Original Article

Histological alteration of the pulmonary alveoli, renal cortex and spleen following exposure to open refuse dump site

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Abstract

Background: Wastes are mainly unwanted products from domestic and industrial sources, which increases due to accelerated industrialization, urbanization, and population growth. Open refuse dumping is the predominant form of waste disposal method in Nigeria and presents huge environmental and health challenges. Hence, this study investigated the effects of long-term exposure of rats to refuse dump sites on histological and serum analysis in three organs; kidney, lungs, and spleen. **Materials and Methods:** Twelve Wistar rats of both sexes were divided into two groups made up of 6 rats in each group. Group A was the controls and group B was the experimental. The experimental rats were exposed to refuse dump fume by keeping them in a research hut built in a refuse dump site for 8 months while the control rats were keep in the Department of Human Anatomy, Niger Delta University, Wilberforce Island, Bayelsa State. **Results:** There was loss of the elastic tissue support for bronchioles, alveolar wall and coalescence of adjacent alveoli as compared with the control. Serum urea concentrations were significantly increased ($P < 0.05$) in exposed rats (9.08 ± 1.58) compared with control rats (5.00 ± 0.32). Similarly, serum creatinine concentrations were significantly increased ($P < 0.05$) in exposed rats (106.20 ± 14.94) compared to Control rats (53.67 ± 5.68). All measured serum electrolytes were significantly altered ($P < 0.05$). Sodium ions (Na^+) (90.17 ± 7.68) and bicarbonate ions (HCO_3^-) (2.33 ± 0.33) were significantly reduced ($P < 0.05$) while potassium ions (K^+) (61.83 ± 6.70) and chloride ions (Cl^-) (117.2 ± 3.08) were significantly increased ($P < 0.05$). **Conclusion:** Our results revealed histological distortion in experimental animals due to exposure to refuse dump site.

Key words: Alveolar, kidney, necrosis, refuse dump sites, spleen, waste

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INTRODUCTION

Adequate management of solid waste is a worldwide challenge. Management of waste is of huge importance for both public and environmental reasons (Porta *et al*., 2009). Waste management includes the generation, collection, processing, transportation and disposal of solid waste (Porta *et al*., 2009). Recycling, sewage treatment, composting, incineration and landfilling are among the foremost methods of waste management (Rushton, 2003). Solid waste may refer to any refuse, litter, or mire from waste handling plant, water supply treatment plant, or air pollution control facility, as well as other cast-off materials, such as solid, liquid, semisolid, or contained gaseous material resulting from industrial, commercial, mining and agriculture operations (RCRA).

In Nigeria however, the predominant method of waste management does not fall within the earlier mention methods, but open dumping of solid waste is most prevalent. Open waste dump sites or refuse dump sites are commonly located in the outskirts of many towns and villages, and sometimes in the vicinity of residential areas. These sites are similar to landfill sites but with little or no sanitary controls.

The practice of living on or near dump sites by scavengers for quick respond to "valuable dumps" is no longer a new trend in the world today especially in developing countries, which has been a concern. Refuse disposal on its part is one of the major environmental problems in most developing countries (Nwigwe, 2008). Historically, dumpsites have been the most common method of unorganized waste disposal and remain so in many places around the world. Most dumpsites are located within the vicinity of living communities and wetlands (Salam *et al*., 2011).

Most people working and living in or near dump sites are partly unaware of the health hazards associated with it (Gwisai *et al*., 2014). Scavengers and refuse dump employees work with little or no protective gadgets against health hazards. Most scavengers are self-employed and do not have any consideration for their health as their earnings are virtually spent on foods. It has been reported that little or no attention has been given to the health risks to which scavengers and dump site employees are exposed (Chattopadhyay *et al*., 2008; Chofqi *et al*., 2004).

The environment which is nature's gift to man has been abused by pollution both on air, land and water (Alabi *et al*., 2014). Literature reveals that open dump sites can emit or release obnoxious odors and smoke that could affect the respiratory system (Uchiyama 2014; El-Gammal *et al*., 2011), cause autoimmune disease (Parks *et al*., 1999), nonmalignant renal (Steenland and Sanderson, 2001) and cardiac disease (Hnizdo and Vallyathan, 2002). Furthermore, pollution of soil and groundwater may lead to direct contamination of indoor air. These may include cases such as seeping of unstable organic chemicals into basements of nearby residents and contamination of home grown vegetables (Abul, 2010). Studies have linked exposure to refuse sites with increase in birth defects and reproductive disorders as well increase in frequency of cancers (Porta *et al*., 2009; Rushton 2003). Nevertheless, direct association of exposure to refuse dump sites with serious health problems is still unclear. Hence, this study has evaluated the long-term exposure of rats to refuse dump sites on kidney function tests, serum electrolyte composition and histological changes in three filtering organs; kidney, lungs, and spleen.

MATERIALS AND METHODS

Animals

Adult Wistar rats of approximately 10 weeks old were obtained from Imo State University, Nigeria. The animals were allowed to acclimatize prior to the start of the experiment.

Dump Site Location

The research was carried out in 2012 in a refuse dump site located along Tombia-Amassoma road in Yenegoa Local Government Area of Bayelsa State.

Experimental Design

A total of 12 Wistar rats of both sexes were divided into two groups made up of 6 rats in each group. Group A was the control group and group B was the experimental group. The experimental rats were caged and kept in a research hut build in a refuse dump site for 8 months while the Control rats were caged and keep in the Department of Human Anatomy, Niger Delta University, Wilberforce Island, Bayelsa. All animals were allowed free access to commercially purchased feed and water *ad libitum*. All animals were handled in accordance with the guidelines for animal research as detailed in the NIH guidelines for the care and use of laboratory animals (NIH Publication 1985).

Histopathology

The rats were sacrificed using anesthesia to induce state of unconsciousness at the end of the 8th month. The lungs, kidneys and spleen were excised, fixed in 10% formal saline, dehydrated in ascending grades of alcohol, impregnated and embedded in paraffin wax. Paraffin sections (5 μm thick) were stained with hematoxylin and eosin method for general histological examination. Slides were viewed under a digital microscope (Leica DM750 with attached ICC50 camera) and digital photomicrographs were taken.

Serum Biochemistry

Blood samples were obtained from the rats by cardiac puncture at sacrifice and were kept for 30 min at room temperature. Blood samples were centrifuged at 5000 rpm for 10 min at room temperature to obtain serum. Kidney function markers (serum urea and creatinine) as well as electrolytes composition (sodium, potassium, chloride, and bicarbonate ions) were determined using commercially available assay kits (Randox Laboratories, UK) following standard methods.

Statistical Analysis

Data were expressed as mean ± standard error of the mean and were analyzed using Student's *t*-test. GraphPad Prism 5 (version 5.03, GraphPad Inc., USA) was the statistical package used for data analysis. Significant difference was considered as $P < 0.05$.

Ethical Approval

This study was approved by the Bayelsa State Ministry of Environment in 2012.

RESULTS

Serum Biochemical Analysis

Serum urea concentrations were significantly increased ($P < 0.05$) in exposed rats (9.08 ± 1.58) compared to control rats (5.00 ± 0.32). Similarly, serum creatinine concentrations were significantly increased ($P < 0.05$) in exposed rats (106.20 ± 14.94) compared with control rats (53.67 ± 5.68) [Figure 1 and 2]. All measured serum electrolytes were significantly altered ($P < 0.05$) following exposure to refuse dump site [Table 1]. Sodium ions (Na^+) (90.17 ± 7.68) and bicarbonate ions (HCO_3^-) (2.33 ± 0.33) were significantly reduced ($P < 0.05$) while potassium ions (K^+) (61.83 ± 6.70) and chloride ions (Cl^-) (117.2 ± 3.08) were significantly increased ($P < 0.05$).

Histopathology of Lungs

The normal histology of the lungs was seen in the tissue sections of the control animals (CN). A distended thin walled pulmonary artery branch lies next to the bronchioles. The epithelial lining of the bronchioles are clearly demonstrated and well arranged. The alveoli were well illustrated with walls consisting of surface epithelium, supporting tissues and blood vessels. Tissue sections of the experimental animals showed loss of the elastic tissue support for bronchioles, as compared with the control. There was loss of the alveolar wall and coalescence of adjacent alveoli. The walls of the alveoli are remarkably atrophic and thin. Most of the alveolar walls have broken down and merged to form spaces considerably larger than normal alveoli [Figure 3].

Histopathology of Kidney

The normal histology of renal cortex was seen in control animals. The renal corpuscles were clearly identified with the glomeruli surrounded by narrow Bowman's spaces. The tubules fill the bulk of the parenchyma between the corpuscles. Occasional interlobar arteries were easily identified. Tissue sections from the experimental animals showed infiltration of fibrous-like materials into the bulk of the parenchyma around the renal corpuscles, the lumen were occluded in most of the tubules. The cytoplasm of the epithelial cells lining the tubules seemed to be swollen. The nuclei of the tubular epithelial cells were markedly reduced and the renal medulla was observed to be disorganized [Figure 4].

Histopathology of Spleen

The normal spleen histology was observed in control animals. The two distinct functional zones were identified; the lymphoid white pulp and hematogenous red pulp [Figure 5]. The white pulp of the spleen was mainly composed of lymphocytes while the red pulp consists of macrophages, plasma cells and many blood cells (erythrocytes, granulocytes, and platelets). The result from the exposed rats showed an overall decrease in cellularity of red pulp compared to the control. On the other hand, there was an increase in cellularity of white pulp. However, many of the cells appeared paler than normal lymphocytes [Figure 5].

DISCUSSION

It was well documented that living in close proximity to landfills may pose serious health challenge, and

Table 1: Serum electrolytes composition

Group/ions	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)
Control	128.70±6.78	32.33±3.10	93.33±5.88	5.17±0.48
Exposed	90.17±7.68*	61.83±6.70*	117.2±3.08*	2.33±0.33*

Values are mean±SEM n=6. *Significant difference (P<0.05) between control and exposed groups. Student's t-test. SEM: Standard error of mean

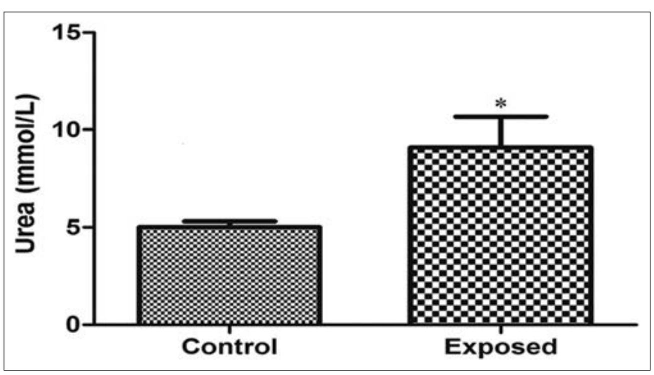

Figure 1: Serum urea level

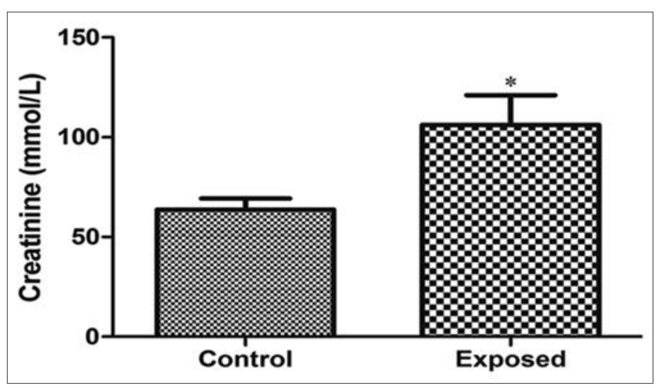

Figure 2: Serum creatinine level

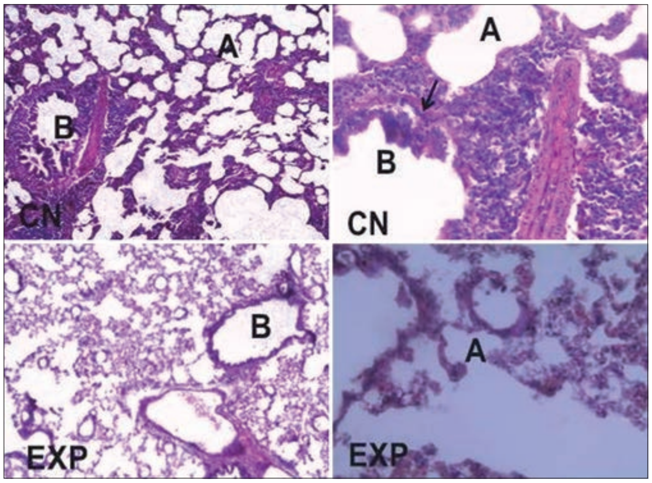

Figure 3: Micrograph of the lungs A section of the lungs of control (CN) rats, bronchioles (b) with its epithelial lining and surrounding smooth muscle layer (arrow). Alveoli (a) are well shown with walls consisting of surface epithelium and supporting tissues. Experimental (EXP) rats showed loss of supporting tissue in the walls of the bronchioles (b). Thin atrophic alveoli (a) resulting in coalescence adjacent alveoli (H and E, ×100 [left] and H and E, ×400 [right])

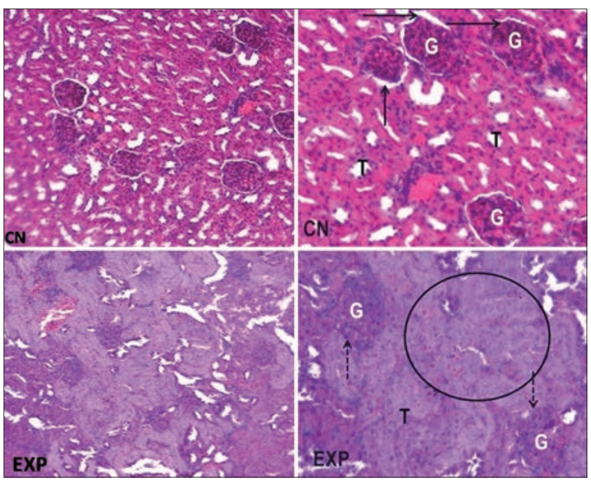

Figure 4: Micrograph of the kidney. Tissue section of the kidney (cortex) of control (CN) rats, glomeruli (G) surrounded by narrow space (arrow). Tissue section of kidney (cortex) from experimental group (EXP) showed infiltration into the parenchyma of the cortex resulted in occluded bowman's spaces (dash arrows) and occlusion of lumen within the tubules. Nuclei of tubular epithelia were markedly reduced (circled) showing necrosis of the cells (H and E, ×100 [left] and H and E, ×400 [rightW)

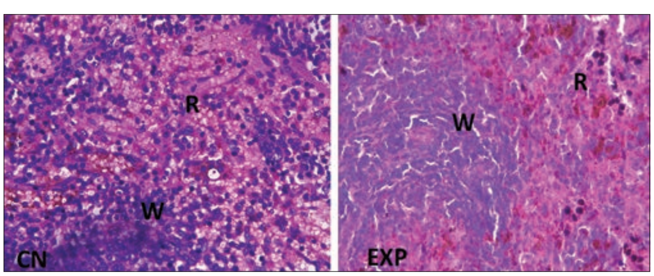

Figure 5: Micrograph of the spleen. Representative of tissue sections from the spleen of control (CN) and experimental (EXP) rats (H and E, ×400). The two major functional regions were identified as white pulp (W) and red pulp (R). It was observed increase cellularity of white pulp and decrease cellularity of red pulp in exposed compared to control

reviews are available (Rushton 2003; Saunders, 2007; Franchini *et al*., 2004; Vrijheid, 2000). However, the direct association of such habitation and development of illnesses seemed difficult to prove.

Damage to the kidney is associated to the decline in renal function which could lead to renal failure. In the present investigation, decreased renal function is clearly evidenced by significant increase in plasma urea and creatinine concentrations in exposed rats. Increased serum urea may point to reduced re-absorption at the renal epithelium (Adedara *et al*., 2012). Similarly, increase in serum creatinine reflects impairment in kidneys, especially for glomerular filtration rate (Adedara *et al*., 2012). Thus the increase in both serum urea and creatine indicate a resulting renal failure in the exposed rats. Furthermore, the present investigation showed significant alteration in Na^+, K^+, Cl^-, and HCO_3^+. These observations are of toxicological significance and may indicate substantial effects on the ion-dependent processes in exposed rats. Altered serum Na^+, K^+, Cl^-, and HCO_3^+, have been implicated in development of renal failure and also metabolic changes associated with respiratory disorder (Hsieh *et al*., 2011; Story *et al*., 2005). In the result of our kidney sections, there were also infiltration of fibrous-like materials into the bulk of the parenchyma around the renal corpuscle, which suggests chronic inflammation within the kidney. The glomerular tufts within the Bowman's capsule are enlarged as a result of the infiltration, thus closing the narrow bowman's space in most of the renal corpuscle. The infiltration is more striking with the tubules around the glomeruli as the lumen are occluded in most of the tubules. The cytoplasm of the epithelial cells lining the tubules seemed to be swollen and thus completely covering the lumen. The nuclei of the tubular epithelial cells were markedly reduced, suggesting necrosis of the tubular epithelial cells. Also, the renal medulla were observed to be markedly disorganized. This was similar to the results obtained by Chibusi *et al*. (2012) where it was reported that exposure to municipal landfill leachate caused tubular necrosis and congestion.

As earlier mentioned, dangerous health conditions can be associated with inhaling even small amounts of pollutants. People who live or work near hazardous waste sites or factories that releases cadmium into the air have suffered serious health impairment such as damaged lungs (Amal and Fawzy, 2013). The results for the lungs in the experimental group corroborated with the studies of Reichrtová *et al.* (1986). Where long-term field exposure of rabbits to a bio-indication station located about 3 km downwind of a disposal site of nickel smelter waste dump revealed histologic abnormalities in the lungs and liver tissues. It was also in line with the study of Zaman (2008) where it was reported that inhaled marble dust damages the cells of the respiratory system. Our findings also agreed with El-Gammal *et al*. (2011) where there was increase in lymphocytic infiltration. Also, persistent changes included, distortion in epithelial lining of bronchioles and alveoli and presence of areas of degeneration after long time exposure to dust. Our results further corroborated the result obtained by Dungworth *et al*. (2001), where they reported morphological changes such as lymphatic infiltration, bronchio-alveolar hyperplasia as well as bronchioles and pulmonary fibrosis in the lungs of mice exposed to silica inhalation.

There have been reports on the alteration of the organs by toxins in our environment. Cadmium exposure has been reported to induce atrophy of the proximal renal tubules (Pratap and Wendelaar Bonga, 1993).

CONCLUSION

In conclusion, this study has shown that long-term habitation in the vicinity of refuse dump sites resulted in damage to major filtering organs like the kidney, lungs and spleen of rats. Since most of these sites hold any type of waste including municipal, industrial and infectious wastes, they may pose serious health challenges to inhabitants, workers and scavengers that are continuously exposed to them. Hence, it is recommended that more sanitary methods of waste management be implemented and sites of waste disposal be situated far away from residential areas to prevent risk of developing illnesses in humans.

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