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Evaluation of haematological parameters, and liver profiles following the administration of leached water in male Wistar rats

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ABSTRACT

Landfills are a significant threat to groundwater worldwide. Leachate is a liquid formed when water passes through waste materials. This study investigated the effect of leached water from the Uyo village road dumpsite on hematological parameters and liver profiles using male Wistar rats. Twenty male Wistar rats weighing 150g-180g were randomly assigned into four subgroups, each consisting of five rats. The control group received 10ml of distilled water, Group 2 received 10ml/kg body weight of the leached water, Group 3 received 20ml/kg body weight of the leached water, and Group 4 received 10ml/kg body weight of boiled/cooled leached water. The administration was done orally for 21 days. Serum was then obtained through centrifugation of the blood samples and used to determine RBC count, hemoglobin level, WBC count, AST, ALT, and ALP levels. Administration of leached water significantly reduced the levels of red blood cells, hemoglobin, and white blood cells, which implies that leached water harms blood indices. The increased levels of ALT, AST, and ALP in rats exposed to leached water imply that leachate can induce hepatic damage, possibly through necrosis caused by its toxic constituents. In conclusion, water bodies surrounding the Uyo village road dumpsite should be tested and treated before consumption, as contamination by leachate can lead to physiological dysfunctions.

Keywords: Leachate, landfill, hematological parameters, liver profiles

1. INTRODUCTION

Landfills have been identified as one of the significant threats to groundwater resources worldwide (Fatta et al., 1999). Groundwater is a valuable resource used chiefly for industry, commerce, agriculture, and most importantly for drinking. Leachate is defined as aqueous effluent generated because of rainwater percolating through wastes, biochemical processes in waste cells, and the inherent water content of waste itself. This leachate accumulates at the bottom of the landfill percolates through the soil and reaches the groundwater (Mor et al., 2006). The composition of

leachate from a landfill varies greatly based on the garbage type and landfill age. Due to the recent sharp rise in population, research on the effects of landfill leachate on surface and groundwater has become increasingly important (Saarela, 2003).

Heavy metals usually non-biodegradable in nature which constituted parts of landfill leachate were able to diminish the quality of surface and groundwater and were poisonous even at lower levels to a biological system (Verma and Dwivedi, 2013). The bio-accumulative, persistent, and toxic nature of heavy metals as well as their carcinogenic and endocrine-disrupting nature has been reported (Kibria et al., 2016). On the other hand, dissolved organic matter, which makes up a sizable amount of leachate, can bind with heavy metals and so affects those metals' bioavailability in aquatic environments (Rikta et al., 2018). The detrimental impact of leached water on human health is a pressing concern, especially in Akwa Ibom State. Existing studies strongly suggest that residents who consume edible products cultivated in water bodies near landfill sites may be at risk of experiencing severe health complications. Thus, this study assesses the impact of leached water from Uyo village road dumpsites on physiological parameters.

2. MATERIALS AND METHODS

Materials

The following materials were used in the course of this research: Animal cages (wooden cages with wire mesh), cotton wool, syringe, petri dish, spectrophotometer, scissors, conducting meter, pH meter, turbidity meter, hand gloves, spatula, refrigerator, masking tape, glass wares (conical flask, test tubes, beaker, volumetric flask, burette, pipette, measuring cylinders), distilled water, culturing media (Nutrient Agar, Macconkey, Eosin-methyl blue (EMB) agar), refrigerator, ketamine, desiccators, sterile plain sample tubes, sterile syringe (5ml), weighing balance.

Experimental Animal Handling

Twenty (20) male rats of the Wistar strain weighing between 150g-180g were obtained from the animal house, Faculty of Basic Medical Sciences, University of Uyo. The animals were bred in standard wooden cages with iron nettings under room temperature (25±20C), relative humidity (50±5%), and a 12-hour light / dark cycle in the animal house of the Faculty of Basic Medical Sciences. The rats were allowed to acclimatize for one week in the experimental room in the animal house. They were housed in metal cages with wire meshed tops. Saw specks dusts were used as bedding to provide warmth and were changed regularly (at two-day intervals). The animals were fed daily with vital feed (Grower's mesh feed) and water ad libitum. Standard laboratory conditions and services were applied throughout the 21 days of the experiment.

Collection of the test sample

Leached water was obtained from a stream close to Uyo Village Road dumpsite in Uyo Local Government of Akwa Ibom State, Nigeria. The sample was taken to the laboratory for analysis.

Experimental design and administration procedures

Twenty (20) Wistar rats weighing between 150g-180g were randomly divided into four groups, each consisting of five rats. Water and feed (grower's mesh) were available ad libitum to all groups. Group 1 served as the control group and received 10ml of distilled water. Group 2 received 10ml/kg body weight of the leached water and feed. Group 3 received 20ml/kg body weight of the leached water and feed. Group 4 received 10ml/kg body weight of boiled and cooled leached water and feed (Table 1).

Table 1 Treatment and groupings

Group	Treatment	No of rats
1	Control (Distilled water)	5
2	Leached water (10ml/kg b.w)	5
3	Leached water (20ml/kg b.w)	5
4	Leached water boiled and cooled (20ml/kg b.w)	5

Procedure for Leachate Water Analysis

The sample was conveyed in a clean glass bottle to the laboratory for analysis based on its physicochemical properties and the constitution of biological organisms. Examined physicochemical parameters such as pH, Electrical conductivity, Temperature (oC), Total Dissolved Solid (TDS), Total Hardness (TH), Salinity, Nitrate (NO-3) Dissolved Oxygen (DO), Phosphate($P0_4$), Sulphate ($S0_3$), Calcium (Ca), Sodium (Na), Chloride (Cl-), Iron (Fe), copper (Cu), Magnesium, Fluoride, and Lead (Pb) were analyzed at the Akwa Ibom State Water Company Limited (AKWCL). An atomic Absorption spectrophotometer was used to determine the concentration of each heavy metal under specific wavelengths. The presence of biological organisms was analyzed through the following steps:

Preparation of the cultural media

Sterilization of the culture media using the pour plate method

Dilution of the leachate sample with distilled water, where 1ml of the diluted sample and 1ml of the culture media were added into Petri dishes for all three-culture media (for Nutrient, Macconkey, and EMB agar).

The Petri dishes were neatly covered and incubated for 24 hours.

The dishes were viewed under the colonic counter, and the biological organisms found were quantified.

The presence of heavy metals and inorganic macro-components such as copper, lead, nitrate, etc, were analysed using the Spectrophotometer. The pH level, turbidity, and conductivity were obtained using the pH meter, turbidity meter, and conductivity meter respectively. The quantitative analysis (titration) method was also used in the determination of some physicochemical parameters such as alkalinity and acidity.

Collection of blood samples for analysis

After 21 days of administration, the rats were sacrificed. Each animal was anaesthetized using 1.5ml ketamine and blood samples were immediately collected through cardiac puncture. 5ml syringes were used for the collection of the blood samples from each animal and transferred into labeled plain sample bottles. Serum was then obtained through centrifugation of the blood samples and used for the determination of the experimental parameters (hematological parameters and liver enzymes) in the laboratory.

Determination of WBC, RBC count, and Haemoglobin level

WBC, RBC, and hemoglobin levels were determined using an automated hematology analyzer (Mindray Hematology Analyser, BC 2300).

Determination of ALT and AST activity

Determination of Alanine amino transaminase (ALT) and Aspartate transaminase activity was done using Agappe kits based on Reitman and Frankel, (1957) procedures.

Determination of ALP activity

Aspartate alkaline phosphate (ALP) levels were assessed using Agappe kits, based on procedures.

Statistical analysis

All data from the control and experimental groups were subjected to statistical evaluation, using the Windows SPSS package (SPSS 22.0). Data was analyzed using one-way ANOVA for significant differences between the control and experimental groups at $p<0.05$. Data were expressed as mean ± standard error of the mean (mean ± SEM).

Table 2 Results of leachate water on RBC count, WBC count, and liver enzyme (AST, ALT, ALP) concentration

Groups	RBC count	WBC count	Hb	AST	ALT	ALP
I	6.65±0.09	4.76±098	14.20±0.11	141.68±1.83	37.60±1.00	364.78±4.27
II	5.45±0.03a	4.65±0.13	13.08±0.16	141.02±1.83	43.36±1.22a	483.32±4.73a
III	1.41±0.02a,b	5.79±0.14a,b	11.00±0.25a,b	121.36±2.98a	47.67±0.53a,b	712.55±3.66a,b
IV	5.09±0.20a,c	6.00±0.16a,b	13.79±0.26	138.90±1.78a	47.27±0.34a,b	635.26±6.58a,b,c

Legend: a= versus group I, b= versus group II, c= versus group III

3. RESULTS

Effect of Leached water on RBC levels

RBC levels were significantly decreased ($p<0.05$) in Group 2 (10mls of leachate water), Group 3 (20mls of leachate water), and Group 4 (10mls of boiled leachate water) compared to Group 1 which served as the control (10 ml/kg of distilled water). Among the experimental groups, RBC levels were significantly reduced ($p<0.05$) in Group 3 when compared with other groups (Table 2 & Figure 1).

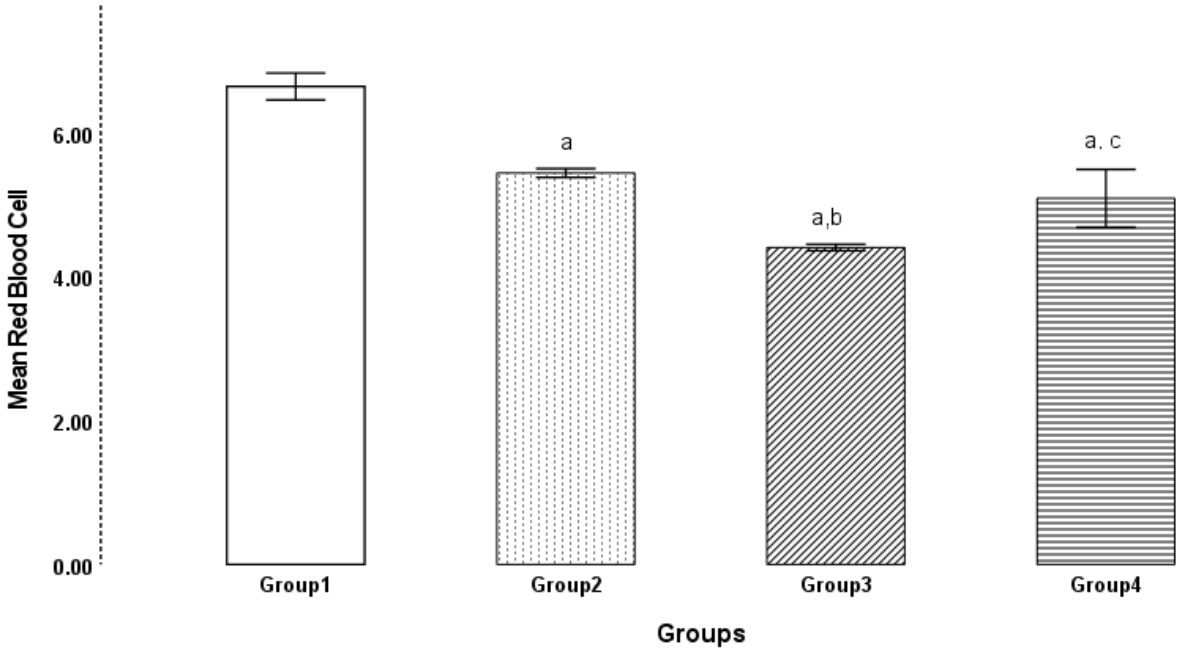

Figure 1 Comparison of RBC Levels in Groups 1, 2, 3, and 4. Values are mean ± SEM. n=5. a = $p < 0.05$ vs Group 1, b = $p < 0.05$ vs Group 2, c = $p < 0.05$ vs Group 3

Effect of Leached water on Hb level

Hemoglobin levels in Group 2, Group 3, and Group 4 were significantly decreased ($p<0.05$) when compared with Group 1 (control). Among the experimental groups, Hb levels significantly decreased ($p<0.05$) in group 3 when compared with group 2, which received 10ml of leachate water. It also increased significantly ($p<0.05$) in group 4 {10mls of boiled leachate water) when compared with group 3 (20mls of leachate water), (Figure 2).

Effect of Leached water on white blood cell

White Blood Cell count significantly increased ($p<0.05$) in Group 3 and Group 4 compared to control Group and Group 2. However, there was no significant difference between the control group and Group 2. Little or no significant difference was also observed in group 4 compared to group 3 (Figure 3).

Effect of Leached water on AST levels

The Aspartate Amino Transaminase levels in Group 2, Group 3, and Group 4 were significantly increased ($p<0.05$) compared to the control (Group 1). This increase was not significant when compared to the treated groups (Figure 4).

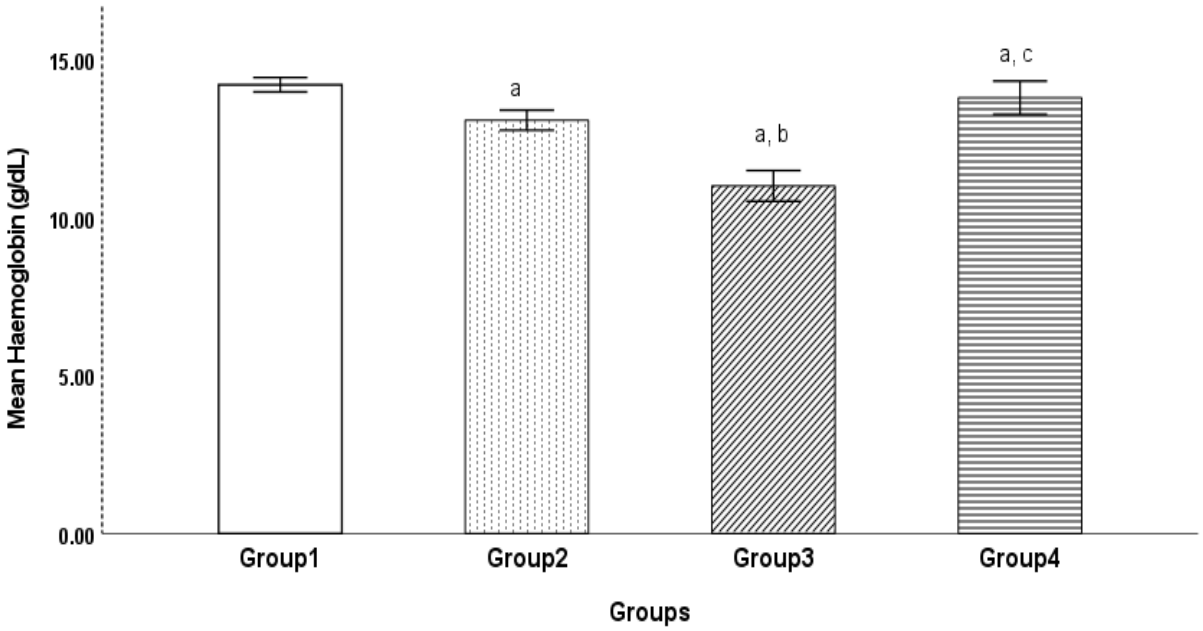

Figure 2 Comparison of Hb Levels in Groups 1, 2, 3, and 4. Values are mean ± SEM. n=5. a = $p < 0.05$ vs Group 1, b = $p < 0.05$ vs Group 2, c = $p < 0.05$ vs Group 3

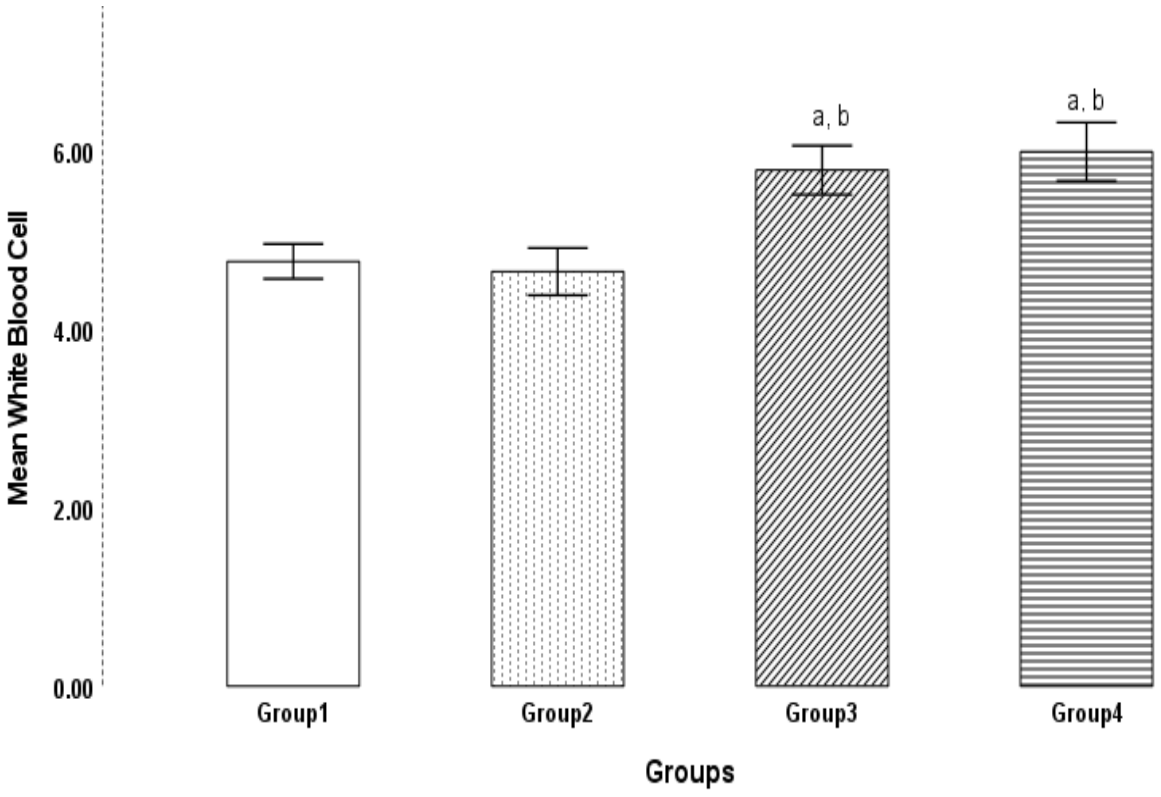

Figure 3 Comparison of WBC Levels in Groups 1, 2, 3, and 4. Values are mean ± SEM. n=5. a = $p < 0.05$ vs Group 1, b = $p < 0.05$ vs Group 2, c = $p < 0.05$ vs Group

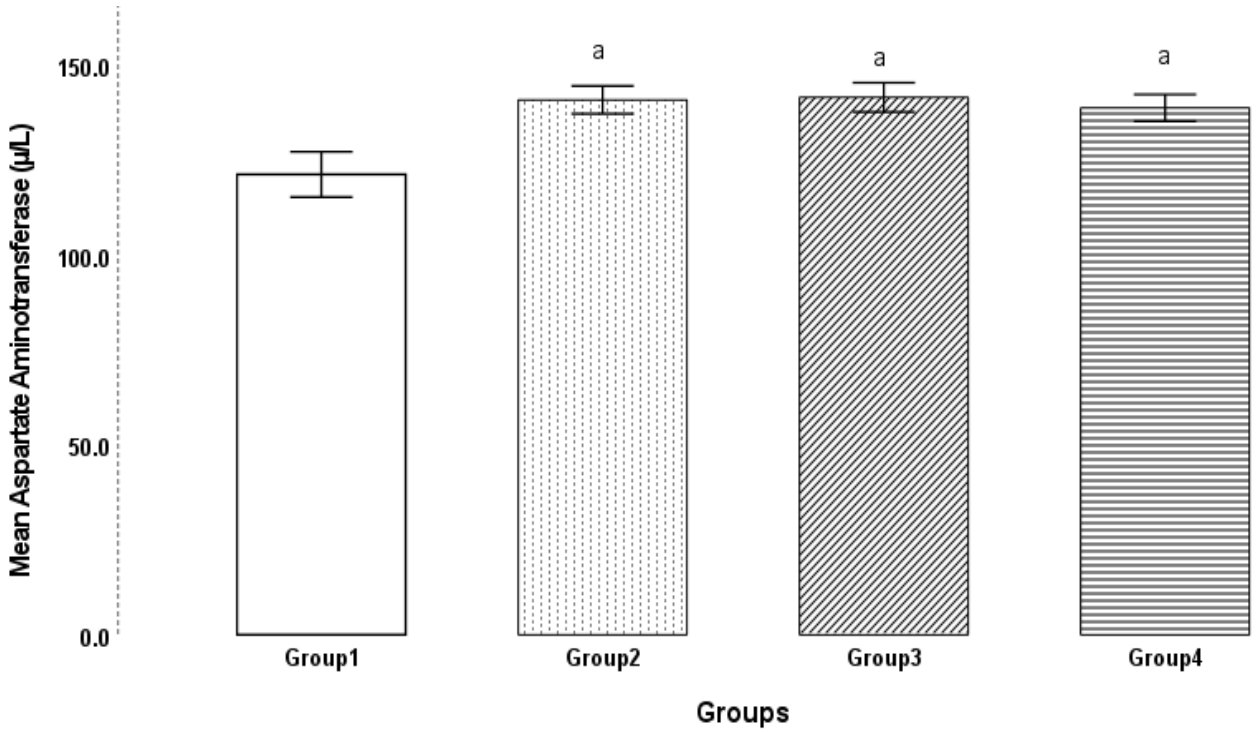

Figure 4 Comparison of AST Levels in Groups 1, 2, 3, and 4. Values are mean ± SEM. n=5. a = $p < 0.05$ vs Group 1, b = $p < 0.05$ vs Group 2, c = $p < 0.05$ vs Group 3

Effect of Leached water on ALT levels

Alanine Amino Transaminase (ALT) levels in Group 2, Group 3, and Group 4 were significantly higher ($p<0.05$) than the control Group. Notably, Groups 3 and 4 exhibited no difference when compared with each other (Figure 5).

Effect of Leached water on ALP levels

Aspartate Alkaline Phosphate levels increased significantly in Group 2, Group 3, and Group 4 when compared to the Control Group. Among the experimental groups, ALP levels were significantly higher ($p<0.05$) in Groups 3 and 4 compared to Group 2 and significantly lower ($p<0.05$) in Group 4 compared to Group 3 (Figure 6).

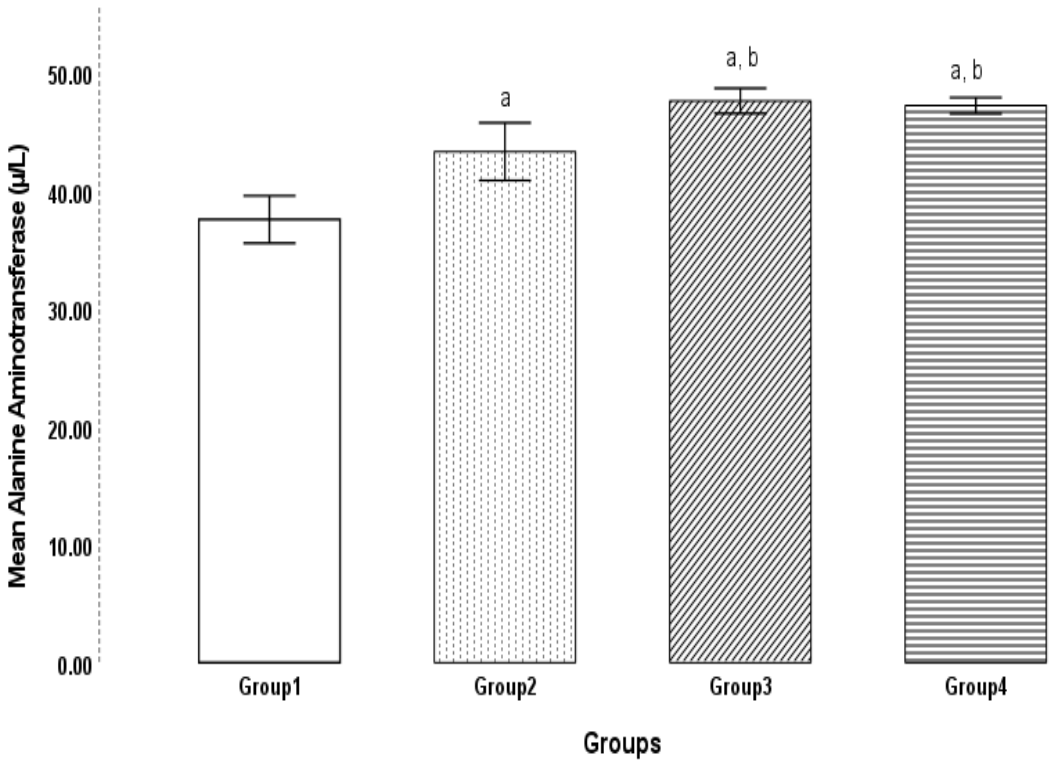

Figure 5 Comparison of ALT Levels in Groups 1, 2, 3, and 4. Values are mean ± SEM. n=5. a = $p < 0.05$ vs Group 1, b = $p < 0.05$ vs Group 2, c = $p < 0.05$ vs Group 3

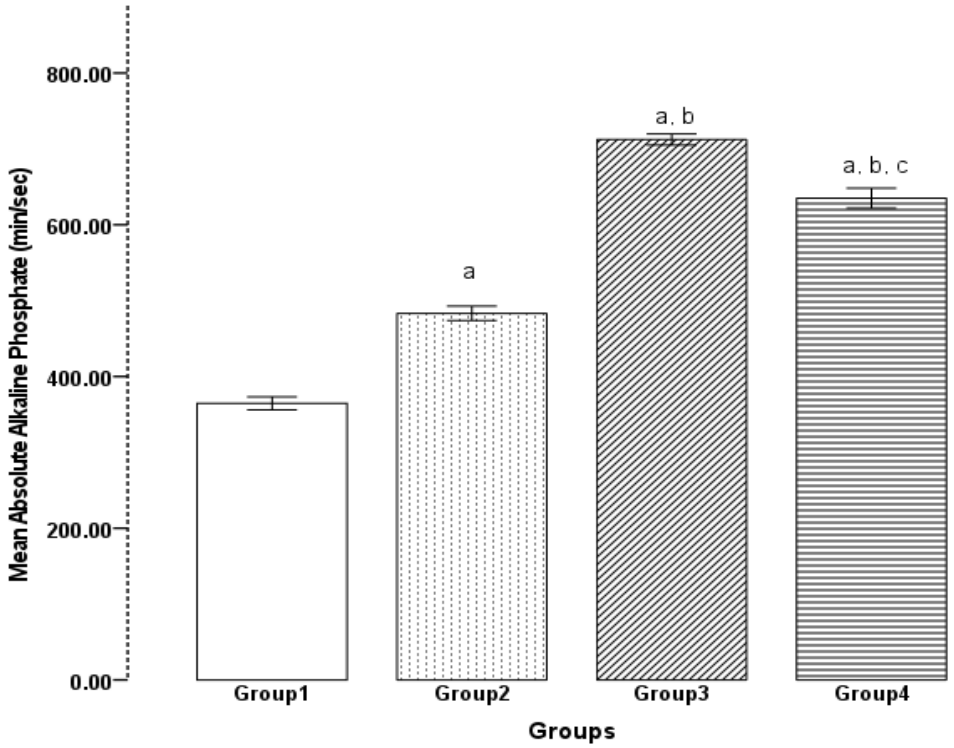

Figure 6 Comparison of ALP Levels in Groups 1, 2, 3, and 4. Values are mean ± SEM. n=5. a = $p < 0.05$ vs Group 1, b = $p < 0.05$ vs Group 2, c = $p < 0.05$ vs Group

4. DISCUSSION

Human exposure to complex combinations of hazardous chemicals is a more common occurrence than exposure to single toxicants (Farombi et al., 2012). The decomposition of solid waste in landfills gives rise to leachate, a dark, noxious liquid with a distinctive foul odor. Studies investigating the toxicity of leachate in mammals are limited but crucial (Bertoldi et al., 2012). The examination of red blood cell counts revealed a notable reduction in the exposed group, with Group 3 displaying the most significant decrease compared to the control group. These findings align with the conclusions drawn by Adeyemi et al., (2006), where the consumption of leachate-contaminated groundwater was implicated in potential health concerns, specifically in the development of anemia. Apart from the toxic elements, the tested leachates may also contain microbes, and opportunistic pathogens. Toxins produced by these bacteria might have played a role in the current observations.

Additionally, it coincides with those of Tewari et al., (2005) and Sang and Li, (2005), wherein municipal solid waste leachate obtained from China and India induced chromosome aberration in mouse bone marrow cells the presence of toxic chemicals in solid wastes leachates may affect blood sensitivity. It has been demonstrated in the present study that leachate ingestion did not only significantly reduce red blood cell levels but also decreased the concentrations of hemoglobin, and this could be a result of heavy metals like Cadmium that can reduce attractive force towards oxygen binding capacity, making erythrocyte more permeable, friable and resulting to damage. The results also collaborate with existing information where continuous exposure to leachate water causes health problems such as sweating, bleeding, stomach disorders, blood disorders, congenital disabilities, and even cancer.

Among the experimental groups, Specifically, Group 3 showed increased white blood cell (WBC) count. These observations suggest potential variations in immune response and blood cell composition among the experimental groups. White blood cells (WBCs) play a crucial role in the immune system, and an elevated WBC count can indicate conditions such as hypersplenism, inflammation, trauma, and stress. A significant increase was observed in AST, ALT, and ALP levels of groups 2, group 3, and group 4 when compared with the control. This finding aligns with the results of a study by Kobayashi et al., (2020), elevated ALT and AST activity in the serum or plasma indicates hepatocellular damage induced by drugs or diseases.

Additionally, findings from Bertoldi et al., (2012) suggested that landfill leachate can instigate oxidative stress in the liver and striatum of rodents. Therefore, the heightened levels of ALT and ALP in rats exposed to leachate imply the potential of leachate to induce hepatic damage, possibly through necrosis caused by its toxic constituents. This result is in line with previous studies by Alimba et al., (2012), which found that rats exposed to the leachates from municipal landfills in Olusosun and Aba-Eku showed increased serum ALT and AST activity.

5. CONCLUSION

Based on the findings of this study it can be inferred that changes in red blood cell counts, hemoglobin levels, and white blood cell counts suggest hematological effects, possibly linked to leachate exposure. Elevated levels of ALT and ALP in groups exposed to leachate suggest potential liver damage, as these enzymes are indicators of hepatic function.

Authors Contributions

Olatunbosun TH: Study design and protocol
Peter IU: Laboratory work
Agogo MK: Development of manuscript for publication
Akpan UP: Analysis of raw statistical data

Ethical approval

Approval was obtained for all experimental procedures from the medical ethics committee of the Faculty of Basic Medical Sciences Ethical Committee of the University of Uyo with the code: UU_FBMSREC_2024_007. The Animal ethical guidelines are followed in the study for experimentation.

Informed consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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