Histopathology Of The Spleen; Platelet Count Of Wistar Rats Exposed To Eliozu Dumpsite Leachate

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***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 histopathology of the spleen; platelet counts 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, the bloods and spleens were collected for Platelet counts were analyzed using Auto-analyzer (Auto-analyzer, Mayamed, England. 2018 model) and histological studies using Haematoxylin and Eosin (H&E), special stain Mason Trichrome. We observed increase in platelet, mean platelet volume, platelet distribution width, platelet large cell volume and procalcitonin, which signifies deleterious effect in platelet counts. The histology results revealed that the Eliozu dumpsite leachate does have effect on the histomorphology of the spleens in experimental animals. Eliozu dumpsite leachate has shown deleterious effect on platelet counts and histomorphology of the spleen 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.

***Index Terms*-** ELIOZU, LEACHATE, PLATELETS COUNTS

I. INTRODUCTION

Dumpsite as an area not constructed by Government or agency for the dumping of solid waste, it is the most economic viable means of municipal solid waste disposal practiced in developing countries (Vaccari, *et al,* 2012). However, fluid effluent known as leachate may drain from such stockpile and contaminate surrounding water body (Chatham-Stephens *et al,* 2014). Exposure of the environment to dumpsite leachate may occur through un-dictated overflow, rainfall and infiltration into the ground water aquifer or nearby surface water. Leachate is now being acknowledged as a potential health danger to both surrounding ecosystems and human populations (Al Sabbagh *et al,* 2012). Studies on leachates from the dumpsite 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. However, benzene, toluene and ethyl benzene may have contributed to the leachate toxicity (Tsai *et al,* 2010). Weleh and co-workers reported concentrations of Copper, Chromium, lead, Arsenic, Cadmium, Manganese, Nickel, ammonia, Chloride and Phosphate in the leachates samples of Eliozu dumpsite above standard permissible limits (Weleh *et al,* 2021). The haematotoxic potentials of heavy metals that may be contained in leachate have been reported in rats. (Afolayan *et al,* 2012). Immunotoxic potentials of raw and simulated leachates from Olusosun municipal solid waste landfill in rats has also been reported (Subhasini *et al,* 2009). Subhasini *et.al* 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 (Subhasini *et al,* 2009). A similar study at Olusosun municipal landfill leachate Ojota Lagos State, Nigeria also reported hepatotoxicity and oxidative stress in rats. Neurologic lesions, neurodegenaration of purkinje cells with lose of dendrites, 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 (Alimba 2016); these reported brain tissue alterations correlate with a significant decrease in body weight gain and Superoxide dismutase activity but increase in absolute and relative brain weight gain, Malondialdhyde concentration and Catalase activity in both brain and serum in treated rats (Alimba 2016)

The Eliozu Dumpsite is located Obio/Apkor local government of Rivers State; it receives untreated deposits of both domestic and industrial wastes from all over Port Harcourt and despite its possible hazardous nature, considering the fact that residents around the dumpsite largely depend on borehole water for consumption. Secondly, despite the fact that diseases of the spleen and platelets (thrombocytes) remains prevalent in our environment, there is paucity of literature on the possible effect of leachate on histopathology of the spleen and platelets (thrombocytes) counts. This study therefore attempts to evaluate the histopathology of the spleen; platelets counts of animals exposed to Eliozu dumpsite leachate using Wistar rats as experimental model. This is owing to the fact that experimental animals and data from the toxicological studies in Wistar rats is a way of forecasting human risk assessment (Green and Weleh, 2021).

II. Materials and Methods

Experimental Animals

Twenty five (25) Wistar rats (10-12) weeks' old, weighing average of 140g were obtained from the Animal House unit of Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria. The animals were cared for according to the general recommendation and provision of the University Ethical committee. Rats were housed in a climate controlled room (12 hours' dark and light cycle), with free access to standard feeding chow and drinking water (*ad libitum)*.

Leachate collection and preparation

Raw leachate sample was collected from leachate well on the dumpsite at Eliozu in Obio/Akpor local government area of River State, Nigeria in clean plastic container. The sample was taken to the laboratory of the Department of Chemistry, University of Port Harcourt where it was filtered and prepared accordingly as described by (Green *et al*, 2020). The different concentrations of the leachate were determined by serial dilution of leachate with distilled water.

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, 1ml of 50% of leachate concentration and 1ml of 100% of leachate concentration respectively. All administrations were given once daily using an oro-gastric cannula for 40 consecutive days. On the 41st day however, the animals were anaesthetized using chloroform vapor, the spleens and blood samples were obtained for histological assay and platelets (thrombocytes) count analysis using auto-analyzer (Auto analyzer, Mayamed, England. 2018 model).

Histopathological Examination

Spleens were collected and fixed with 10% formalin, embedded in paraffin, sectioned and stained with hematoxylin-eosin (H&E) and Mason trichrome for histochemical analysis. Experienced pathologist performed pathological evaluation.

As the name implies, three dyes are employed selectively staining muscles, collagen fibres and erythrocytes. The general rule in trichrome staining is that the less porous tissues are coloured by the smallest dye molecule, whenever a dye of large molecular size is able to penetrate, it will always do so at the expense of the small molecule. Others suggest that the tissue is first stained with the acidophilic tissue components. Then when treated with the phosphor acids, the less permeable components retain the red, while the red is pulled out of the collagen; blood was collected by direct cardiac puncture after chloroform anesthesia for determination of platelet parameters. About 4ml of blood was obtained from each animal, Platelets and platelet indices were also determined.

Ethical Clearance

The researcher sought for Ethical approval and obtained it from the University of Port Harcourt Research Ethics committee on 3rd July, 2018, with approval reference number as UPH/R&D/REC/04.

III. RESULTS

Table 1: Effect of Eliozu dumpsite leachate on Platelet counts

PARAMETERS	Group 1 (commercial bottle water)	Group 2 (Near-by borehole water 1km)	Group 3 (10% EDL)
Platelet ($\times 10^9$)	213.40±13.2	360.40* ±8.3	254.40±5.4
Mean platelet volume (fl)	8.38±0.12	8.5* ±0.14	8.70±0.11
Platelet Distributive Width (%)	7.96±0.67	10.24* ±0.56	10.08* ±0.13
Procalciton in (%)	0.23±0.04	0.32±0.04	0.34±0.15
Platelet Large Cell Ratio (%)	13.22±3.63	20.48* ±1.61	20.14* ±1.02

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

Table 1: shows the results of Platelets and Platelets indices, with platelet (PLAT) ($\times 10^9$/l) result expressed as Mean±S.E.M for control, BHW, EDL10%, EDL50% and EDL100% are 213.40±13.15, 360.40*±.35, 254.40±5.35, 337.80*±6.53 and 381.00*±36.00, respectively, with a significant ($p<0.001$) increase in experimental groups except EDL10% when compared with control group.

The Mean Platelet Volume (MPV) (fl) result also expressed as Mean±S.E.M for control, BHW EDL10%, EDL50% and EDL100% as in Table 1 are 8.38±0.12, 8.5*±0.14, 8.70±0.11,

8.65*±0.10 and 8.80*±0.17 respectively with statistically significant (P<0.001) increase in groups BHW, EDL50%, EDL100%, compared with the control group.

Table1 also shows the result of Platelet Distribution Width (PDW) (%) expressed as Mean±S.E.M for groups of control, BHW, EDL10%, EDL50% and EDL100%, respectively; 7.96±0.70, 10.20*±0.56 10.08±0.13, 10.10*±0.25, 11.32*±0.16, with statistically significant (p<0.001) increase in the experimental groups except in EDL10% group when compared with the control group.

The Procalcitonin (PCT) (%) result shown in the Table 1 is expressed as Mean±S.E.M for groups control, BHW, EDL10%, EDL50% and EDL100% have values as 0.23±0.04, 0.32±0.04, 0.34±0.15, 0.30*±0.01 and 0.24±0.00 respectively. Paradoxically, there is significant increase (p<0.001) in EDL50% compared with the control group.

Platelet Large Cell Ration (PLCR) (%) result shown in Table 1 and expressed as Mean±S.E.M for groups; control, BHW, EDL10%, EDL50% and EDL100% are thus 13.22±3.63, 20.48*±1.61 20.14*±1.02, 24.02*±1.81 and 26.68*±1.30, the result shows significant high value (p<0.001) in experimental groups when compared with the control group.

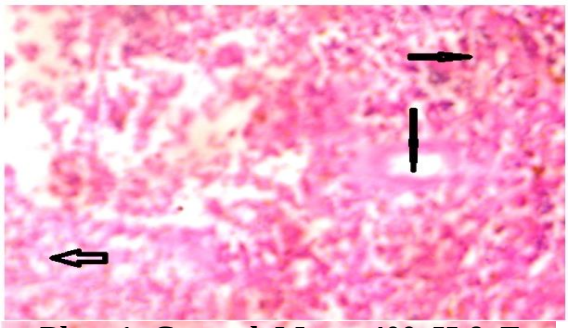

Plate 1: Control, Mag x 400, H & E

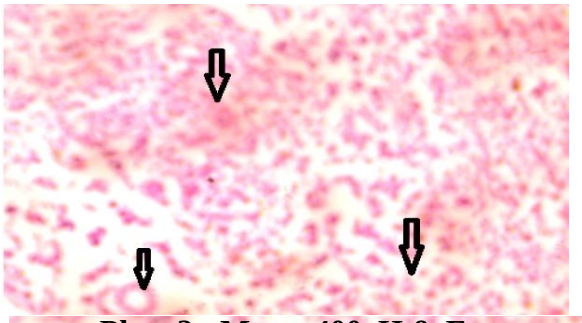

Plate 2: Mag x 400, H & E

Plate 3: Mag x 400, H & E

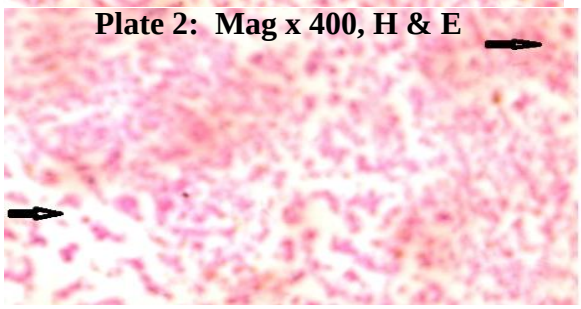

Plate 4: Mag x 400, H & E

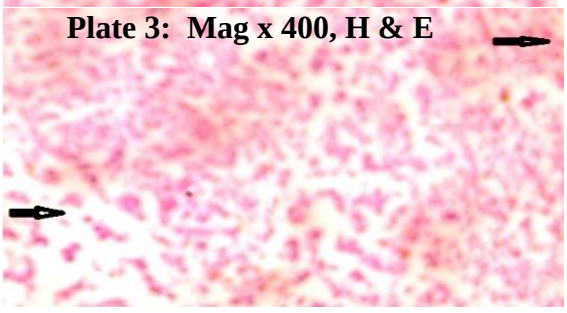

Plate 5: Mag x 400, H & E

Plates 1: shows result of a normal histo-architecture of spleen of animals exposed to commercial bottle water to when stained with H&E, the splenic white and red pulp shows normal compared with the experimental groups. Plate 2: shows result of splenic tissue of animals exposed to boreholes water 1km from the dumpsite, with reduced lymphocyte in the white pulp and vacoulations in the spleen while compared with the group 1, which is the control. Plates and show distorted splenic tissue of animals exposed to 10%, 50% and 100% of EDL with reduced lymphocyte in white pulp and severe hyperemia in red pulp and sinusoids.

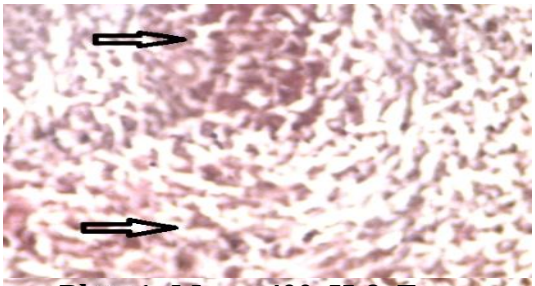

Plate 1: Mag x 400, H & E

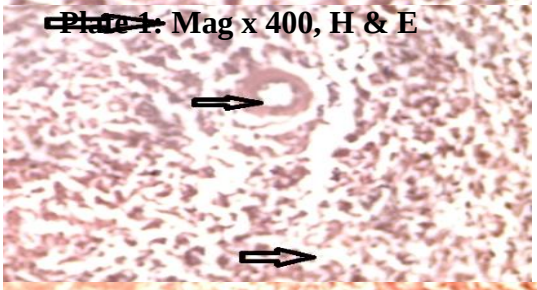

Plate 2: Mag x 400, H & E

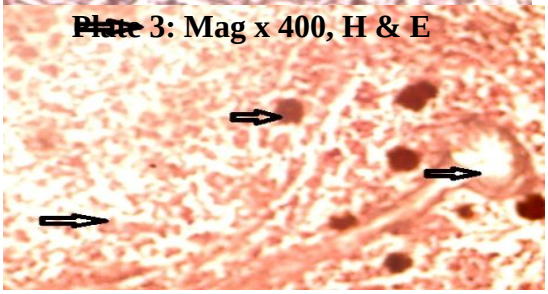

Plate 3: Mag x 400, H & E

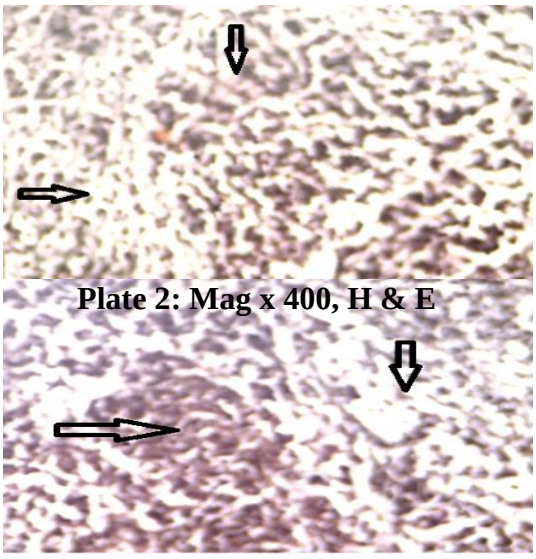

Plate 4: Mag x 400, H & E

Plate 5: Mag x 400, H & E

Plate 6 shows photomicrograph of spleen stained with Masson's Trichrome in group 1 (control) commercial bottle water manifesting black stain around the white and red pulp indicating normal histochemistry of spleen when compared with experimental group. Plate 7 also shows photomicrograph of spleen stained with Masson's Trichrome in animals in group 2 exposed to borehole water 1km from dumpsite manifesting mildly reduced stain and deposits of collagen which is an indication of splenic disruption histochemically. Plates8and9 show photomicrograph of spleens stained with Maason's Trichrome in animals exposed to 10%, 50% and 100% concentrations of EDL, manifesting deposits of collagen, noodles of deposit of collagen indicating lymphagioma when compared with the control group.

IV. DISCUSSION

Platelet or thrombocytes are the blood cells fragments that lead to the formation of blood clots; they are dynamic blood particles that interact with each other as well as with leucocyte and endothelial cells. Dynamic platelets readily get activated/ inhibited by many endogenous and exogenous stimuli. They initiate primary hemostasis by attaching strongly to the damaged vessel wall. Platelets start to change its shape by the formation of pseudopods when intracellular Ca^{2+} concentration exceeds a specific threshold. Oxidative stress is associated with superoxide anion [reduction and nitric oxide synthesis as well as production of reduced glutathione]. Hyperglycemia exerts oxidative stress which can implement altered platelet function (Ceriello *et al*., 1995).

Increased platelet concentration observed in the blood of rats exposed to leachate contaminated underground water may be attributed to the presence of heavy metals, among others in Eliozu dumpsite leachate and near-by borehole water sample. High level of platelets may arise from ingestion of high level of Lead; this is in agreement with the work by (Adeyemi *et al* 2006) on the effect of leachate –contaminated groundwater on the growth and blood of Albino rats. Increased platelet counts could lead to blood clots developing in the blood vessels which can lead to stroke, embolisms and infarction (Damoah, 2010).

Mean platelet volume is machine generated average size of platelet. Increased in Mean Platelet Volume value is correlated with the event of myocardial infarction (Martin *et al*., 1991).

Significant increase in Platelet Distribution Width, Procalcitonin, Platelet Large Cell Ratio was observed in our study and this is similar with a work by (Akkani *et al*., 2016) on alcoholics as compared with non-alcoholics, this could be as a result of effect of lead (Pb) in the leachate and near-by borehole water, this result is similar with earlier reports by (Okediran *et al*., 2019), (Mugahi *et al*., 2003), effect of chronic lead acetate intoxication on blood indices of male adult rats, investigation on sub-chronic lead intoxication on blood indices of male rats. While Tapu *et al*., 2014 reported decreased in the level of platelets and its indices in workers involved in lead-acid battery manufacturing plant in the first 14days. This is in variance with our study, the variance could be as a result of mode of exposure and the time duration or concentration of the toxicants.

The alteration of platelet indices might be the effect of leachate on intracellular calcium ion (Ca^{2+}) homeostasis either mimicking calcium ion (Ca^{2+}) action or antagonizing calcium ion (Ca^{2+}) dependent cellular functions and activations of protein

kinase C this is a mechanism of action similar with that proposed by (Theron *et al*., 2012) and also its actions on endothelial tissue injury, decreased level of nitric oxide, tissue plasminogen activator and an increased level of plasminogen activator inhibitor. -

The histopathologic assessment of the spleen revealed that the most serious alterations occurred in both the red and white pulp, the highest concentration of Eliozu dumpsite leachate showed a total loss of normal splenic histostructure with vacuole formation. Alteration of the splenic histostructure will unavoidably impair tissue functions. Our result is similar with studies conducted on rats by (Mesure *et al.*, 2015, Deveci 2006). Vacoulations increased with concentration of leachate exposure, this finding also complies with study on the rats and carps by (Green *et al* 2022) in a work, the histopathological manifestation of effects of Eliozu dumpsite leachate on reproductive tract of wistar rats, where they deposited that the dumpsite leachate caused deleterious effect on the cytostructure of the reperoductive tract of female wistar rats.

In addition, depletion of the splenic follicles was common and is in consistence with a work by (Adel *et al*., 2016) in the Ameliorative role of Grape Seed Extract on Cadmium Induced Splenic toxicity in Albino Wistar Rats, suggesting leachate as lymphocytic toxic agent that can cause necrosis of lymphocytes of the splenic white pulp.

Histochemically, Mason Trichrome describes the alterations in connective and soft tissue components in terms of sclerotic lesions, perivascular fibrosis and scar formation, which indicates direct damage to important organs as a result of chemical and mechanical injury. (Cabibi *et al*.,2015). In this study, we observed nodules of lymphoid and fibres in the Eliozu dumpsite leachate exposed animals which are also known as lymphagioma or splenic fibrosis. This report is in variance with an earlier report by (Khurram *et al*., 2019) on the oral toxicity of arjuanolic acid on heamatological, biochemical and histopathological investigation on female Spraque Dewley rats, reason for this discrepancy could be as a result of the physicochemical constituents of Eliozu dumpsite leachate. The lymphagioma which is a benign tumor of the spleen involving lymphatic endothelium present itself as a solitary nodule, this is similar with a report by (Dinesh and Sambit 2013).

V. CONCLUSION

In conclusion, this study shows that Eliozu dumpsite leachate have high concentrations of both the physical and chemical properties when compared with the NESREA standard. The concentration dependent alterations in the studied platelet parameters and structural alteration in the histomorphology of spleen are indications that Eliozu dumpsite is very toxic. Our study reports the potential toxicological effects of Eliozu dumpsite leachate and urges municipal authorities to intervene in public interest.

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