bottles, to be used for haematological parameters.

2.5 Tissue Preparation

The livers of the rats were excised, washed with normal saline solution and weighed. 1 g of the washed liver was homogenized in 5 ml homogenizing buffer (7.86 g of 50 mM Tris-HCl and 11.2 g of potassium chloride dissolve in 900ml of distilled water, pH 7.4) using mortar and pestle. The homogenates were centrifuged at 3000 rpm for 15 minto obtain the clear supernatant. The resulting homogenates were kept at 4ºC for biochemical parameters.

2.6 Preparation of Bone Marrow Aspirate

The femur and humorous bone of the sacrificed experimental mice were dissected and both the proximal and distal ends were removed by using the modified method of Saha, et al. [10]. Briefly, citrate buffer solution of pH 6.0 was injected gently into one end of the shaft. This process was followed to flush out the bone marrow through the opposite end into eppendoff tube to prepare and centrifuge at 3000 rpm for 15 minutes to obtain the clear supernatant and pellet and were kept at 4°C for biochemical analysis.

2.7 Determination of Haematological Parameters

Haematological parameters were assessed by flow cytometry (direct current method) using suitable cell packs according to the manufacturer's specification for the desired cell population on the SYMEX KX-21N autoanalyzer.

2.8 Biochemical Examination

The concentration of 8-hydroxyldeoxylguanosine (8-OHdG) in liver homogenate, bone marrow cell and bone marrow supernatant were measured using immunoenzymatic colorimetric method of DiaMetra, Italy (2008) and total protein concentration was determined in the serum and tissue homogenates by the Biuret (colorimetric) method [11] using FORTRESS Total Protein kit.

2.9 Statistical Analysis

Results are presented as Mean ± SD. Paired Student's t-test was used to compare variations amongst groups. The minimum level of significance was considered at $p<0.05$. Statistical analysis was carried out using a software program (GraphPad Prism Ver. 5; GraphPad Software, San Diego, CA).

3. RESULTS

3.1 Effects of Post Benzene Exposure on Haematological Parameters

There was a significant decrease ($P<0.05$) in red blood cell counts of groups post exposed to benzene at week 0 (8.19×10^3 mm^3), week 3 ($7.38 \times 10^3 mm^3$) and week 6 (5.02×10^3 mm^3 ; Figure 1) compared to the control (11.3×10^3 mm^3). Similarly, there was a significant ($P<0.05$) decrease in white blood cell counts of groups post exposed to benzene at week 0 (9.85×10^9/L), week 3 (4.46×10^9/L) and week 6 (4.42×10^3 mm^3; Fig. 2) compared to the control (12.0×10^9/L). Platelet counts were significant ($P<0.05$) decrease in groups post exposed to benzene at week 0 (636×10^9/L), week 3 (563×10^9/L) and week 6 (510×10^9/L) (Fig. 3) compared to the control (981×10^9/L)

3.2 Effect of Post Benzene Exposure on 8-Hydroxydeoxyguanosin (8-OHdG) Concentration

There was a significant increase ($P<0.05$) in liver 8-hydroxydeoxyguanosin concentration of group post exposed to benzene for 0 (0.61 ng/mg protein), 3 (0.59 ng/mg protein) and 6 (0.35 ng/mg protein) weeks respectively (Fig. 4) compared to the control. Production of liver 8-OHdG reduced during the post-exposure period; this it decreased between 4% and 43% from initial week to third week and third week to sixth week, respectively. There was also significant ($P<0.05$) increase in generation of 8-OHdG by

bone marrow cell in groups post exposed to benzene for 0 (0.29 ng/mg protein), 3 (0.46 ng/mg protein) and 6 (0.71 ng/mg protein) weeks (Fig. 5) compared to the control (0.1651 ng/mg protein). The generation of 8-OHdG by bone marrow cell in groups exposed benzene increase during post-exposure period, (between 37% and 58% at week 3 and week 6, respectively). Similarly there was a significant ($P<0.05$) increase in 8-OHdG concentration in bone marrow supernatant groups post exposed to benzene for 0 (3.75 ng/mg protein), 3 (3.90 ng/mg protein) and 6 (4.50 ng/mg protein) weeks respectively, compared to the control (0.242 ng/mg protein; Fig. 6).

3.3 Effect of Benzene Exposure on Blood Film

The histological study on blood film and bone marrow smear revealed different morphological changes in blood cells structures. The photomicrograph of blood film of benzene exposed group showed promyelocyte and myeloblast compared to normal control, promyelocyte (10-20 m) is slightly larger than a blast. Its nucleus, although similar to a myeloblast shows slight chromatin condensation and less prominent nucleoli. The cytoplasm contains striking azurophilic granules or primary granules. These granules contain

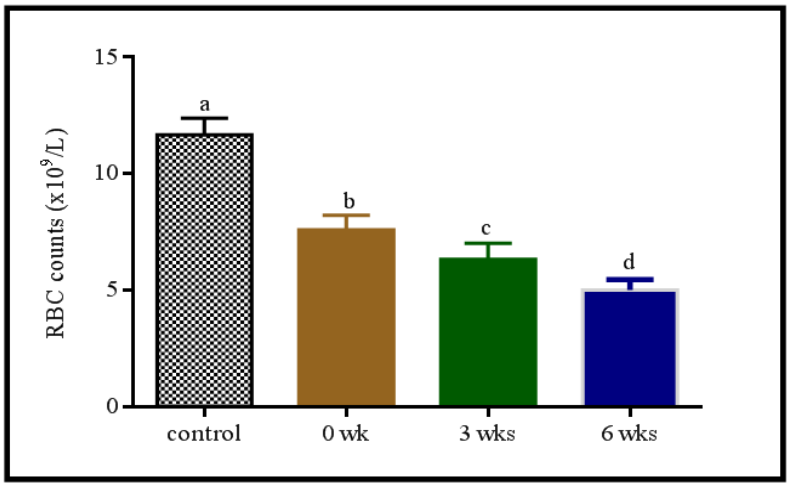

Fig. 1. Effects of post treatment on red blood cell counts

Each value is Mean ± SD. Bars with different alphabets ($^{a, b, c, d}$) are significantly different from each other at $p<0.05$

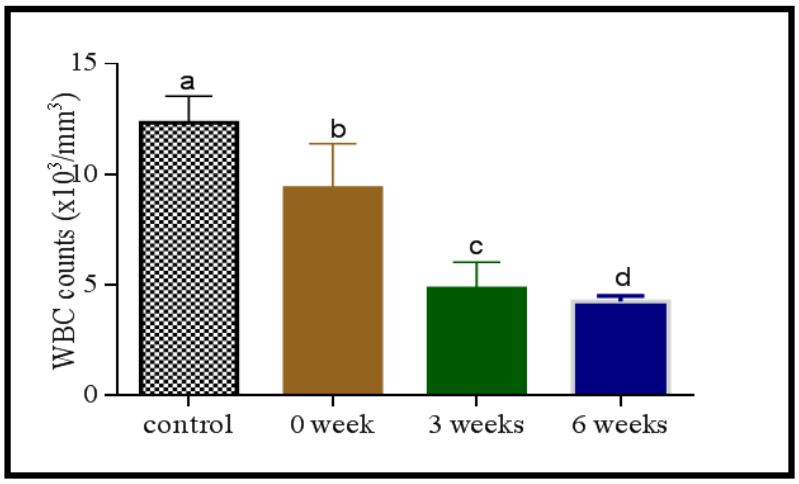

Fig. 2. Effects of post treatment duration of benzene on white blood cell counts

Each value is Mean ± SD. Bars with different alphabets ($^{a, b, c, d}$) are significantly different from each other at $p<0.05$

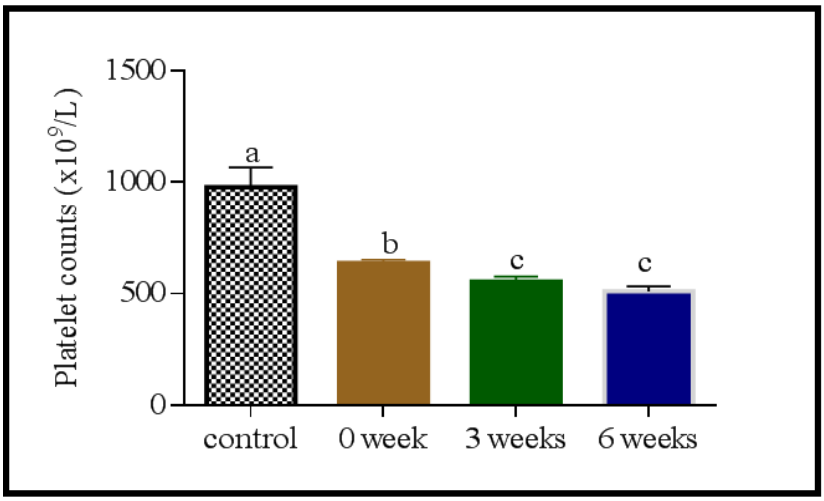

Fig. 3. Effects of post treatment duration of benzene on platelet counts

Each value is Mean ± SD. Bars with different alphabets ($^{a, b, c, d}$) are significantly different from each other at p<0.05

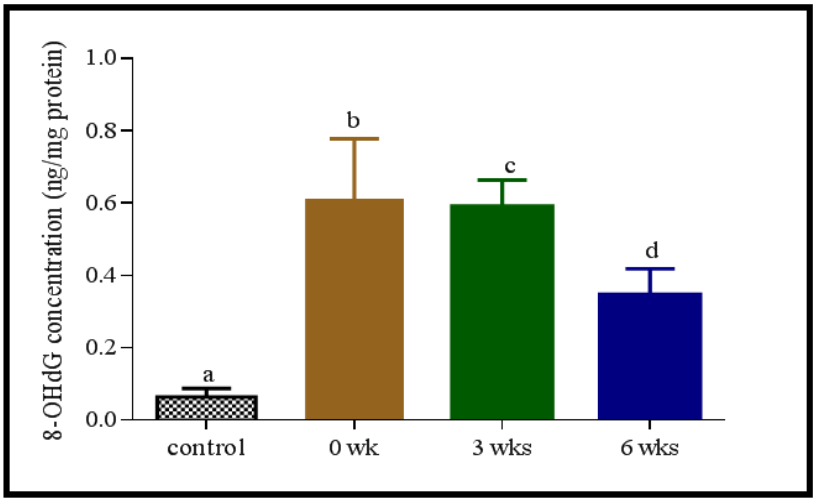

Fig. 4. Effects of post treatment duration of benzene on liver 8-OHdG concentration

Each value is Mean ± SD. Bars with different alphabets ($^{a, b, c, d}$) are significantly different from each other at p<0.05

myeloperoxidase, acid phosphatase, and esterase enzymes. Normally no promyelocytes are seen in the peripheral blood. Myelocytes (10-18m) are slightly smaller than promyelocytes and have eccentric round-oval nuclei, often flattened along one side. The chromatin is fine but shows evidence of condensation. Nucleoli may be seen in early stages but not in the late myelocyte.

The photomicrograph of blood films of the exposed group also shows hypersegmented neutrophil compared to normal control. Neutrophils are generally the most abundant white cell. They are round, are 10–14 µm wide, and contain a lobulated nucleus with two to four lobes connected by a thin chromatin thread.

4. DISCUSSION

A study has revealed that blood is the most important tissue in the body by which metabolic changes are reproduced [12], any changes in blood parameters and indices have always been considered to be the most reliable indicator of toxic effects of any substance, such as drugs and heavy metals. Changes in some haematological

parameters and indices were recorded in rats post expose to benzene.

The principal function of WBCs as phagocytes is to defend the body against invading microorganisms or is xenobiotic by ingesting and destroying them and, hence, contributing to cellular mechanism [13]. The decrease in total WBC suggests a decrease in immune system in fighting foreign substances [14]. These findings were consistent with studies in which leucopoenia was shown to be the most sensitive effect on blood cells associated with benzene exposure [15,16]. A similar conclusion was reported by Tsai, et al. [17] in a large study on 1,200 employees exposed to benzene. The mechanism underlying reduction in WBC is direct cytotoxicity of benzene metabolites on the production of WBC in the bone marrow. Benzene is known as immunotoxic and blood cell carcinogen, inducing anaemia, and blood formula modification [18].

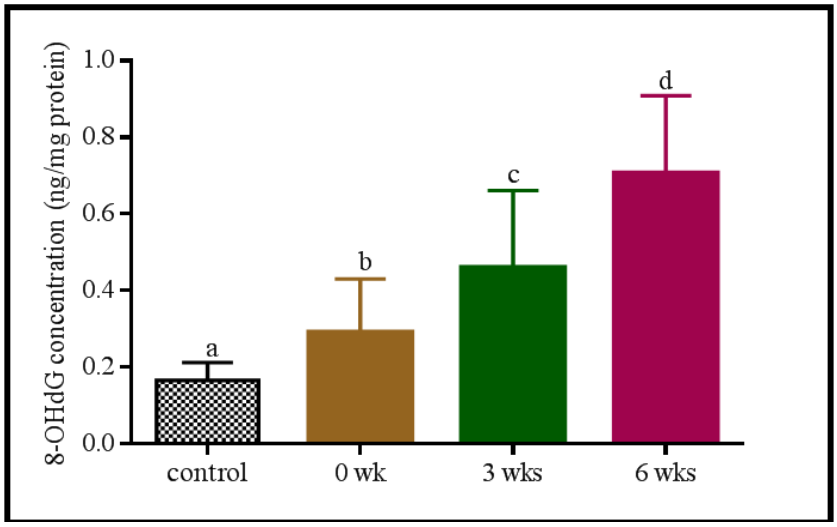

Fig. 5. Effects of post treatment duration of benzene on bone marrow cell 8-OHdG concentration

Each value is Mean ± SD. Bars with different alphabets ($^{a, b, c, d}$) are significantly different from each other at p<0.05

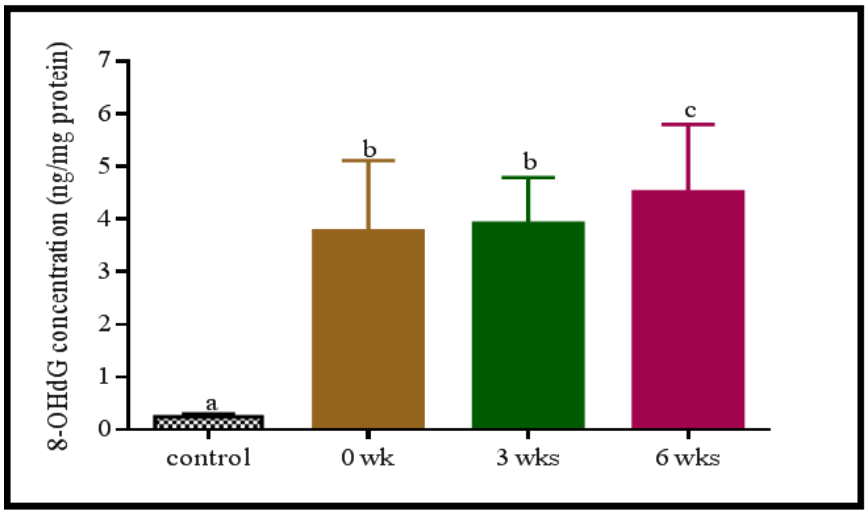

Fig. 6. Effects of post-treatment duration on bone marrow supernatant 8-OHdG concentration

Each value is Mean ± SD. Bars with different alphabets ($^{a, b, c, d}$) are significantly different from each other at p<0.05

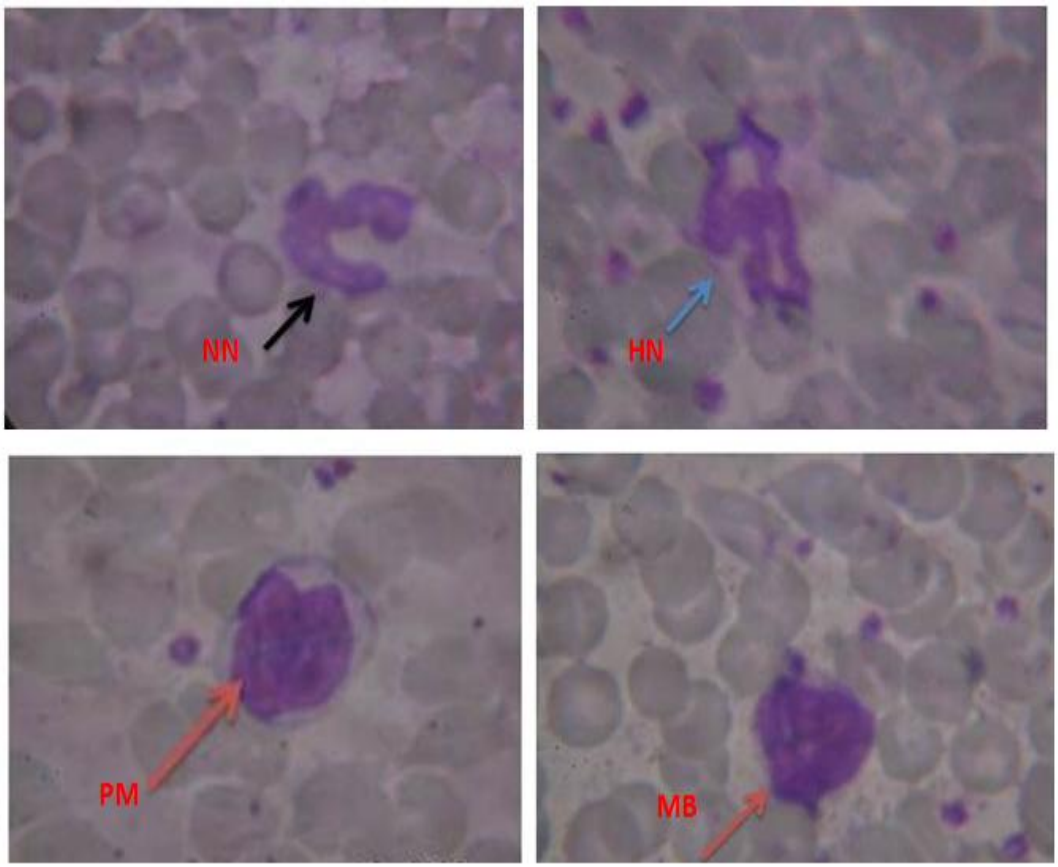

Fig. 7. Photomicrograph of blood film of rat showing Normal Neutrophil (NN), Hypersegmented Neutrophil (HN), Promyelocyte (PM) and Myeloblast (MB) (Leishman stain X1000)

KEY: NN (Normal Neutrophil), HN (Hypersegmented Neutrophil), PM (Promyelocyte), MB (Myeloblast)

The RBC values showed a significant reduction in benzene post expose groups. The RBC reduction was duration dependent. This was consistent with the study of Sun, et al. [19] who reported a decrease in hematopoietic stem cells, reduced burst-forming unit-erythroid (BFU-E), committed progenitors granulocytes-erythroid-monocyte-megakaryocyte (CFU-GEMM) and granulocyte- macrophages (CFU-GM) progenitors in mice with subchronic benzene exposure. This result indicated that significant hematotoxicity was successfully induced by benzene exposure rat within 0, 3 or 6 weeks of post-exposure. The observed reduction in RBC may be attributed to the cytotoxic effects of benzene. Oxidative stress may be induced by benzene with its effect on red cell membrane, this could possibly have accounted for the susceptibility of the red cell membrane to oxidative attack giving way to hemolysis. In studies carried out by Shakirov and Farkhutdinov [20], exposure or contact with chemicals in the oil-refining industry have been established to caused alterations in the red cell adenyl and blood monooxygenase system. They suggested that such an effect could alter the integrity of the red cell membrane to cause cellular haemolysis. Therefore, the result of this study agreed with their claim.

This studied showed decreased in platelet counts in benzene post exposed rats. Megakaryocytes are large bone marrow cells which are responsible for platelet production [21]. Suppression of megakaryocytes or bone marrow suppression in benzene exposed rats decreased the production of platelets leading to thrombo-cytopenia. The severity of suppression is directly proportional to post-exposure duration in benzene exposed rats in the present study. Low platelet count observed in benzene exposed rats suggested that the process of clot-formation will

be prolonged resulting in excessive loss of blood in the case of injury as reported by Gresele et al. [21].

Reduction in the values of RBC, WBC and platelet content as recorded in this study is suggestive of anaemic conditions which agree with the report of Eyong, et al. [22] on the hematotoxicity of petroleum product. The haemopoietic system, in response to this likely anaemic condition, may have flooded the system with reticulocytes which can carry oxygen to meet the body's demand as earlier stated. Benzene is among other toxicants, suppresses the immune system, and causes disruption or suspension of haematopoiesis [23]; which corroborate the results in this study. It has also been established that the toxic constituents of benzene are activated in the bone marrow, where these substances exert cytotoxic effects that could be mediated through disturbance in DNA function [24]. The resultant bone marrow depression is characterized by inadequate production of red cell and other formed elements. This is in line with the findings in this study, as post exposure to benzene showed a significant reduction of RBC from the control value.

When DNA is oxidized, a number of damage products such as base damage, sugar damage, and protein-DNA crosslinks are formed [25]. One of these products, 8-OHdG, has been proposed to be an indicator of oxidative damage in DNA both in vitro and in vivo. Our results showed a significant increase in liver and bone marrow 8-OHdG levels compared to the control. This may be largely attributed to a high number of mitochondria and higher metabolism of liver cells [26]. Also, higher production of ROS in the liver cells is associated with a high density of mitochondria [27]. The quantity of 8-OHdG generated from DNA was dependent on the 8-OHdG levels in the DNA, almost all 8-OHdG in the DNA was released as 8-OHdG by rat liver homogenates. Therefore, generation of 8-OHdG correlated with the degradation of DNA. The majority of benzene metabolism occurs in liver where cytochrome P-450 oxidizes benzene to phenol, catechol, hydroquinone and benzoquinone [27]. These phenolic metabolites and muconic acid are then transported from the liver to bone marrow. High accumulation of benzene and its metabolites over a long period in the bone marrow resulted in increased DNA lesion in benzene post exposed group. Furthermore, the chronic and progressive nature of benzene hematotoxicity suggests genomic reprogramming that would result in aberrant gene expression inducing extensive apoptosis in bone marrow resulting to increase generation of 8-OHdG [28] as observed during the post-treatment duration of benzene in the study

5. CONCLUSION

The present study has provided insight into the hematotoxicity of benzene and the degree of alteration of the integrity of haematological responses. Evidence from our study suggested that elevated levels of 8-OHdG in liver and bone marrow compare to control would be a sign of increased oxidative stress, impaired antioxidant defence or inadequate repair of oxidative damaged DNA.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The protocol of the study was approved by the Local Ethical Committee for animal experimentation of the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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