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Research Article

Oxidative Stress and Changes in Antioxidative Defence System in Erythrocytes of Female Rats Exposed to 2, 5-Hexanedione

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Abstract
Exposure to xenobiotics such as 2,5-Hexanedione (2,5-HD) can cause oxidative stress in the erythrocytes. 2,5-Hexanedione is a neurotoxic metabolite of *n*-Hexane widely used as solvent in several industries. The current study was designed to investigate the effects of 2,5-HD on the oxidative stress and antioxidant markers in the erythrocytes of female Wistar rats. The rats were randomly divided into four study groups of eight (8) rats per group. The control group was administered only tap water, while the remaining three groups were exposed to 0.25, 0.5, and 1.0% 2,5-HD respectively. Thereafter, markers of oxidative stress and antioxidant defence system were assayed in the erythrocytes. Results indicated elevated concentrations of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in the 2,5-HD-treated rats compared with the control ($p < 0.05$). In addition, the activities of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione-*S*-transferase (GST) significantly ($p < 0.05$) increased compared with the control. These results imply that treatment of female rats with 2,5-HD for 21 days is accompanied by induction of oxidative stress and changes in the antioxidative defence system of the erythrocytes.

Keywords: 2,5-Hexanedione; Erythrocytes; Oxidative stress; Antioxidative defence system

INTRODUCTION

Several environmental toxicants have the potential to induce oxidative stress in erythrocytes (Prabu and Sumedha, 2013). 2,5-Hexanedione (2,5-HD) is a metabolite of *n*-Hexane, and methyl-*n*-butyl ketone exposures, widely used as solvents in vanishes, inks, shoemaking, and oil extraction industries (Couri et al., 1982; Cheng et al., 2012). Although, the neurotoxicity of 2,5-HD (Carratu et al., 1995; Lehning et al., 1995), as well as its inhibiting effects on certain glycolytic enzymes such as enolase and phosphofructokinase (Sabri et al., 1979; Howland et al., 1980; Griffiths et al., 1981) has been reported, studies on its effects on the erythrocytes' oxidative stress-antioxidants relationship are limited in the literature.

The biconcave, disc-shaped, and high concentration of haemoglobin in the erythrocytes, make them to function mainly in oxygen delivery to tissues. Reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl and superoxide radicals can easily penetrate erythrocytes' membrane and induce oxidative damage (Lynch and Fridovich, 1987; Denicola et al., 1998; Tsukimori et al., 2005) due to the presence of high levels of polyunsaturated fatty acids in the membrane (Hatherill, et al., 1991; Eritsland, 2000). Although, ROS is involved in normal cellular signalling, however, at concentrations higher than the physiological range, it has been implicated in several disease states (Halliwell and Gutteridge, 1999; Droge, 2002), including erythrocytic diseases such as anaemia.

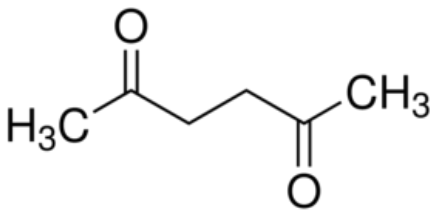

Figure 1:
Chemical structure of 2,5-Hexanedione

In order to forestall the damage caused by ROS, erythrocytes are equipped with an array of both enzymatic and non-enzymatic antioxidants such as catalase, superoxide dismutase, glutathione-S-transferase, reduced glutathione, and vitamins. These antioxidants play active role in preventing the overproduction of ROS, thereby maintaining the integrity of the erythrocytes' membrane (Halliwell and Gutteridge, 1999; Farombi et al., 2003; Abolaji et al., 2013). In the present study, we hypothesized that 2,5-HD exposure in rats may cause an imbalance between the erythrocytes' antioxidant defence systems and ROS, and hence, induce

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oxidative damage in the erythrocytes. Since erythrocytes are useful models to comprehend the vulnerability of membranes to oxidative damage induced by various toxicants (De Flora, 1993; Hamidi and Tajerzadeh, 2003), this study was carried out therefore, to investigate erythrocyte oxidative stress and antioxidative defence status in female rats exposed to 2,5-HD

MATERIALS AND METHODS

Chemicals

The 2,5-Hexanedione (purity ≥ 98%), epinephrine, glutathione (GSH), 5',5'-dithio-bis-2-nitrobenzoic acid, hydrogen peroxide (H_2O_2), thiobarbituric acid, and 1-chloro-2,4-dinitrobenzene (CDNB) were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade, and were obtained from the British Drug Houses (Poole, Dorset, UK).

Animals and Treatment

Thirty two (32) female Wistar rats (230-250 g) were obtained from the Central Animal House Facility of the College of Medicine, University of Ibadan, Nigeria, and housed under controlled conditions of 12 h light/dark cycle, temperature 23 ± 2^{o}C. The rats were allowed free access to tap water and feed manufactured by Ladokun Feeds Limited, Ibadan, Nigeria.

After a period of one week of acclimatization, the animals were randomly divided into four study groups of eight (8) rats per group. While the control group received only tap water, the remaining three groups were exposed to 0.25, 0.5, and 1.0% 2,5-HD respectively. Animals were handled in accordance with the guidelines of the National Institute of Health (NIH) for Laboratory animal care and use.

Blood Collection and preparation of erythrocytes

At the end of the three weeks treatment period, the rats were fasted overnight, and sacrificed by cervical dislocation. Blood was collected by cardiac puncture, allowed to clot, and centrifuged at 3,000 x *g* at room temperature for 15 min to obtain the erythrocytes. The erythrocytes were washed three times with ice-cold phosphate buffered saline (PBS: 145 mM NaCl, 1.9 mM NaH_2PO_4 and 8.1 mM Na_2HPO_4), and centrifuged. The erythrocyte pellets obtained were thereafter re-suspended in PBS (0.1 M, pH 7.4) at 1:9 dilutions, and used for the evaluation of erythrocytes biochemical assays.

Biochemical Assays

Protein content of erythrocytes was determined according to the method of Lowry et al. (1951) using bovine serum albumin as a standard. Hydrogen peroxide generation was determined by the method of Wolff (1994). Lipid peroxidation (LPO) was assayed as malondialdehyde (MDA), according to the method described by Farombi et al. (2000) and Buege and Aust (1978). Catalase activity was assessed using the method of Clairborne (1995), while SOD activity was determined by measuring the inhibition of autoxidation of epinephrine (pH 10.2) at 30°C based on the method of Misra and Fridovich (1972). In addition, while GST activity was determined by the method of Habig et al. (1974), GPx activity was assayed by the method of Rotruck et al. (1973), and GSH level was assessed using the method of Jollow et al. (1974).

Statistical Analysis

All values are expressed as the mean ± standard deviation. Levels of statistical significance were determined using the one-way analysis of variance, followed by Students' t-test. $P < 0.05$ was considered significant.

RESULTS

Results of erythrocytes oxidative stress markers

Figures 2 and 3 depict the effects of 2,5-HD on oxidative stress markers (hydrogen peroxide and malondialdehyde respectively) in the erythrocyte of female Wistar rats exposed to 2,5-HD. The results indicated that 2,5-HD caused significant ($p < 0.05$) increases in erythrocytes H_2O_2 and MDA concentrations in all the exposed rats when compared with the control animals.

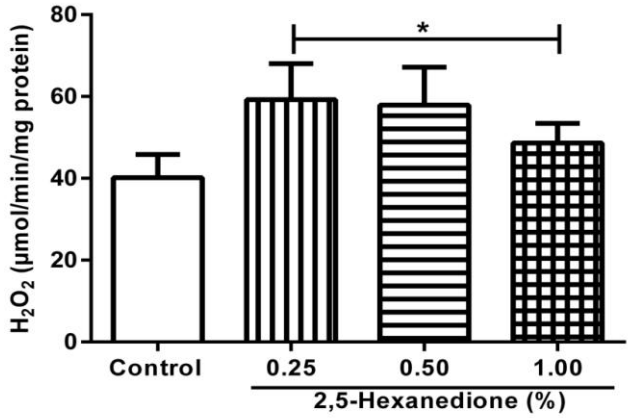

Figure 2
Effect of 2,5-HD on erythrocyte hydrogen peroxide (H_2O_2) generation in female Wistar rats after 21 days of exposure. Data are presented as mean ± SD of 8 rats per group. Values with asterisks (*) indicate significant difference from control at $p < 0.05$

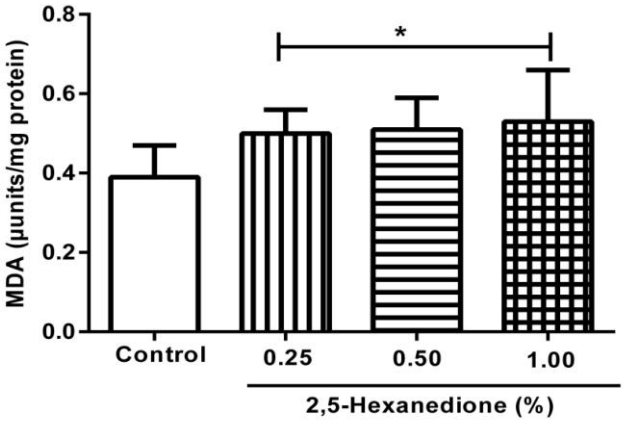

Figure 3
Effect of 2,5-HD on erythrocyte malondialdehyde (MDA) level in female Wistar rats after 21 days of exposure. Data are presented as mean ± SD of 8 rats per group. Values with asterisks (*****) indicate significant difference from control at $p < 0.05$

Results of erythrocytes antioxidant markers

In Figure 4-7, the data show the effects of 2,5-HD on the activities of erythrocytes antioxidant enzymes. The activities of SOD, catalase, GPx and GST were significantly ($p < 0.05$) elevated in the erythrocytes of the exposed rats compared with those of the control animals. In addition, erythrocytes glutathione level after exposure of female rats to 2,5-HD indicated that there was no significant change in the levels of glutathione in all the 2,5-HD-treated rats compared with the control.

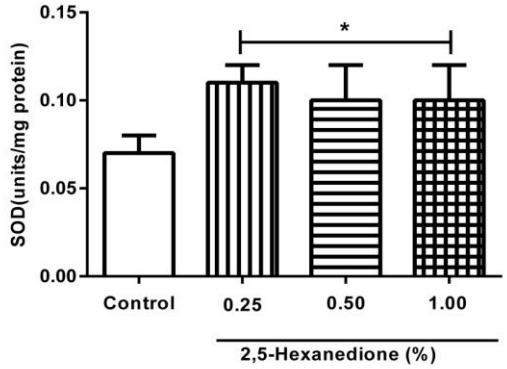

Figure 4
Effect of 2,5-HD on erythrocyte superoxide dismutase (SOD) activity in female Wistar rats after 21 days of exposure. Data are presented as mean ± SD of 8 rats per group. Values with asterisks (*****) indicate significant difference from control at $p < 0.05$.

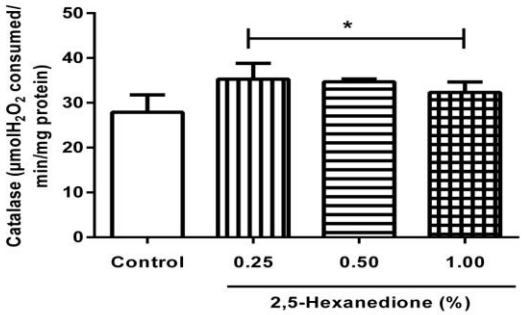

Figure 5
Effect of 2,5-HD on erythrocyte catalase activity in female Wistar rats after 21 days of exposure. Data are presented as mean ± SD of 8 rats per group. Values with asterisks (*****) indicate significant difference from control at $p < 0.05$.

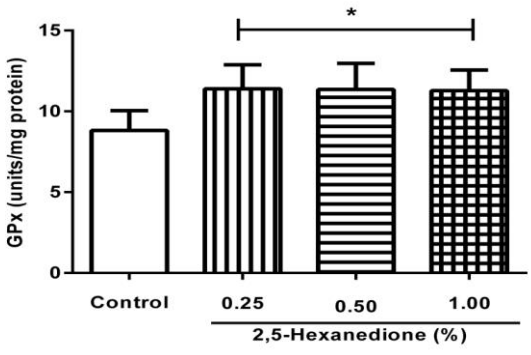

Figure 6
Effect of 2,5-HD on erythrocyte glutathione peroxidase (GPx) activity in female Wistar rats after 21 days of exposure. Data are presented as mean ± SD of 8 rats per group. Values with asterisks (*****) indicate significant difference from control at $p < 0.05$.

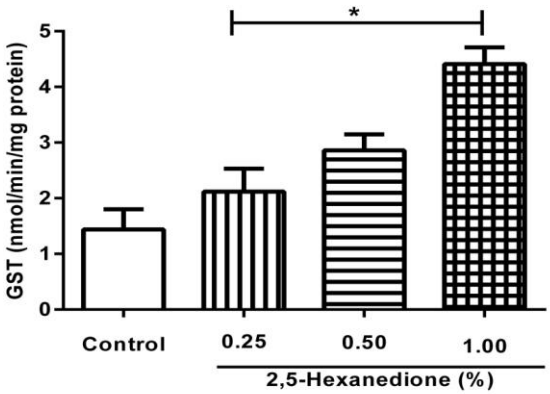

Figure 7
Effect of 2,5-HD on erythrocyte glutathione-S-transferase (GST) activity in female Wistar rats after 21 days of exposure. Data are presented as mean ± SD of 8 rats per group. Values with asterisks (*****) indicate significant difference from control at $p < 0.05$.

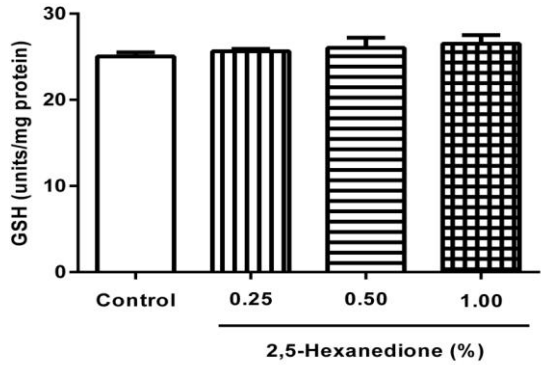

Figure 8
Effect of 2,5-HD on erythrocyte glutathione level in female Wistar rats after 21 days of exposure. Data are presented as mean ± SD of 8 rats per group. Values with asterisks (*****) indicate significant difference from control at $p < 0.05$

DISCUSSION

Exposure to xenobiotics such as 2,5-HD can exacerbate oxidative stress in the erythrocytes due to their role as oxygen transporter. In this study, erythrocyte was used as a model to assess oxidative/antioxidative defense status following exposure of female rats to the neurotoxicant 2,5-HD. The results of this study indicated changes in the concentrations of the oxidative stress and the antioxidant markers in the erythrocytes of 2,5-HD-treated rats.

According to our data, the concentrations of oxidative stress markers H_2O_2 and MDA were elevated in the erythrocytes of 2,5-HD-exposed rats with respect to the control animals. This therefore, implies induction of oxidative stress in the treated rats. Hydrogen peroxide is capable of reacting with superoxide radicals (Haber-Weiss reaction) in the presence of iron (Fenton reaction), to generate highly reactive hydroxyl radicals ($OH^{\bullet}$). Indeed, hydroxyl radicals can oxidize polyunsaturated fatty acids in erythrocyte membranes to induce lipid peroxidation (Halliwell and Gutteridge, 1999) resulting in increased level

of MDA as noted in this study. Due to the absence of nucleus and other organelles in the erythrocyte, its plasma membrane is the target of peroxidation, which reduces the hydrophobic nature of the lipid bilayer, thereby altering interaction of lipids and proteins, and eventually impairs erythrocyte homeostasis. Lipid peroxidation, as observed in this study therefore, is due to the elevated level of H_2O_2, which attacked polyunsaturated fatty acid in the erythrocytes, thereby generating MDA as a by-product.

Erythrocytes possess an array of antioxidants that can prevent or reduce oxidation by ROS, through a direct or indirect mechanism of action. In this investigation, the activities of SOD, catalase and GPx significantly increased in the 2,5-HD-exposed rats. Superoxide dismutase is the first line of defence enzyme against the deleterious effects of oxyradicals that damage erythrocytes membrane structure. Catalase, in conjunction with GPx, helps to scavenge the hydrogen peroxide generated by SOD by converting it to water, and molecular oxygen in order to protect erythrocyte membranes from lipid peroxidation. In the presence of oxidative stress, catalase is the most adaptive antioxidant enzyme that plays a major role in cell defence against oxidative damage (Pidaran and Leelavinothan, 2007; Valko et al., 2007; Patlolla et al., 2009; Guzin et al., 2011). The observed increases in erythrocytes SOD, catalase and GPx activities therefore, suggest erythrocytes adaptive response to 2,5-HD-induced elevations of oxidative stress markers as noted in our study.

Furthermore, we observed higher activity of GST in the 2,5-HD-exposed rats when compared with the control animals. This implies the occurrence of oxidative stress conditions, since GST is also considered as an oxidative stress marker **(**Neefjes et al., 1999). In the erythrocytes, GST catalyses the conjugation of electrophilic molecules with GSH in order to detoxify xenobiotics. In addition, the elevated activity of GST may be due to increased lipid peroxidation following exposure of rats to 2,5-HD as reported in this study (Natasa et al., 2008).

In conclusion, 2,5-HD caused changes in antioxidant enzyme activities, and induced oxidative stress as evidenced by the elevated levels of hydrogen peroxide and lipid peroxidation by-product (MDA) in the erythrocytes. Due to this imbalance between the erythrocytes antioxidant enzyme activities, and the oxidative stress markers in the 2,5-HD-exposed rats, the mechanism of toxicity of 2,5-HD is connected with oxidative damage, which can consequently initiate impairment of erythrocytes functions.

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