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Sperm functional parameters and erythrocytes oxidant–antioxidant imbalance during municipal landfill leachate treatment withdrawal in rats

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ABSTRACT

Adequate information on how leachates affect hematological and reproductive functions is necessary to help in linking causality with predictable response. The present study investigated the effects of Olushosun municipal landfill leachate (OMLL) exposure and withdrawal on sperm characteristics and erythrocytes oxidant–antioxidant balance in rats. Adult male Wistar rats were exposed to 0%, 12.5% and 25% OMLL in drinking water for 28 days. One-half of the rats in each group were sacrificed on day 29 while the remaining one-half stayed an additional 28 days without treatment. OMLL exposure significantly decreased sperm functional parameters, disrupted antioxidant systems with concomitant elevation in hydrogen peroxide and malondialdehyde levels in erythrocytes and sperm. Following withdrawal of treatment, OMLL-mediated decrease in sperm count and daily sperm production were reversed to near control. However, erythrocytes and sperm oxidative damage, increased sperm abnormalities, decreased epididymis weight, sperm progressive motility and testicular sperm number persisted and were consistent with results obtained from rats sacrificed immediately after OMLL treatment. Collectively, OMLL-induced irreversible oxidative damage to erythrocytes and sperm in rats within the time course of investigation. These findings highlight potential adverse effects of OMLL on individuals unduly exposed to leachates contaminated substances.

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1. Introduction

Waste could be classified as industrial, biomedical and municipal solid waste (Bhargav et al., 2008). The disposal of waste to landfill remains the most common method of waste management worldwide (Siddique et al., 2009). Leachates are liquid effluents produced by the decomposition of wastes or by interaction of wastes with rain water. Leachates from landfills which are not adequately managed may pose a severe environmental impact. Hazardous wastes are those that hold a considerable potential threat to human health or environment when improperly treated, stored, transported, disposed off or otherwise managed (Farombi et al., 2012). In Nigeria, there is growing community concern about the general health and environmental risk likely to be posed by the rapid increase in the annual waste volume on landfills which are generally not controlled and managed in accordance to international

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standards of operations (Momodu et al., 2011; Olorunfemi, 2011; Farombi et al., 2012; Odewabi et al., 2013).

Unlike developed countries, waste segregation is not a common practice in Nigeria where solid wastes mostly consist of a mixture of household, industrial and medical wastes (Odewabi et al., 2013). The high moisture content of municipal solid wastes, due to the hot and humid weather in Nigeria, favors the proliferation and breeding of pathogenic microbes (Ogwueleka, 2009). The relative high levels of toxic heavy metals such as cadmium, cobalt, lead, copper and iron in contaminated groundwater has been attributed to the uncontrolled disposal of lead acid batteries and spent petroleum products (Oyeku and Eludoyin, 2010). Bacterial contamination of drinking water been implicated in poisoning, carcinogenesis, heart diseases and teratogenic abnormalities (Sia Su, 2008). Recently, the signs of poor health characterized by reduced hemoglobin and packed cell volume as well as reactive lymphocytosis and eosinopilia in Nigerian waste workers was reported (Odewabi et al., 2013). Data on male-factor infertility showed that Nigeria has approximately 12 million infertile persons (Giwa-Osagie, 2003).

Free radicals generated during biochemical redox reactions in the body act as pro-oxidants resulting in cell injury. However, several cellular defense mechanisms present in the erythrocytes and sperm cells prevent ROS buildup and subsequently protect against oxidative damage (Veena et al., 2007; Adedara et al., 2013a). The severity of oxidative damage depends on the extent of disruption in normal redox state within the cells (Adedara and Farombi, 2012a). Cellular toxicity is temporary if a cell can regain its original functional state after overcoming small perturbations whereas more severe oxidative stress can cause permanent toxicity. The transportation of leachates constituents through blood to the liver for metabolism may damage the erythrocytes which is highly vulnerable to lipid peroxidation due to their high content of polyunsaturated lipids. Although our laboratory recently showed the involvement of free radicals in municipal landfill leachates-induced toxicity in boar sperm *in vitro* (Adedara et al., 2013b), there is still much to understand about the mechanism and the possible reversibility of biochemical disturbances associated with hematotoxicity and spermatotoxicity of Olushosun municipal landfill leachate (OMLL) from Ojota in Lagos State of Nigeria.

Owing to the inadequacy in the waste management practice currently operated in Nigeria and the great potential for human exposure to leachates via groundwater and food contamination, adequate information about possible adverse cellular effects of environmental pollution is of public interest. The present study, as a follow-up of our previous studies, elucidated the precise nature of OMLL toxicity by determining the sequential changes in the sperm characteristics and erythrocytes oxidant–antioxidant balance upon withdrawal of treatment in experimental rats.

2. Materials and methods

2.1. Chemicals

Thiobarbituric acid (TBA), glutathione (GSH), epinephrine, hydrogen peroxide (H_2O_2), 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), and 1-chloro-2,4-dinitrobenzene (CDNB) were purchased from Sigma Chemical Co. (St Louis, MO, USA). All other reagents were of analytical grade and were obtained from the British Drug Houses (Poole, Dorset, UK).

2.2. Collection of leachate

The toxic effects of leachate from Olushosun landfill, Ojota, Lagos State, Nigeria were investigated in this study. Raw leachate collected from leachate wells (holes in the ground) was thoroughly mixed and filtered to remove debris. The physicochemical characteristics of the leachate used in the present investigation were the same with our previously published data (Farombi et al., 2012). Twenty-five percent (25%) leachate was prepared from the homogenous mixture according to a standard procedure (ASTM, 1992) as modified by Adedara et al. (2013b). Briefly, 250 mL of the raw sample was made up to 1000 mL of distilled water (v/v), mechanically shaken for 1 h and allowed to settle for 30 min before filtering with a 2.5-μm filter (Whatman No. 42) to remove the suspended particles. The resulting filtrate was finally centrifuged at 600 × *g* for 15 min at room temperature. The pH of the supernatant fluid was measured before storage at 4 °C until use. The sample was labeled Olushosun Municipal Landfill Leachate (OMLL).

2.3. Animal model and experimental protocol

A total of 36 sexually matured adult male rats (10 weeks, ≈185 g) of Wistar strain procured from the Department of Biochemistry, University of Ibadan, Ibadan, Nigeria, were used for this study. They were housed in plastic cages placed in a well-ventilated rat house, provided rat chow and water *ad libitum* and subjected to natural photoperiod of 12-h light:12-h dark. The ethic regulations were followed in accordance with National and institutional guidelines for the protection of animal welfare during experiments (PHS, 1996). The rats were then randomly divided into three groups of 12 rats per group. Olushosun municipal landfill leachate (OMLL) was diluted with distilled water and made available as drinking water to the rats at concentrations of 12.5% and 25% for 28 days. Corresponding group of rats drank distilled water for 28 days and served as control. One-half of the rats from each group were sacrificed by cervical dislocation on day 29, and the remaining half stayed for an additional 28 days without treatment before they were sacrificed. In each case, the testes and epididymides were quickly removed, weighed and placed on an ice bath. The body weights of rats were taken before OMLL treatment and prior to sacrifice.

2.4. Erythrocytes preparation

The erythrocytes from control and OMLL-exposed rats were prepared according to previously published study (Adedara et al., 2013a). Briefly, 5 mL of the blood was collected from the retro-orbital venous plexus of the rats, before sacrifice, into vials containing heparin as an anticoagulant. The erythrocytes were sedimented by centrifugation at 4000 × *g* for 10 min at 4 °C within 1 h of collection and the plasma was removed. The erythrocytes were subsequently washed three times with

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