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Hepatotoxic Effect of Port Harcourt Eliozu Landfill Leachate in Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author IIW designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OAG and DVD managed the analyses of the study. Author LCB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Studies have reported deleterious effect of landfill leachate and their surrounding water sources around the world. None has been reported on the Port Harcourt (Eliozu) landfill. This therefore necessitates this study that evaluated the hepatotoxic effects of Port Harcourt (Eliozu) landfill in wistar rats. 25 wistar rats were grouped into five of five rats each. Group 1 served as the control and received 1 ml of commercial non-carbonated bottled water; Group 2 served as negative control and received 1 ml of water obtained from borehole about 1 km from the landfill; while Groups 3, 4 and 5 received 1 ml of 10% of leachate concentration, 1 ml of 50% of leachate concentration and 1 ml of 100% of leachate concentration respectively once daily for 90 days. After the treatment period, the rats were sacrificed and 5 ml of blood was collected via cardiac puncture. The blood samples were prepared and used for the determination of serum concentrations of liver enzymes. The rats were also dissected and the liver harvested for histochemical analysis using the periodic

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acid Schiff stain. Result showed the serum concentrations liver enzymes significantly increased in the rats exposed to the leachate and water from a nearby borehole. Also histochemistry examination showed a marked distortion of the hepatic cells and cirrhotic change in the test groups compared to control. This study therefore concludes that exposure to Port Harcourt Eliozu landfill leachate (PELL) may cause significant hepatotoxicity in rats.

Keywords: Leachate; Port Harcourt; histochemistry; landfill.

1. INTRODUCTION

Landfilling is the most economic viable means of municipal solid waste disposal practiced in developing countries [1]. However, liquid effluent also called leachate may drain from such stockpile and contaminate surrounding water body [2]. Exposure of the environment to landfill leachate may occur through uncontrolled overflow, rainfall and infiltration into the underlying ground water aquifer or nearby surface water. Leachate is now being recognized as a potential health risk to both surrounding ecosystems and human populations [3]. Studies on leachates from the dump site of Delta State Teaching Hospital Oghara reported high concentrations of heavy metals [4]. Also, Characterization and toxicological evaluation of leachates from Air Hitman Sanitary Landfill in Malaysia using *Pangasius sutchis* and *Clarias batrachus* concluded that leachate contained significant quantities of Ammonia, dissolved organic matters, some semi-volatile organic compounds and monocyclic aromatic hydrocarbons which are very toxic to both species of fishes; and this may be used as indicators of leachates contamination in freshwater. Ammonical-nitrogen present in the leachate is considered the principal cause of the fish mortality. However, benzene, toluene and ethyl benzene may have contributed to the leachate toxicity [5]. Chibuisi and co-workers reported concentrations of Cu, Cr, Pb, As, Cd, Mn, Ni, Sulphate, ammonia, Chloride and Phosphate in the leachates samples of Olusosun and Aba-Eku municipal landfill leachate above standard permissible limits [6]. The haematotoxic potentials of heavy metals that may be contained in leachate have been reported in fishes [7,8]. Immunotoxic potentials of raw and simulated leachates from Olusosun municipal solid waste landfill in rats has also been reported [9]. Subhasini et al. [9] reported alterations in the biochemical and Histopathological profile of the liver in distillery soil leachate treated Swiss Mice at concentrations 5%, 10% and 20% of leachate [10]. A similar study at Olusosun municipal landfill leachate Ojota Lagos State, Nigeria also reported hepatotoxicity and oxidative stress in rats [11]. Neurologic lesions, neurodegenaration of purkinje cells with lose of dentrites, perineural vacoulations of the neuronal cytoplasm (spongiosis) and neuronal necrosis in the brain of wistar rats exposed to Olusosun and Aba-Eku municipal landfill leachate has also been studied [6]; these reported brain tissue alterations correlate with a significant decrease in body weight gain and SOD activity but increase in absolute and relative brain weight gain, MDA concentration and CAT activity in both the brain and serum of treated rats [6].

The Port Harcourt (Eliozu) landfill (PELL) is one of the biggest landfills in Rivers State; it receives untreated deposits of both domestic and industrial wastes from all over Port Harcourt and despite its possible direct or indirect hazardous nature, no study has been done on its toxicological effects. Also considering the fact that residents around the landfill largely depend on borehole water around there for drinking. This study therefore attempts to evaluate its hepatotoxic effects in wistar rats.

2. MATERIALS AND METHODS

2.1 Preparation of Experimental Animals

25 Wistar rats weighing about 200 g were obtained from the animal house unit of Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria and acclimatized for 14 days. The animals were maintained under standard laboratory conditions of 12 hours' dark and light cycle with free access to drinking water and standard rodent chow (*ad libitum)*. The animals were handled in accordance with the guideline for the Care and use of laboratory animals.

2.2 Leachate Collection and Preparation

Raw leachate sample was taken from leachate well on the landfill at Eliozu in Port Harcourt River State, Nigeria in a cleaned 5 litres plastic containers. The sample was taken to the laboratory of the Department of Chemistry, University of Port Harcourt where it was filtered using glass wool and whatmann No.42 filter paper to remove suspended particles. It was then centrifuged at 3000 rpm for 10 minutes, the supernatant was considered as stock samples (100%) and labeled as Port Harcourt (Eliozu) landfill leachate (PELL). Other concentrations were then prepared by diluting with 10% and 50% of distilled water.

2.3 Experimental Design

Following two weeks of acclimatization, the rats were divided into five groups of five rats each. Group 1 served as the control and received 1 ml of commercial non-carbonated bottled water; Group 2 served as negative control and received 1 ml of water obtained from borehole about 1 km from the landfill; while Groups 3, 4 and 5 received 1 ml of10% of leachate concentration, 1 ml of 50% of leachate concentration and 1 ml of 100% of leachate concentration respectively. All administrations were given once daily using an oro-gastric cannula for 90 consecutive days. On the 91st day however, the rats were anaesthetized using chloroform and 5 ml of blood sample was collected via cardiac puncture. The blood samples were prepared and used for the determination of serum concentration of liver enzymes. The rats were also dissected and the liver harvested and processed for histological and histochemical analysis.

2.4 Determination of Serum Concentration of Liver Enzymes

The liver enzymes were determined by the colorimetric method [12]. The absorbance of Aspartate Transferase (AST) in the sample was read at 540 nm in the calorimeter after 5 minutes of' incubation. The enzyme activity was obtained from the enzyme activity/absorbance chart by matching the absorbance obtained.

Determination of alkaline phosphatase (ALP) present in serum act upon AMP-buffered sodium thymiolphthalein monophosphate and addition of an alkaline reagent stops enzyme activity and simultaneously develops a coloration of pink which is measured at 590 nm.

Alanine Amminotransferase (ALT) catalyzes the transfer of an amino group from alanine to α-ketoglutarate at pH 7.4 and temperature 37°C to form pyruvate and glutamate. Pyruvate reacts with 2,4-dinitrophenyhydrazine (DNTP) to form 2,4-dinitrophenylhydrazone in a red-brown colour alkaline medium. The intensity of the colour measured at 530 nm is directly proportional to the enzyme activity. The mixture and the absorbance are read at 540 nm; enzyme activity was obtained from the enzyme activity/absorbance chart matching the absorbance obtained.

2.5 Staining and Histochemical Analysis of Liver Tissue

The processed liver tissues from all groups were fixed in 10% formaldehyde, the tissue processing was done using routine paraffin wax processing technique and the prepared 5μ thick section were mounted on slides and stained with Hematoxylin and eosin (H&E). Periodic acid Schiff (PAS) stain was also done following the established protocols. The PAS stain is a histochemical reaction in which the periodic acid oxidizes the Carbon to Carbon bond forming dialdehyde which react with the fushsin-sulphurus acid (metabisulphate and hydrochloric acid) and yields an insoluble magenta coloured compound on the tissue.

2.6 Statistical Analysis

Data obtained were subjected to statistical analysis using SPSS one-way analysis of variance (ANOVA) was used to compare the various groups and the significant differences were set at a $p<0.05$ and are indicated on the Tables with asterisk.

3. RESULTS AND DISCUSSION

3.1 Result Presentations

At the end of data analysis, mean values of results are presented in the Tables below; while histochemistry stained plates are presented with magnification of X400.

3.2 Discussion

It has been long known that the elevation of liver enzymes in serum may signify serious damage to hepatic tissues [13,14,15]. The present study showed significant increase in the serum activities of AST, ALT and ALP in rats treated with 100% PELL for 90 days (Table 1). These enzymes are localized in the periportal hepatocytes, showing that their actions in oxidative phosphorylation and gluconeogenesis presumably increase as a result of cellular membrane damage and leakage [11]. This underlines their use as biochemical

Table 1. Effect of Port Harcourt landfill leachate (PELL) on liver function test

Parameters	Group 1 (non-carbonated bottle water)	Group 2 (borehole water)	Group 3 (10%PELL)	Group 4 (50%PELL)	Group 5 (100%PELL)
Aspartate Transferase (µl/L)	134.24±1.26	166.74±2.23*	150.04±1.98*	156.04±1.02*	188.30±3.99*
Alanine Transaminase (µl/L)	7.97±0.43	16.71±0.90*	11.46±0.30*	16.10±0.84*	23.74±0.33*
Alkaline Phosphatase (µl/L)	75.60±3.07	131.8±11.18*	103.96±6.74*	119.54±12.6*	156.0±2.19*

*Results presented as mean±SEM, *= significant difference compared with group 1 ($p<0.05$)*

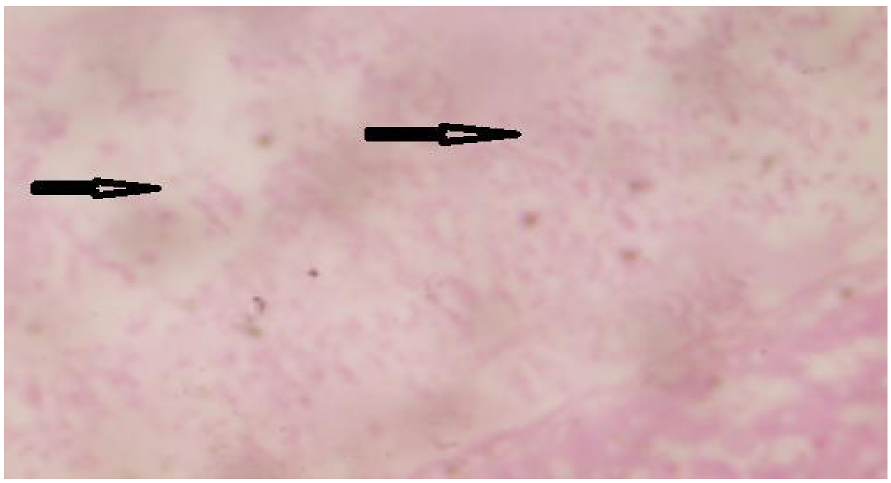

Plate 1. Photomicrograph of the liver tissue MAG X400 PAS stain group 1 (commercial non-carbonated bottle water) showing reduced affinity to PAS stain

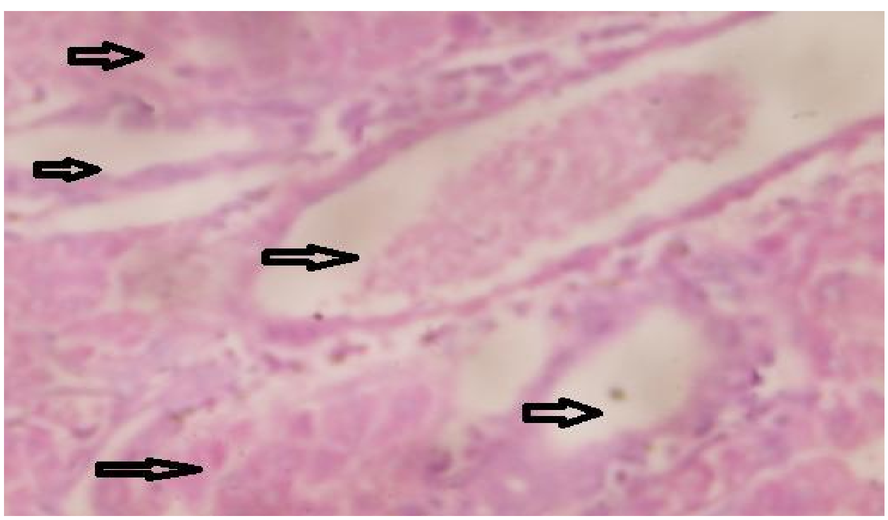

Plate 2. Photomicrograph of liver tissue MAG X 400 H&E stain group 1 control (commercial non-carbonated bottle water). Showing the normal liver architecture. Hepatocyte (HC), hepatic artery (HA), portal vein (PV), sinusoid (VS) and bile duct (BD)

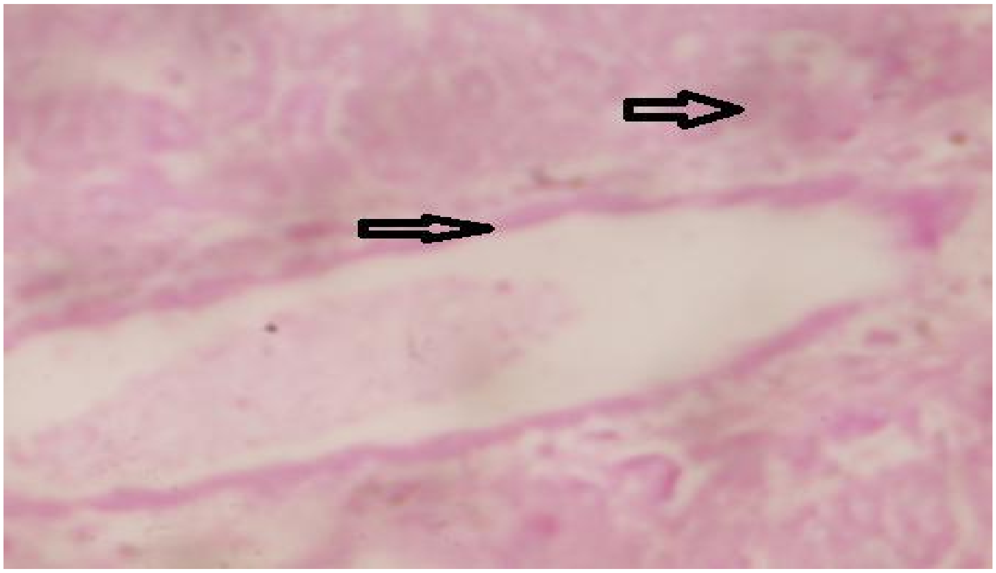

Plate 3. Photomicrograph of the liver tissue MAG X400 PAS stain group 2 (near-by borehole water) showing increased affinity to PAS stain

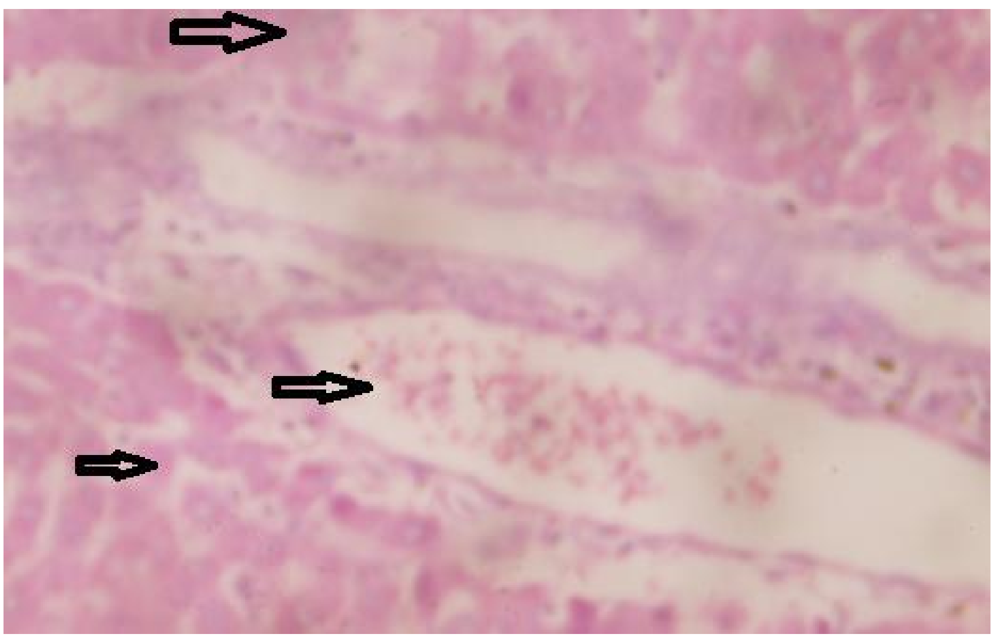

Plate 4. Photomicrograph of liver tissue MAG X 400 H&E stain group 2 (water from near-by borehole). Showing the mildly distorted liver architecture. Hepatocyte (HC), sinusoid (VS) and patent central vein (CV)

Markers for hepatic damage [16]. Therefore, increase levels of AST and ALT in circulation is an indication of hepatic damage after PELL exposure. Even though there are various enzymatic and non-enzymatic systems developed by hepatocyte to cope with the reactive free radicals, when a conditions of oxidative stress is established, the defense capacities against ROS become insignificant [17]. The increased in liver enzyme activities seen in this study is consistent with a study on the alterations in biochemical and Histopathological profile of the liver in distillery soil leachate treated Swiss mice [18].

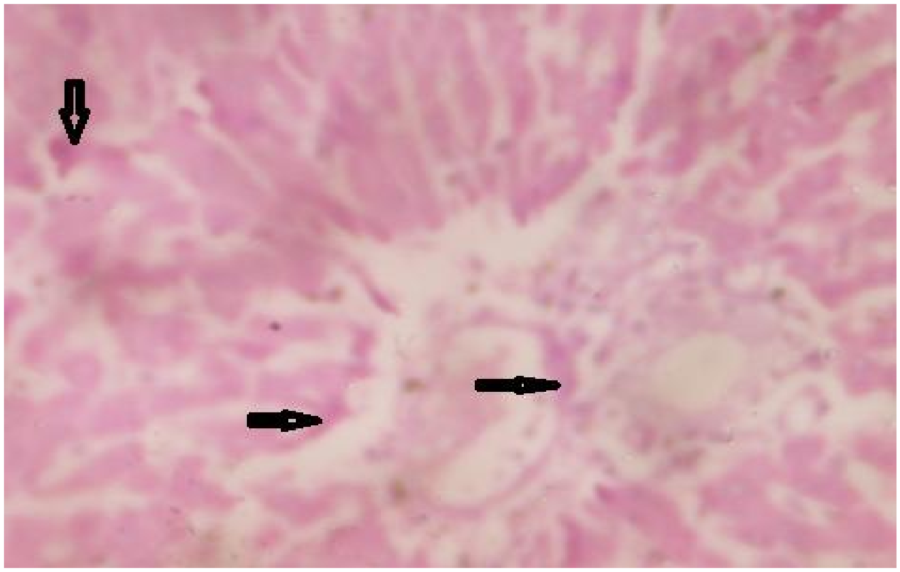

Plate 5. Photomicrograph of the liver tissue MAG X400 PAS stain group 4 (PELL100%) showing excess affinity to PAS stain

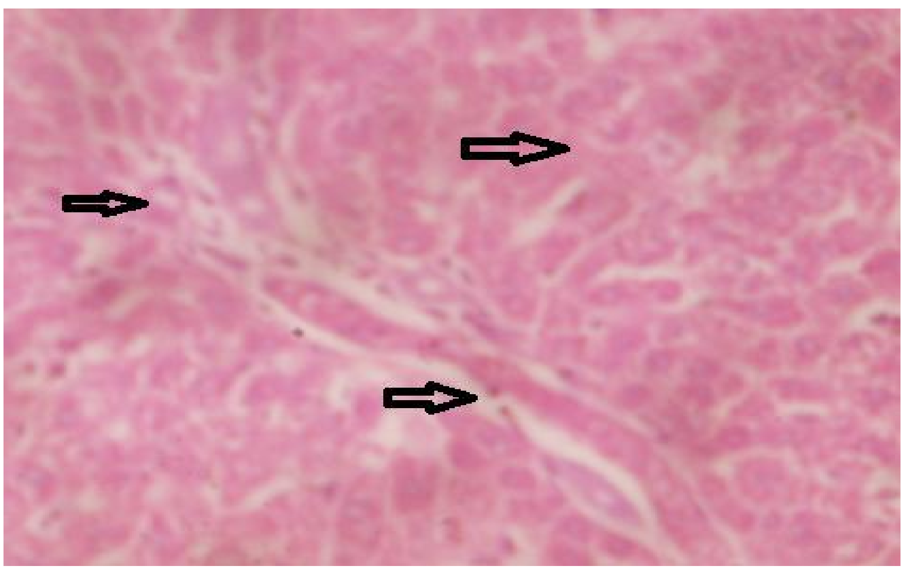

Plate 6. Photomicrograph of liver tissue MAG X 400 H&E stain group 5 (100% PELL). Showing distorted liver architecture. Necrosis of hepatocyte, congested central vein, sinusoids containing inflammatory cells

The elevated liver enzymes correspond with the histopathological examination of the liver tissues in this study. Histopathological examination is useful in identifying the types of alterations caused by xenobiotics and is perceived as the most sensitive end point for detecting organs toxicity [14]. From the present study, the liver of the 100% PELL treated group appeared to be at risk of significant injury. This is manifested in the distorted hepatic architecture, necrosis of the hepatocyte and inflammatory cells (Plates 5 and 6). The PAS stain, showed massive deposit of glycogen in the hepatocyte forming pseudolobules which has been previously reported be a marker of cirrhotic change [19]. Heavy metals, inorganic and microbes found in the PELL may have caused the observed alterations in the liver. It could also be as a result of oxygen deficiency or the presence of reactive oxygen species (ROS) [20]. Hepatic necrosis with inflammatory cells observed in this study has been reported previously [21]. Thus, increased in serum transaminases observed in this study lend credence that PELL caused loss of physiological integrity. The findings of the inflammatory responses to tissue damage as shown in congestion, necrosis, support the free radical generation mechanism. This is in compliance with a report on the cadmium bioaccumulation and toxicity in tilapia fish [21].

4. CONCLUSION

From the result of this study, we conclude that exposure to landfill leachate and (or) drinking water from a nearby source may cause significant hepatological toxicity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval for this study was sought and obtained from The University of Port Harcourt Research Ethics Committee on the 28th of September, 2017 with the approval reference number: UPH/R&D/REC/04. So the study was performed in accordance with the ethical standards and guidelines for the care and use of laboratory animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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