Assessment of the Cytomorphology of the Cerebral Cortex of Wistar Rats Exposed to Eliozu Dumpsite Leachate

Abstract

The cerebral cortex is the outermost layer of the cerebrum and plays a critical role in various cognitive, sensory and motor functions. It is highly folded and has distinct regions responsible for different functions. Leachate refers to the liquid that forms when water percolates through waste materials, such as landfills and dumpsite picking up various organic and inorganic compounds along the way. It is a complex mixture of chemicals, including heavy metals, volatile organic compounds (VOC) and other potentially harmful substances. Eliozu dumpsite is one of the biggest dumpsite in Port Harcourt metropolis. It receives deposits of both domestic and industrial wastes from Port Harcourt, these wastes are dumped untreated, and thus may pose serious environmental risks to inhabitants in the area. The present study focuses on the assessment of the cytomorphology of the cerebral cortex exposed to Eliozu dumpsite leachate using Wistar rats as model. Twenty five (25) male Wistar rats were divided into five groups of five animals each; the leachate was collected from the dumpsite and water from near-by borehole also collected. Group 1 which served as control group received 1ml of commercial bottle water, group 2 received 1ml of borehole water 1kilometer from the dumpsite, groups 3, 4 and 5 received different concentration of the leachate in 10%, 50% and 100% for ninety days, the animals were sacrificed after being anesthetized with chloroform vapor, the brains were collected and put in 10% formaldehyde, sectioned and stained with haematoxylin and eosin (H&E), special stain Gorgon and Sweet for histopathological studies. The slides were placed in a light microscope and analyzed by a consultant Anatomy pathologist. The results revealed that the Eliozu dumpsite leachate does have deleterious effect on the cytomorphology of the cerebral cortex. Eliozu dumpsite leachate has shown deleterious effect on cerebral cortex which is an indication of toxicity that may cause brain death to the experimental animals. Therefore, it is recommended that continued research, education and public awareness are essential to promote sustainable waste management practices and safeguard the integrity of the cerebral cortex.

Keywords: haematoxylin, cerebral cortex, leachate, hepatic toxicity

Introduction

Leachate refers to the liquid that forms when water percolates through waste materials, such as landfills and dumpsite picking up various organic and inorganic compounds along the way. It is a complex mixture of chemicals, including heavy metals, volatile organic compounds (VOC) and other potentially harmful substances (Weleh *et al.,* 2020).

"The cerebral cortex is the outermost layer of the cerebrum and plays a critical role in various cognitive, sensory and motor functions. It is highly folded and has distinct regions responsible for different functions. Structurally, the cerebral cortex is composed of six layers, numbered from the outermost (Layer I) to the deepest (Layer VI). The cortex is divided into four lobes: the frontal, parietal, temporal and occipital lobes each associated with different functions. A number of incidences have been reported previously where leachate had contaminated the surrounding soil and polluted the underground water acquifer or nearby surface water" (Al Sabbagh *et al*., 2012). "Study also reported that exposure to dump site leachate and (or) drinking water from a nearby source may cause significant hepatic toxicity in Wistar rats" (Subhasini *et al* ., 2009]. "Also, haematological toxicity has been reported in solid waste workers in Port Harcourt Nigeria; which was attributed to exposure to chemicals in solid wastes" (Weleh *et al*., 2021). "Some earlier reports stated that exposure to leachate cause the decrease in gonado-somatic index value and shrinking oocyte diameter of female Nile Tilapia" (Zulfahmi *et al*., 2019). "Eliozu dumpsite is one of the biggest dumpsite in Port Harcourt metropolis. It receives deposits of both domestic and industrial wastes from Port Harcourt, these wastes are dumped untreated, and thus may pose serious environmental risks to inhabitants in the area and entire Port Harcourt population directly or indirectly" (Green et al., 2021). "Despite its potential hazardous nature to Port Harcourt metropolis, no study has been done on the assessment of the cytomorphology of the cerebral cortex exposed to Eliozu dump site leachate on human population living around the vicinity. Residents near the dumpsite are oblivion of the possible contamination of water sources from leachate" (Ogbonna *et al*., 2016, Weleh *et al*., 2021). Thus, the need for the present research . This study therefore, assess the cytomorpology of cerebral cortex of wistar rats exposed to Eliozu dumpsite leachate and is part of a more extensive assessment of the potential toxicological effects of this leachate apparently ignored by municipal authorities in Port Harcourt, Nigeria

2. MATERIALS AND METHODS

2.1 Collection of Leachate

Raw leachate was collected from leachate well at Eliozu dump site in Port Harcourt, Nigeria. The leachate sample was taken to the laboratory in a clean plastic container, where it was filtered using Whatmann No. 42 filter paper to remove suspended particles. The filtrate was centrifuged at 3000 rpm using Techmel and Techmel USA (model: 80-2) for 10 minutes and the supernatant fluid obtained was considered as stock samples (100%) and labeled as Eliozu dumpsite leachate (EDL) and stored at 4°C. The following concentrations were thus determined, 10%, 50% by dilution of the leachate with distilled water.

2.2 Experimental Animals

Twenty-five (25) Wistar rats with average weight of 170 g were obtained from the animal house unit of Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria. The animals were acclimatized for 14 days under standard laboratory conditions of 12- hour dark and light cycle with free access to standard feeding chow and drinking water *ad libitum*. The animals were treated according to the guide for the Care and use of laboratory animals.

2.3 Experimental Design

Following two weeks of acclimatization, the rats were divided into five groups of five Wistar rats each. Group 1 served as the control and received 1ml of commercial bottled water; Group 2 received 1ml of water obtained from borehole about 1km from the dumpsite; while Groups 3, 4 and 5 received 1ml of 10% of leachate concentration, 50% of leachate concentration and 100% of leachate concentration respectively. All administrations were given once daily using an orogastric cannula for 90 consecutive days. On the 91st day however, the animals were anaesthetized using chloroform vapor and the brain harvested for histological assessment.

2.4 Histopathological Examination

Immediately the brain tissue samples from the experimental model were collected, the brain tissues were immersed in a fixative solution (4% paraformaldehyde) to preserve the cellular structures, after fixation, the tissue samples were dehydrated, embedded in paraffin blocks and sectioning into thin slices (about 5-10µ thick) using microtome. The staining method involved the use of hematoxylin and eosin (H&E) which allows visualization of overall tissue structures and Gorgon and Sweet special stain was used for the histochemical assessment. Trained pathologist was consulted for the accurate assessment and interpretation of cytomorphological changes in the cerebral cortex exposed to Eliozu dump site leachate.

Results

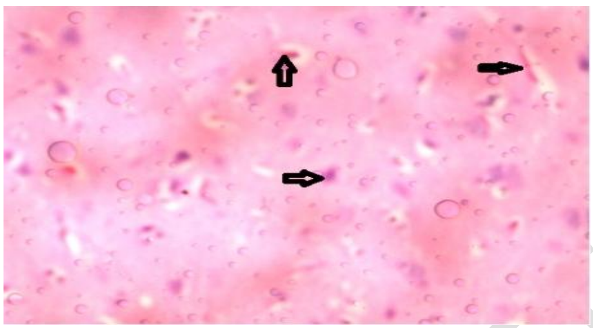
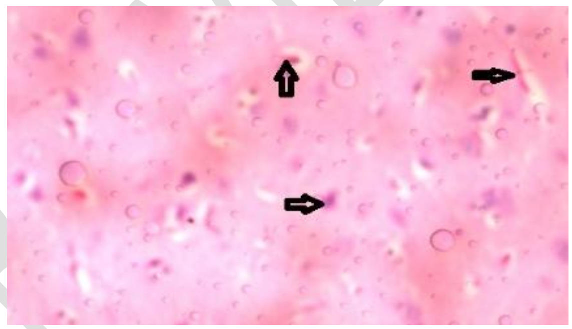


Plate 1



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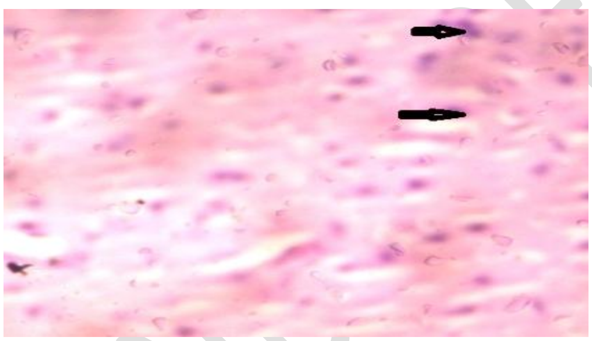

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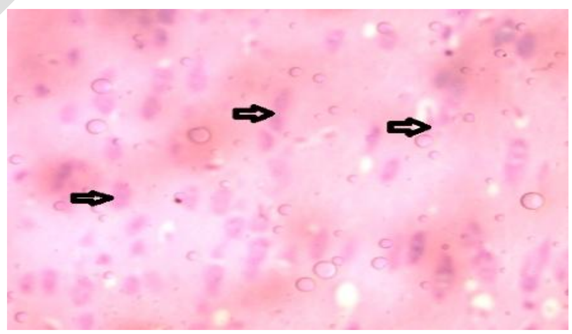

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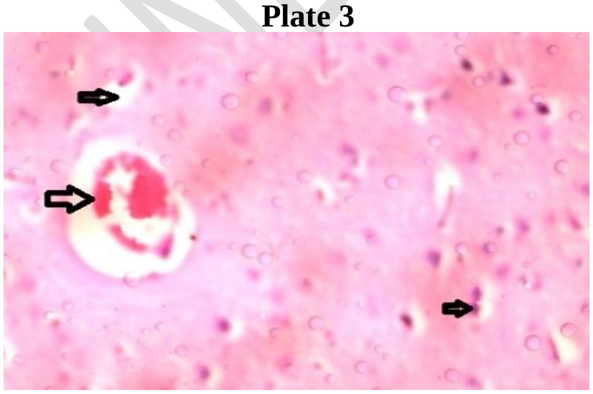

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Plate 6

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Plate 8

Plate 9

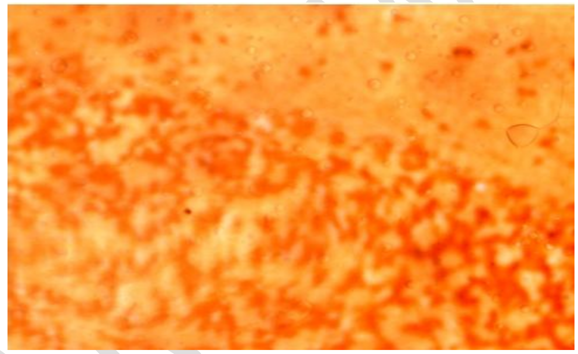

Plate 10

Plate 1: Photomicrograph of cerebral cortex Mag X400 H&E group 1 (commercial bottle water) showing normal cerebral cortex with pyramidal cells, Astrocytes and blood vessels.
Plate 2: Photomicrograph of cerebral cortex Mag X400 H&E group 2 (near-by borehole water 1km). Showing normal cerebral cortex with pyramidal cells, Astrocytes and blood vessels.
Plate 3: photomicrograph of cerebral cortex Mag X400 H&E group 3 (EDL10%) showing diffused vacoulation of the neuronal cytoplasm (spongiosis)
Plate 4: photomicrograph of cerebral cortex Mag X400 H&E group 4 (EDL50%) showing neuronal necrosis with proliferating astrocytes
Plate 5: photomicrograph of cerebral cortex Mag X400 H&E group 5 (EDL100%) showing vacuolation of the neuronal cytoplasm, inflammation of neuron and thrombosis
Plate 6: photomicrograph of cerebral cortex Mag X400 Gordon and Sweet stain group 1 (commercial bottle water) showing normal cerebral cortex with pyramidal cells and Astrocytes
Plate 7: photomicrograph of cerebral cortex Mag X400 Gordon and Sweet stain group 2 (near-by borehole water 1km) showing high concentration of reticular fibres in cerebral cortex
Plate 8: photomicrograph of cerebral cortex Mag X400 Gordon and sweet stain group 3 (EDL 10%) showing high concentration of reticular fibres in cerebral cortex
Plate 9: photomicrograph of cerebral cortex Mag X400 Gordon and sweet stain group 4 (EDL 50%) showing high concentration of reticular fibres in cerebral cortex
Plate 10: photomicrograph of cerebral cortex Mag X400 Gorgon and sweet stain group 5 (EDL 100%) showing high concentration of reticular fibres in cerebral cortex

Discussion

In developing countries paucity of suitable technological choices could be the reason solid waste are dumped [Veccari *et al*., 2012]. Exposure to leachate causes health risk. The physicochemical properties of Eliozu dumpsite leachate is higher compared with the accepted values of National Environmental Standard Regulations Enforcement Agency as described by (Weleh *et al*., 2021).

The neurologic lesions in the cerebral cortex of wistar rats exposed to eliozu dumpsite leachate observed in this present study suggest neurotoxic oxidative damage. Neuronal necrosis observed in the treated rats is as a result of disruption of structural and functional integrity of cell membrane. It is possible that pro-oxidant metals (Arsenic) in the leachate reacted with lipid hydroperoxides of the brain (cerebral cortex) cell membrane to interfere with the structural integrity of the neuronal membrane; this is in consonance with reports of (Chaltopadhyay *et al*., 2002, Jomova *et al*., 2010).The occurrence of the inflammatory neurons, neuronal necrosis and proliferating astrocytes associated neurodegeneration in the leachate treated rats suggests reactive oxygen species formation and subsequent disruption of metabolic enzyme. These may have activated neuronal accidental cell death (necrosis) and or neuronal programmed cell death (apoptotic) pathway. (Wright and Baccarelli 2007, Alimba *et al*., 2016). Inflammatory cells are associated with spongiosis, neurodegeneration of the pyramidal cells and swollen endothelial cells. The presence of the oedema suggests obstruction of cellular fluid flow in the brain cells. (Park *et al*., 2009, Lanning *et al*., 2002). Neurodegeneration in the pyramidal cells may alter impulse conduction or transmission to higher place that will enhance motor coordination of the body (Gartner and Hiatt, 2001).

Histochemical stain for the cerebral cortex is to identify reticulin fibers which are essential for the recognition of stroma that is produced by cells of mesodermal origin (meningiothelial cells). Reticulin is one such component that forms an integral part of stroma skeleton; it is present under basement membrane, on the surface of adipose tissue (Neil *et al* 2011). Reticulin fibers (collagen III) are supporting fibers measuring 20nm in diameter, these fibers provide support for the follicular dendritic cells and important for the functioning of the astrocyte. (Roozendal *et al*., 2009). In our present study, we observed that there was abundant quantity of Gorgon and sweet stain in the Port Harcourt (Eliozu) dumpsite leachate exposed rats while compared with the control. This study is similar with earlier report by (Beatriz and Lopez, 2019) on diagnostic histochemistry in neuropathology where reticulin fibers are used to determine fibrous tumor components of glial tumor or if neoplastic tissue is present in the parenchyma of the nervous system. (Vanderbberg 1993).The prominent reticulin deposition or desmoplasia indicates glial or mixed glioneuronal tumors like pleomorphic xanthoastrocytes, desmoplastic ganglioglioma (Kepes *et al*., 1979, Kepes 1993).

Conclusion

The cerebral cortex is a vital part of the brain responsible for complex cognitive functions. Exposure to Eliozu dumpsite leachate with its toxic constituents poses serious threats to the cerebral cortex and overall brain health. Understanding and addressing these potential hazards through effective management practices and regulations will be crucial in ensuring the well-being of individuals and the environment. Continued research, education and public awareness are essential to promote sustainable waste management practices and safeguard the integrity of the cerebral cortex.

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