Toxicological Impact of Eliozu Metropolitan solid waste Dumpsite leachate on Biochemical Parameters and Renal histomorphology.

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Abstract

The 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 deleterious substances. Eliozu metropolitan solid waste dumpsite is one of the biggest dumpsite in Port Harcourt metropolis. The dumpsite receives deposits of both domestic and industrial solid wastes from Port Harcourt, these wastes are stockpiled untreated, and thus may pose serious environmental risks to inhabitants in the metropolis. The present study focuses on the toxicological assessment of the biochemical parameters and renal histomorphology of wistar rats exposed to Eliozu metropolitan solid waste dumpsite leachate. Twenty-five (25) male Wistar rats were divided into five groups of five animals each; the leachate was collected from the stockpile and water from proximal 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 blood samples were collected for biochemical assay using standard methods and the kidneys were harvested and put in 10% formaldehyde, sectioned and stained with haematoxylin and eosin (H&E), periodic acid Schiff (PAS) was used as special stain for histopathological studies. The slides were placed in a light microscope and analyzed by a consultant Anatomy pathologist. The results revealed that the Eliozu metropolitan solid waste dumpsite leachate have deleterious effect on the biochemical parameters indicating renal damages, this was confirmed by the morphological changes in the renal histomorphology. In conclusion, Eliozu metropolitan solid waste dumpsite leachate has shown to have toxic effect on the renal biomarkers and histo-structure. 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 kidneys.

INTRODUCTION

The Eliozu metropolitan solid waste dumpsite is one of the biggest solid waste dumpsite in Rivers State; it receives untreated deposits of both domestic and industrial wastes from all over Obio/Akpor (Weleh *et a*l., 2021) and despite its possible direct or indirect health

risk, no study has been done on its toxicological impacts on biochemical parameters and renal histmorphology also considering the fact that residents around the dumpsite largely depend on borehole water around there for drinking.

Dump site is the most secular possible means of solid waste disposal practiced in growing countries (Vaccari *et al*., 2012). However, fluid also called leachate may drain from such cumulus and contaminate surrounding water body (Chatham-Stephens *et.al*.,2014). Exposure of the environment to dumpsite leachate may occur through uninfluenced overflow, rainfall and infiltration into the sunjacent ground water aquifer or nearby surface water. Leachate is now being recognized as a potential health risk to both surrounding ecosystems and human populations (Chatham-Stephens *et.al*.,2014). Studies on leachates from the dump site of Eliozu reported high level of heavy metals, microbes, chemical oxygen demand, biological oxygen demand and ammonia. (Weleh *et al.,*2021). A work on the leachate from medical dump site of Delta State Teaching Hospital Oghara reported high concentrations of heavy metals [Nwaka *et al.,* 2018]. 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]. The haematotoxic potentials of heavy metals that may be contained in leachate have been reported in fishes (Afolayan *et al.,* 2012). Immunotoxic potentials of raw and simulated leachates from Olusosun municipal solid waste landfill in rats has also been reported (Olujimi *et al.*, 2016). (Subhasini *et al*. 2012) 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. A similar study at Olusosun municipal landfill leachate Ojota Lagos State, Nigeria also reported hepatotoxicity and oxidative stress in rats [Olujimi *et al*.,2016]. 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 Eliozu dump site leachate (Weleh *et.al*., 2023), thus, concluding that Eliozu dump site leachate poses serious threat to the cerebral cortex and the overall brain health using wistar rats as experimental model. Further research also reported in a work, histopathology of the spleen; platelet count of wistar rats exposed to Eliozu dumpsite leachate stated concentration dependent alteration in the histomorphology of the spleen and studied platelets parameters (Weleh *et al.,* 2023). The evaluation of the histomorphology of small intestine of wistar rats exposed to Eliozu dumpsite leachate shows that it caused deleterious effect on the histomorphology of the small intestine (Weleh *et al.,*2023) This study therefore attempts to evaluate the toxicological impact of Eliozu dumpsite leachate on biochemical parameters and renal cytostructures using Wistar rats.

MATERIALS AND METHODS

2.1 Preparation of Experimental Animals

Twenty-five (25), Wistar rats of about 12-14 weeks old and weighing between 140-160g 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 dumpsite at Eliozu in Obio/Akpor, local government of Rivers State, Nigeria in a clean plastic container. 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. The required concentrations were obtained according to (Weleh *et al* 2020)

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 bottled water; Group 2 served as negative control and received 1 ml of water obtained from borehole about 1 km from the dumpsite; 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. 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 biochemical parameters. The rats were also dissected and the kidneys harvested and processed for histological and histochemical analysis.

2.4 Determination of Biochemical parameters

The method is titrimetric method, where carbondioxide (CO_2) is released from plasma bicarbonate with dilute Hydrochloric acid, excess is titrated against sodium hydroxide using phenyl red as an indicator. The principle of urea determination is that serum urea is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction. Creatinine in alkaline solution reacts with picric acid to form a coloured complex whose amount formed is directly proportional to the creatinine concentration. The method is colorimetric,

2.5 Histopathology Analysis of kidneys Tissue

The processed kidneys 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 fushsinsulphurus acid (metabisulphate and hydrochloric acid) and yields an insoluble magenta colored compound on the tissue. The slides were analyzed by a consultant pathologist.

2.6 Statistical Analysis

Data obtained were subjected to statistical analysis using SPSS version 22, 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 Table below; while histology and histochemistry stained plates are presented with magnification of X400.

Table 1: Effect of Port Harcourt dumpsite leachate on Biochemical parameters

PARAMETRES	Group 1 (bottle water)	Group 2 (Near-by borehole water 1km)	Group 3 (10%EDL)	Group 4 (50%EDL)	Group 5 (100%EDL)
Bicarbonate (mmol)	24.34±1.02	29.36* ±0.37	28.96* ±1.03	29.06* ±1.07	29.66* ±0.10
Urea (mmol)	7.22±0.32	7.38±0.86	5.01* ±0.38	8.04±0.48	8.22±0.41
Creatinine (mmol)	134.8±2.96	161.0* ±6.23	135.60±4.50	142.60±6.56	168.0* ±4.74

*= significant difference compared with Group 1 ($p<0.05$)

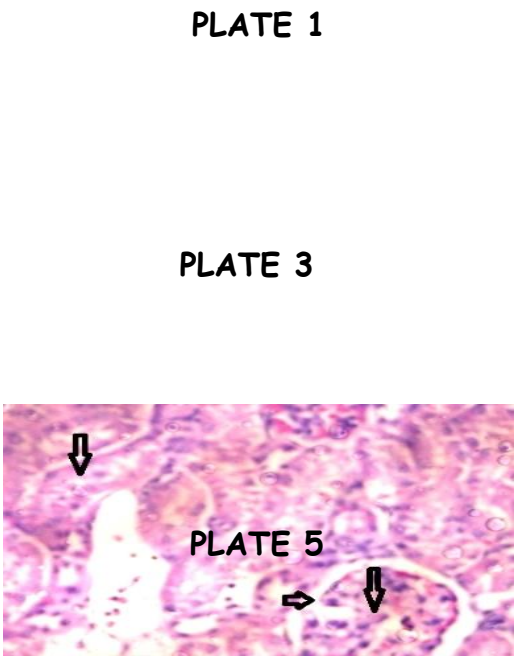

Plate1: Photomicrograph of kidneys tissue Mag X 400 H&E stain Group 1 control (commercial bottle water). Showing normal kidneys arc GT), **Plate2:** Photomicrograph of kid 2 (N orted kidneys architecture, ma GT), n's capsule (BC). **Plate3**: Pho H& orted kidneys architecture, ma GT), 's capsule (BC). **Plate 4:** Pho &E ys architecture, manifesting wit ena . **Plate 5:** Photomicrograph of 5 (1 nanifesting with partitioned glo) an hotomicrograph of kidneys

tissue stained with H&E of animals in group 1 commercial bottle water, showing normal kidneys architecture with glomerular tuft, renal tubules and Bowman's capsule when compared with the experimental groups. Plate 2 also shows kidneys tissue stained with H&E of animals in group 2 exposed to borehole water 1Km from the Dumpsite, manifesting mildly distorted kidneys tissue with partitioned glomerular tuft, collapsed renal tubules and patent Bowman's capsule when compared with the control group Plates 3, 4 and 5 also show kidneys tissues stained with H&E of animals exposed to 10%, 50% and 100% concentration of EDL, with manifestation of partitioned glomerular tuft, collapsed renal tubules, patent and occluded Bowman's capsule when compared with the control group **Plate 6:** photomicrograph of kidney Mag X400 PAS stain group1 (commercial bottle water), showing normal glomeruli and PAS positive tubules characterized by magenta coloured accumulation **Plate 7**: photomicrograph of kidney Mag X400 PAS stain group2 (nearby borehole water 1km), showing normal glomeruli and PAS positive tubules characterized by magenta coloured accumulation **Plate 8:** photomicrograph of kidney Mag X400 PAS stain group3 (EDL 10%), showing distorted glomeruli and mildly PAS positive tubules characterized by faint magenta coloured accumulation **Plate 9:** photomicrograph of kidney Mag X400 PAS stain group4 (EDL 50%), showing distorted glomeruli and reduced PAS stain in the tubules **Plate 10:** photomicrograph of kidney Mag X400 PAS stain group5 (PEDL 100%), showing distorted glomeruli and reduction in PAS stained tubules

3.2 Discussion

Dump sites in developing and emerging countries suffer from the lack of leachate treatment and caption facilities; causing the contamination of water bodies and soil and threatening human health. (Alam *et al*., 2008, Al Sabbagh *et al.,* 2012).

The present investigation shows alteration in some kidneys biochemical parameters and histostructures. Therein, alteration in serum biochemical is an imperative indicator for toxicity in identifying target organs and general health status of animal. Significant elevation in bicarbonates compared with the control is indication of serum electrolyte alteration.

The statistically significant increase in bicarbonate observed in this study could be as a result of one or some of the pathophysiological pathway. There was significant increase in serum creatinine and urea. Creatinine is the breakdown product of creatine which is pertinent part of the muscle. The test to evaluate the serum creatinine is more sensitive indicator of renal function than the blood urea nitrogen (Nabil *et al.,* 2013). If the renal function is abnormal, the creatinine level will increase in the blood as a result of decreased excretion of creatinine in the urine (Hecht., 2006).The increase in the serum creatinine caused by constituents of leachate suggests that the kidney destruction which may result from renal lesions decreased perfusion of the kidney obstruction of lower urinary tract or due to deranged metabolic process caused by metals, our result is similar with the report of (Abd El Rahiem *et al*., 2007), who reported that lead acetate increased serum creatinine level compared to the control group and similar results have been reported by some researchers (Abdel-Razik *et al*.,2007)

Significant increase in urea was observed in 10% EDL and non-significant increase in the rest experimental groups this is similar with report of (Dioka *et al.,* 2004) who reported that the exposure of human subjects to lead in petrol increased the concentration of urea in a non-significant manner as compared to unexposed subjects. The ionic mechanism of action of the chemical constituents of Eliozu dumpsite leachate like lead, arsenic, iron, manganese, is due to their ability to substitute other bivalent or monovalent cations such as sodium, chlorine, potassium, etc. thus affecting various fundamental physiological and biological processes of the body damaging cellular components, this is similar to a proposed mechanism by (weleh *et al*.,2023).

In the study, the disintegration of the cytoplasmic membrane of the glomeruli and kidney tubules were observed, disruption of the cellular membrane has been reported as one of the consequences of the ingestion of oxidized lipid (Ologan, 2002, Jimoh and Odutuuga 2004).

This is as a result of peroxidation of membrane phospholipid due to free radicals. The disintegration of cytoplasmic membrane might likely lead to disruption in the filtration and concentration of the urine, it may also affect the fluid and electrolyte balance as well as regulation of total body homeostasis.

As observed in this study, chemical components (e.g Iron and Manganese) of the Eliozu metropolitan solid waste dump site leachate exposed experimental rat model caused kidney dysfunction; this supports reports which showed that elevated level of iron is capable of inducing multiple changes in renal-tubular epithelial functions. The effect of Iron could be due to diminished expression of beta-1-integrin subunit and impaired proliferation (Jacob and Ganiyu 2016).

High level of manganese can bind either to adenosine Triphosphate (ATP), or to protein directly, thereby, causing conformational changes (Huang and Lin, 2004). In addition, reports have shown that elevated dose of manganese can damage kidneys (inflammation and urinary tract in high fed rats) (Ponnapakkpan *et al*., 2003)

In the present study, it has been shown that collectively, the necrosis of the tubular epithelia cells and injuries induced by EDL could be linked to individual synergetic or antagonistic interaction of heavy metals with renal biomarkers this is similar to a report by (Akintunde *et al*., 2016, Akinntunde and Oboh, 2015).

We further observed progressive glomeruloangiosclerosis, this could be as result of the presence of lead which was reported to cause tubule-interstitial changes leading to kidney remodelling and glomeruloangiosclerosis. (Kuttubay and Oguz, 2007).

Histochemical observations in the kidney manifested considerable amount of carbohydrates (glycogen) in the cytoplasm of the kidney cells of control rats when stained with Periodic Acidic Schiff (PAS) which gave a red or magenta colour. Treated rats manifested decreased in carbohydrates in the kidney. Marked reduction of carbohydrates was observed in 100% EDL.

Disturbances in carbohydrates metabolism as observed in the animals under the effect of EDL suggested to be achieved via modifying the activities of the enzymes of glycolytic pathway. This is similar with the report of (Mehadavaswami *et al*., 2001). The impairment of the magenta colour is a testament damaged kidneys structure which may be due to some toxic compounds present in leachate, such as bacteria, heavy metals and polycyclic aromatic hydrocarbons (PAHs), which have been shown to have direct cytotoxic effect on cells (Green *et al*., 2022) this can disrupt the cytostructure of the kidneys and impair their ability to function properly.

Conclusion

Eliozu dump site leachate contains chemicals that have toxicological impacts on biochemical processes and renal histomorphology. Understanding and addressing potential hazard through proper waste management and practice, leachate treatment measures are pertinent for safeguarding the health of individuals and preventing adverse effects on renal function. Continued research in this area is essential to further elucidate the specific mechanisms of toxicological impact and develop effective strategies for prevention and mitigation.

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