EVALUATION OF THE HISTOMORPHOLOGY OF SMALL INTESTINE EXPOSED TO ELIOZU DUMPSITE LEACHATE USING WISTAR RATS AS EXPERIMENTAL MODEL

ABSTRACT

Eliozu dumpsite is one of the biggest dumpsite in Port Harcourt metropolis. 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 histomorphology of the small intestine 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 (40) days, the animals were sacrificed after being anesthetized with chloroform vapor, and the small intestine harvested for histological studies using Haematoxylin and Eosin (H&E), special stain Alcian Blue for histochemistry. We observed tissue vacoulation and sclerosis in the experimental animals which indicate deleterious effect on thehistomorphology of the small intestine in the experimental animals. Eliozu dumpsite leachate has shown deleterious effect on the histomorphology of the small intestine which is an indication of toxicity that may cause ailments to the experimental animals. Therefore, it is recommended that further studies aimed at corroborating this finding be carried out especially on humans and residents around dumpsite should always embark on health medical check-up.

INTRODUCTION

Municipal solid waste (MSW) disposal constitutes environmental challenge globally (Vincenzo *et al*., 2017). Landfilling and /or open dumping is the preferred method of waste disposal in developing countries due to lack of suitable technology (Vaccari *et al*., 2012,Guerrero et al., 2013). This therefore could lead to environmental pollution and health risk to immediate community; because liquid containing significant concentration of undesirable materials may drain from the stock piled materials and contaminate underground water or nearby water sources. The water that drains from the stockpile of dumpsites is regarded as leachate; it may contain toxic compounds that may collate in water bodies and make them toxic to the body system when taken [Chatham-Stephens *et al*., 2014, Alam *et al*.,2008, Al Sabbagh *et al*., 2012]. Studies have reported that leachates samples of Aba-Eku landfill in Lagos and Eliozu landfill in Port Harcourt Nigeria, contain heavy metals and microbes [Ogbonna *et al*., 2016,Weleh *et al*., 2020]. The study

also reported that exposure to landfill leachate and (or) drinking water from a nearby source may cause significant hepatological toxicity in Wistar rats [Weleh *et al*.,2020]. Also, haematological toxicity has been reported in solid waste workers in Port Harcourt Nigeria; which was attributed to exposure to chemicals in solid wastes [Nwoke *et al.,* 2017]. The hepatotoxic effect of Eliozu dumpsite leachate has been studied, where the researcher deposited that the leachate has toxicological effect on the liver function parameters and the histoarchitecture of the liver (Weleh *et al*., 2020), further study was reported in a work, where the Eliozu Dumpsite leachate destroyed the histology of the reproductive tract of animals exposed to the leachate. (Green *et al*., 2022).

Interference with the morphology of the small intestine may lead to several ailments. The biological systems such as the gastrointestinal tract system are affected by environmental factors like pollutants. 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). There is however scanty literature on the effect of Eliozu dumpsite leachate on the histomorphology of small intestine; hence, the need for this study that aimed at evaluating the histomorphology of small intestine exposed to eliozu dumpsite leachate using Wistar rats as experimental model.

MATERIALS AND METHODS

Collection of Leachate Raw leachate fluids were collected from leachate well at Eliozu dumpsite in Obio/Akpor, Nigeria. The leachate sample was taken to the laboratory in clean and dry plastic containers, 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.

Experimental Animals

Twenty-five female Wistar rats weighing between 120-140 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 drinking water and standard rodent chow ad libitum. The animals were treated according to the guide for the Care and use of laboratory animals

Experimental Design

The rats were randomly assigned into five groups of five (5) rats each. Group 1 served as a control and orally received 1 ml of bottled water, group 2: Orally received 1 ml of water from a borehole close to the dumpsite. Groups 3, 4 and 5 received 1 ml of 10%, 50%, and 100% leachate concentrations orally for 30 days. At the end of the leachate administration, the rats were anaesthetized using chloroform vapor and small intestine immediately collected for both histology and histochemical assays.

Histopathological Examination

Small intestines were harvested and processed for histological and histochemical analysis,the processed organs 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 Hematoxyline and Eosin (H&E). Alcian Blue special stain was used for histochemistry which Principle is to differentiate between neutral and acidic muco-substances, probably the best pan-mucin stain available and the slides were evaluated by a consultant pathologist.

Results

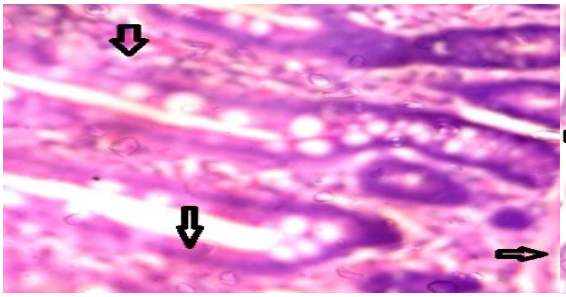


Plate1: Mag X400 H&E

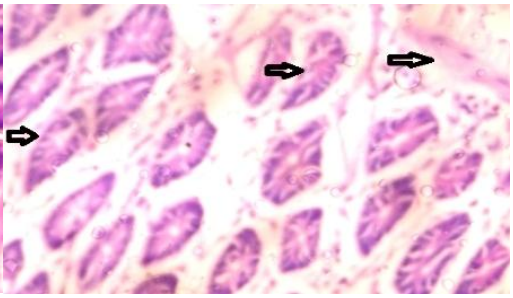


Plate 2: Mag X400 H&E

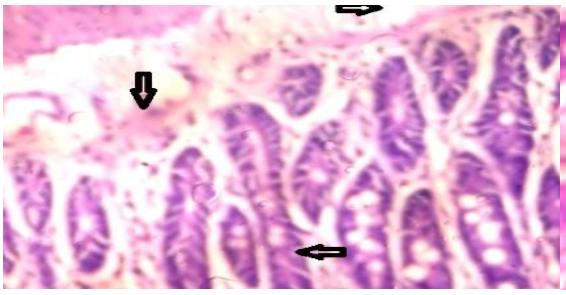
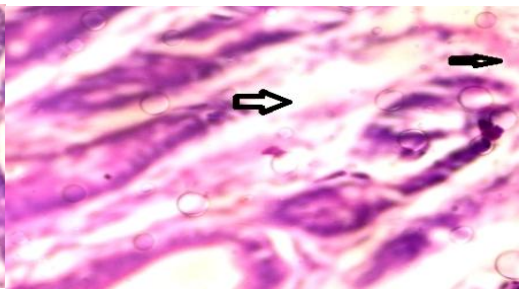


Plate 3: Mag X400 H&E



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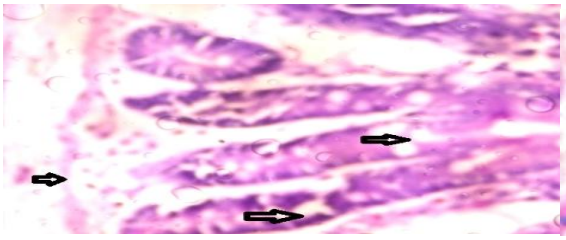

Plate 5: Mag X400 H&E

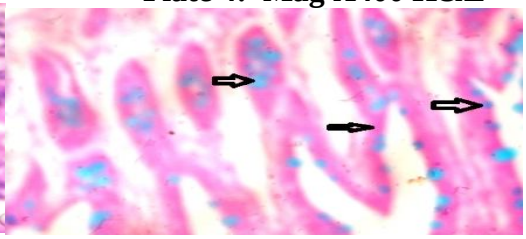

Plate6: Mag X400 H&E

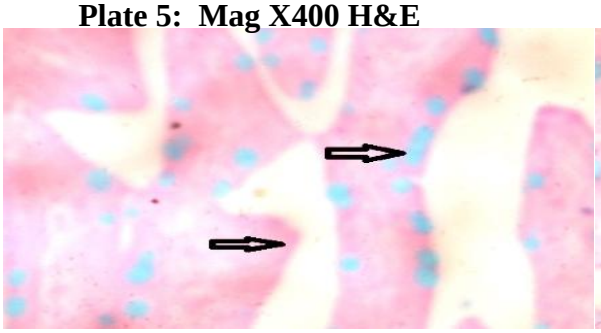

Plate 7: Mag X400 H&E

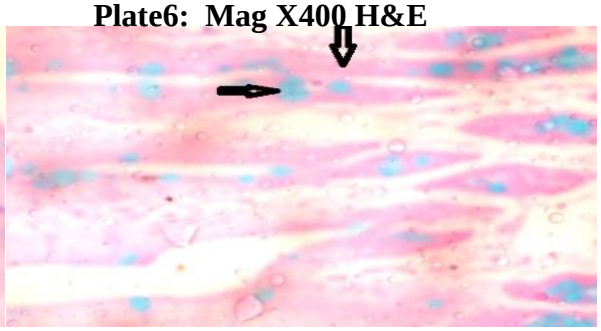

Plate 8: Mag X400 H&E

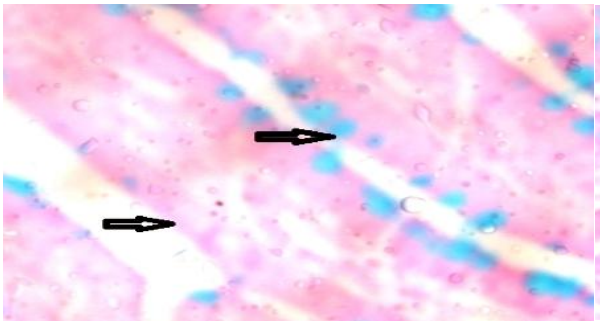

Plate 9: Mag X400 H&E

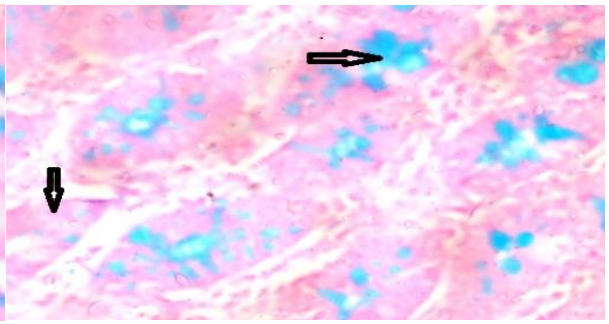

Plate10: Mag X400 H&E

Plate1 shows the photomicrograph of small intestine stained with H&E, from animals in group 1 (control), bottle water manifesting normal histology of small intestine with villi, goblet cells and glands. Plate 2 also shows photomicrograph of small intestine stained with H&E from animals in group 2 (borehole water 1 km from the dumpsite) manifesting histologically distorted small intestine with disrupted muscular mucosa and villi.Plates3, 4.and 5, show photomicrograph of H&E stained small intestine in group of animals exposed to 10%, 50% and 100% concentrations of Eliozu dumpsite leacahte manifesting serious disruption of the histology of small intestine when compared with control group. They show disrupted muscular mucosa, vacoulations in the submucosa.Plate 6 shows photomicrograph of small intestine stained with Alcian Blue 2.5 animals from group 1 (control) commercial bottle water manifesting histochemically normal small intestine with mucopolysaccharides and acidic mucins, goblet

cells show blue, acid mucin blue, cytoplasm shows pink coloration.Plate 7 also shows photomicrograph of Alcian Blue 2.5 stained small intestine of animals in group 2 (borehole water 1km from dumpsite) manifesting mildly enlarged goblet cells with blue colour and reduced pink coloration of the cytoplasm an indication of disrupted small intestine.Plates 8, 9 and 10 also show the photomicrograph of Alcian Blue 2.5 stained small intestine of animals exposed to 10%, 50% and 100% concentrations of Eliozu Dumpsite Leachate manifesting damaged small intestine with enlarged goblet cells blue colour, reduced coloration of pink in the cytoplasm.

DISCUSSION

The municipal solid waste (MSW) final disposal represents an environmental challenge globally (Vincenzo *et al*., 2016) since landfilling and or open dumping is still most preferred solution. In developing countries (Vacearo *et al*., 2012, Guerrero *et al*.,2013) lack of suitable technological choices means environmental pollution and risk for health of population due to the exposure to toxic compounds caused by the release of leachate (Chathan *et al*., 2014). 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 paucity of data on waste generation and its effect on health environment around Port Harcourt metropolis continue to be a pivotal problem thus inhibiting exact regulation. There is very little known about leachates in Port Harcourt, so efforts to put exposure levels to protect humans often prove very ineffective. Worthy of note is that pollution decreases the quality of life in different area and affects health and life span (Grover and Kaur 1999).

After analysis of the basic properties and chemical composition of leachates in Port Harcourt Eliozu dumpsite, it shows that landfill leachate is a mixture and contains varieties of contaminants; there are general underlying pollutants common to all landfill effluents (Anshu and Poulrag 2015).

In the present study, we observed alteration in the histoarchitecture of the small intestine of the Eliozu dumpsite leachate treated animals, the small intestine of the control group has shown normal intestinal histoarchitecture. The mucosa forms a series of finger-like villi, each showing lamina propria, blood capillaries etc. the simple columnar mucosal epithelium contains absorptive goblets cells, crypts of Liberkuhn in the mucosa and Brunner's glands.

Distortions of the villi were also prominent in leachate treated animals, absorptive goblet cells and paneth columna epithelium including cytoplasmic disruption leading to formation of mucous vacuoles.

There have been scanty reports on the alterations of the cellular architecture in the small intestine of animals in response to leachate toxicity. We observed in this study that oral administration of EDL resulted in significant histopathological abnormalities in the small intestine as evident by atrophy musculature, disintegration of sub-mucosal and connective tissue and disruption of serosa to different extent.

The mucosa epithelium cells showed cytoplasmic vacuolization, necrotic nuclei indicated by clumped nuclear material and nuclear pycnosis, this is similar with earlier report that leachates disrupt kidney and liver (Alimba *et al*., 2012). It is similar with another finding that stated that aluminium chloride exposure was detrimental to the histology of small intestine (Buraimoh 2012), it is also similar with the report that Aluminium chloride exposure was detrimental to the histology of the kidney of wistar rats (Buraimoh and Ojo 2012).

Histochemically, the goblet cells of the control group stained positive with the Alcian Blue (pH 2.5) stain, with blue coloration. This is an indicator for sulfated mucin substance which are very important in digestion and subsequent absorption, this comply with an earlier report on the histology and histochemistry study of the small intestine of the striated scope Owl (Otus Scors Brucei) (Saffer *et al*., 2016).

In this study, we observed abnormal mucus deposition in the enlarged goblet cells in the intestine of the EDL-treated animals this could be as a result of a proposed mechanism by (Zhang and Wang 1984) that the alteration could be disturbances in lipid inclusions and fat metabolism in response to toxic substances resulting in cytoplasmic vacuolization. This is also similar with the report of (Animesh *et al* 2009).

Possible mechanism of actions of some heavy metals in Eliozu dumpsite leachate

The mechanisms of some heavy metals' toxicity are established. Lead toxicity occurs due to the ability of lead (Pb) metal ions to replace other bivalent cations such as Calcium ions (Ca^{2+}) Magnesium ion (Mg^{2+}), ferrous ion (Fe^{2+}) and monovalent cations like sodium ion (Na^{+}), which

ultimately disturbs biological metabolism of the cell. These ionic mechanisms cause significant alterations in biological processes such as adhesion, intra and extracellular signaling, protein folding, maturation, apoptosis, ionic transportation, enzyme regulation and release of neurotransmitters this similar with a mechanism proposed by (Flora *et al*., 2008).

Mercury can cause disruption of membrane potential, interrupt intracellular calcium homestasis, damage the tertiary and quaternary protein structure and alter cellular function. It can also intervene with the process transcription and translation, resulting in the disappearance of ribosomes and the eradication of endoplasmic reticulum and the activity of natural killer cells. Cellular integrity is also affected, causing free radical formation (Jaishanker *et al*., 2017, Bernhoft 2012).

Conclusion

From our study, we conclude that exposure to the eliozu dumpsite leachate and the consumption of the nearby borehole water with significant heavy metals and microorganism have caused the histopathological alterations in gastrointestinal tracts (small intestines) of animals and we recommend that Government and envirmonetal regulatory agencies facilities for treating Eliozu dumpsite leachate.

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