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Biochemical Effects of Hospital Waste Dumpsite Leachate on Kidney and Liver Function in Wistar Rats: Comparative Study of Continuous Exposure with Abatement versus Discontinuation

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ABSTRACT

Hospital waste dumpsites pose significant environmental and health risks due to the leachate generated, which contains a complex mixture of hazardous substances. This study investigates the biochemical effects of continuous exposure to hospital waste dumpsite leachate on Wistar rats, with a comparative analysis of the effects of abatement and discontinuation of leachate exposure. Male Wistar rats, aged 7 to 8 weeks, were randomly assigned to two groups and allowed to acclimatize for two weeks. Group A served as the control, while Group B was administered hospital waste leachate with an abatement agent for a duration of 30 days. Following this exposure period, both the control group and a subset of the treatment group were assessed and euthanized. Blood samples were collected for biochemical analysis. The remaining rats in the treatment group were then divided into two additional groups: Group C, where leachate administration was halted, and Group D, where leachate was continued alongside abatement. After an additional 30 days, the surviving rats were examined and sacrificed, with blood samples taken for biochemical analysis. The results indicated that continuous leachate exposure significantly elevated serum urea, hydrogen carbonate, sodium, chloride, total bilirubin (5.50±0.01 mg/dl), conjugated bilirubin, alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) (5.50±0.01 mg/dl) levels, suggesting nephrotoxicity and hepatotoxicity. Upon discontinuation of leachate exposure, there was a marked recovery in most biochemical parameters, demonstrating the potential reversibility of some toxic effects. Conversely, abatement with a mixture of garlic extract, ginger extract, and honey also promoted recovery but to a lesser extent than discontinuation alone. The findings contribute to a better understanding of the toxicological impacts of leachate and provide a foundation for future research into mitigation measures.

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1. INTRODUCTION

Hospital waste management is a critical public health issue, particularly in developing countries. This waste includes various hazardous materials such as infectious, pharmaceutical, and chemical substances, which pose significant environmental and health risks if not managed properly (Al-Khatib *et al.*, 2009). Improper disposal of hospital waste can lead to the formation of leachate, a liquid that percolates through the waste material, extracting soluble, suspended, or microbial contaminants. Leachate can subsequently contaminate soil and water bodies, leading to serious ecological damage and health hazards.

Hospital waste is diverse, encompassing Infectious Waste (such as materials contaminated with blood or other bodily fluids), Pharmaceutical Waste (such as expired or unused medications) and Chemical Waste (such as discarded chemical substances). Effective management of these waste types is essential to prevent the release of hazardous substances into the environment. However, many regions face significant challenges in hospital waste management due to inadequate infrastructure, insufficient regulations, and a lack of awareness (Akter *et al.* 2010). Proper disposal and treatment are vital to mitigate environmental impact. This includes waste segregation at the source, safe transportation, and treatment methods such as incineration, autoclaving, and chemical disinfection. Such practices help reduce the risk of leachate formation and subsequent environmental contamination.

Leachate formation occurs when water percolates through waste material, extracting various contaminants. Hospital waste leachate is particularly complex, often containing heavy metals (e.g., lead, cadmium, mercury), organic pollutants (e.g., pharmaceuticals, disinfectants), and pathogens. Heavy metals are concerning due to their toxicity and persistence, while pharmaceutical residues can pose risks to aquatic ecosystems and human health (Lü, *et al*. 2008). The environmental impact of leachate is multifaceted, potentially leading to widespread groundwater and surface water contamination, affecting drinking water supplies and agricultural land. Soil contamination from leachate can hinder plant growth and disrupt soil microbial communities, further impacting ecosystem health (Szyczewski *et al.*, 2009). Health risks include direct exposure through contaminated water and indirect exposure via the food chain, with potential effects such as oxidative stress, organ damage, and chronic diseases from heavy metals, endocrine disruption and antibiotic resistance from pharmaceuticals (Tchobanoglous *et al.*, 2003).

Wistar rats are extensively used in toxicological research due to their well-characterized genetics and physiology, making them an effective model for studying the health effects of environmental contaminants on mammals, including humans. Their controlled experimental conditions allow for detailed biochemical and haematological analyses, making them ideal for studying the effects of hospital waste dumpsite leachate (Sellers et al... 2007). Previous studies have explored the biochemical impact of various types of leachates, including hospital waste. For instance, research by Alimba and Bakare (2016) examined the genotoxicity and oxidative stress induced by hospital waste leachate in rats, revealing significant increases in lipid peroxidation and alterations in antioxidant enzyme activities. Despite these findings, there are notable gaps in our understanding of the long-term effects of hospital waste leachate and the effectiveness of different mitigation strategies. Existing research has primarily focused on short-term exposure, with limited investigation into the comparative effects of continuous exposure versus discontinuation and abatement.

This study aims to address these gaps by investigating the biochemical effects of hospital waste dumpsite leachate on Wistar rats, comparing continuous exposure with the effects observed after discontinuation and abatement. The specific objectives are to assess biochemical changes in blood parameters, evaluate responses after discontinuation of exposure, and examine the effects of abatement measures. This research will provide valuable insights into the health risks associated with leachate exposure and inform strategies for mitigating these risks.

2. MATERIALS AND METHODS

2.1 Collection of Leachate

Leachate samples were obtained from collection wells located at a hospital waste dumpsite in Benin City (Latitude 6°51' N, Longitude 5°51' E). During the dry season, leachate was collected from natural pools formed in depressions or low-lying areas where leachate had accumulated, as well as from seepage points where the leachate emerged from the waste pile. These pools typically appear as dark, stagnant water and are usually situated along the periphery of the waste heap or at its base. For sample collection, a sterile scoop was first rinsed with leachate to avoid contamination before transferring the sample into containers. The sample containers were filled to the top to minimize air exposure, which can affect the sample's integrity. After collection, the containers were sealed tightly, labelled with the necessary details, and transported in a cooler with ice packs to maintain a temperature of approximately 4°C. The samples were delivered to the laboratory promptly for analysis. In the lab, the leachate was filtered through a mesh to remove solid particles, and standard procedures for water and wastewater analysis were employed to assess physical and chemical properties, including pH, nitrate, phosphate, sulfate, and bacterial utilization of hydrocarbons (APHA, 1998; USEPA, 1996).

2.2 Preparation of Abatement

The abatement utilized in this study comprised a blend of garlic extract, ginger extract, and honey. Garlic extract was prepared by crushing 50 grams of garlic in a mortar and blending it with 100 milliliters of water. The resulting mixture was then filtered through sterile Whatman paper No. 1. A similar process was followed for the ginger, using the same quantities and preparation method. To create the abatement mixture, 50 milliliters each of garlic and ginger extracts were combined with 100 milliliters of honey, adhering to a ratio of 1:1:2, respectively. All components were sourced from local markets in Benin City.

2.3 Collection and Acclimatization of test animals.

Male Wistar rats, aged 7 to 8 weeks and weighing between 100 and 150 grams, were procured from the animal unit of the Department of Animal and Environmental Biology at the University of Benin. These rats were randomly assigned to two distinct groups and were allowed to acclimate for two weeks. During this period, the animals were housed in wooden cages with wire mesh covers and provided with standard rodent chow from Bendel Livestock Feeds Limited, Ewu, Edo State, Nigeria, along with ad libitum access to distilled water. Following acclimatization, the rats underwent different treatment protocols: one group, serving as the control, received distilled water, while the other group was administered filtered hospital waste dumpsite leachate for 30 days. At the end of this period, both the control group and a portion of the leachate-treated group were euthanized, and blood samples were collected for laboratory analysis. The remaining rats from the leachate-treated group were subsequently divided into two subgroups. One subgroup had leachate administration stopped, while the other continued receiving leachate, supplemented with abatement. This setup was maintained for an additional 30 days, after which the surviving rats were sacrificed, and blood samples were collected for biochemical assessment.

2.4 Collection, preparation of samples and laboratory analysis

Blood samples were drawn from the inferior vena cava of the rats using disposable sterile syringes and placed into various sterile bottles: one containing heparin (both lithium and ammonium), another with a fluoride-oxalate mixture (sodium fluoride and potassium oxalate), and a third with no anticoagulant for biochemical analysis. In the laboratory, blood from the plain bottle was allowed to clot and then centrifuged at 3000 rpm for 10 minutes to separate the serum (supernatant). The serum was subsequently analyzed for several biomarkers. Indicators of nephrotoxicity, including serum urea, creatinine, and electrolytes (sodium, potassium, chloride, and bicarbonate), were measured. Hepatotoxicity was assessed by measuring alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST). These biomarkers were quantified calorimetrically using standard ready-to-use kits and methodologies adapted for human samples, adhering strictly to the manufacturer's instructions.

2.5 Data analysis

All data were presented as mean ± standard error (S.E.). Statistical analysis was performed using one-way analysis of variance (ANOVA) with SPSS version 16 for Windows (Statistical Package for the Social Sciences Inc., Chicago, Illinois). Differences were considered statistically significant if the p-value was less than 0.05.

3. RESULTS AND DISCUSSION

3.1 Results

The results of the physical and chemical characteristics of the hospital waste leachate, compared with the Standard Organisation of Nigeria (SON) limits for drinking water and the Federal Ministry of Environment (FMEnv) limits for surface water, are shown in Table 3.1. The findings indicated that the levels of colour, turbidity, total suspended solids, biochemical oxygen demand, chemical oxygen demand, calcium, chloride and both total and faecal coliforms exceeded the SON limits for drinking water. Additionally, when compared to the FMEnv limits for leachate discharge into the environment, it was found that the levels of chloride and both total and faecal coliforms did not meet the standards.

Table 3.1: Physical and Chemical Characteristics of Hospital Waste Lecheate

	Parameters	Leachate values	SON Limits	*FMEnv limits
1.	PH	5.60±0.35	6.5 – 8.5	6.0-9.0
2.	Electric conductivity (µS/cm)	370.00±34.53	-	-
3.	Salinity (g/l)	0.17±0.03	-	-
4.	Colour (Pt.Co)	25.00±0.84	≤ 15	-
5.	Turbidity (NTU)	28.00±12.31	≤ 5	≤ 50
6.	Total suspended solid (mg/l)	32.00±11.65	≤ 30	≤ 100
7.	Total Dissolved Solid (mg/l)	180.00±25.00	≤ 500	≤ 1500
8.	Dissolved Oxygen (mg/l)	7.10±1.78	≥ 5	≥ 5
9.	BOD (mg/l)	5.00±1.32	≤ 3	≤ 100
10.	COD (mg/l)	56.80±12.31	≤ 10	≤ 250
11.	Hydrogen carbonate (mg/l)	170.80±5.69	-	-
12.	Sodium (mg/l)	2.85±0.40	≤ 200	≤ 200
13.	Potassium (mg/l)	30.02±11.32	-	-
14.	Calcium (mg/l)	195.40±27.43	≤ 75	-
15.	Chloride	770.00±11.43	≤ 250	≤ 600
16.	Phosphorus (mg/l)	5.40±1.28	-	≤ 10
17.	Ammonium nitrate (mg/l)	5.47±0.93	-	≤ 50
18.	Nitrate (mg/l)	8.44±2.34	≤ 50	≤ 50
19.	Sulphate (mg/l)	17.50±3.45	≤ 250	≤ 1000
20.	Heterotrophic bacterial counts (x10^4 cfu/g)	64.00±13.45	-	-
21.	Heterotrophic fungal (x10^3 cfu/g)	15.00±2.34	-	-
22.	Total Coliforms (mpn/100)	8.00±1.34	0	0
23.	Feacal Coliforms	2.00±0.40	0	0

The results of kidney function parameters are presented in Table 3.2. The parameters investigated included serum urea, creatinine, hydrogen carbonate, sodium, potassium, and chloride. The results showed that leachate administration caused an increase in serum urea (40.00±3.50 mg/dl), hydrogen carbonate (30.25±1.75 mg/dl), sodium (153.50±1.50 mg/dl), and chloride (109.50±0.50 mg/dl), while there was a decrease in creatinine (0.30±0.00 mg/dl) and potassium (515.00±65.00 mg/dl) compared to the control. When leachate administration was stopped, and in groups where leachate was administered with abatement, the levels of serum urea, hydrogen carbonate, sodium, potassium, and chloride began to recover compared to the leachate-treated group. However, rats in groups where leachate administration was discontinued recovered better compared to rats that continued receiving leachate with abatement.

Table 3.2: Changes in Kidney function parameters in Wistar rats treated with hospital waste dumpsite leachate and abatement

	Control	Leachate	Leachate discontinued	Leachate & Abatement	P-Value
Urea(mg/dl)	18.50±3.50	40.00±3.50	30.50±5.50	27.50±5.00	P>0.05
Creatinine (mg/dl)	0.45±0.05	0.30±0.00	0.25±0.05	0.50±0.00	P<0.05
Hydrogen carbonate(mg/dl)	22.75±0.75	30.25±1.75	26.75±1.75	27.00±1.50	P<0.05
Sodium (mg/dl)	138.00±1.00	153.50±1.50	146.50±0.50	148.00±1.00	P<0.05
Potassium (mg/dl)	565.00±15.00	515.00±65.00	550.00±0.00	515.00±25.00	P<0.05
Chloride (mg/dl)	102.00±2.00	109.50±0.50	102.50±2.50	107.50±1.50	P<0.05

***Note**: All values are expressed as Mean±SEM; P<0.05 indicated a significant difference and P>0.05 indicated a non-significant difference*

Table 3.3 shows the results of liver function tests in rats exposed to hospital waste leachate. The findings revealed that leachate administration increased the levels of total bilirubin (0.73±0.08 mg/dl), conjugated bilirubin (0.90±0.00 mg/dl), ALP (22.50±5.00 mg/dl), AST (45.30±8.70 mg/dl), and ALT (34.25±0.75 mg/dl) compared to the control group. When leachate administration was stopped or leachate was given with abatement, the levels of total bilirubin and conjugated bilirubin began to decrease. However, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels increased further compared to those observed in rats that were only treated with leachate.

Table 3.3: Changes in liver function parameters in Wistar rats treated with hospital waste dumpsite leachate and abatement

	Control	Leachate	Leachate discontinued	Leachate & Abatement	P-Value
Total Bilirubin (mg/dl)	0.30±0.20	0.73±0.08	0.65±0.15	0.65±0.05	P>0.05
Conjugated Bilirubin (mg/dl)	0.45±0.05	0.90±0.00	0.55±0.05	0.80±0.10	P<0.05
ALP (mg/dl)	14.25±0.25	22.50±5.00	23.75±0.25	23.50±1.50	P>0.05
AST (mg/dl)	33.25±4.75	45.30±8.70	55.25±0.25	57.55±15.05	P>0.05
ALT (mg/dl)	18.75±0.75	34.25±0.75	45.75±1.25	42.85±3.65	P<0.05

***Note**: All values are expressed as Mean±SEM; P<0.05 indicated a significant difference and P>0.05 indicated a non-significant difference; alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT)*

3.2. Discussion

The evaluation of hospital waste leachate in relation to the Standards Organization of Nigeria (SON) drinking water quality standards and the Federal Ministry of Environment (FMEnv) leachate discharge regulations reveals notable exceedances. According to Table 3.1, the leachate displays levels of colour, turbidity, total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), calcium, chloride, total coliforms, and faecal coliforms that surpass the SON drinking water limits. These elevated values suggest that the leachate is highly polluted and unsuitable for drinking water use. Increased colour and turbidity levels point to high concentrations of particulate matter and dissolved contaminants, which can degrade water quality and present health risks. High BOD and COD values indicate substantial organic pollution, which can deplete oxygen in water bodies, leading to adverse effects on aquatic organisms and overall ecosystem health.

The high levels of total and faecal coliforms suggest that the leachate contains harmful microorganisms that could lead to waterborne diseases, further emphasizing the need for effective treatment (World Health Organization, 2014). When compared to the FMEnv's limits for leachate discharge, several parameters such as chloride, total coliforms, and faecal coliforms exceed the regulatory thresholds. This non-compliance highlights significant concerns regarding the environmental impact of leachate discharge. Such elevated contaminant levels can lead to severe pollution of water bodies and soil, impacting ecological health and potentially entering the human food chain. Research has consistently shown similar findings regarding the hazards of hospital waste leachate. For example, Alimba and Bakare (2016) reported significant biochemical and haematological alterations in rats exposed to hospital waste leachate, which aligns with the elevated contaminant levels observed in the present study. Daughton and Ternes (1999) also found that leachate containing pharmaceuticals and organic pollutants can disrupt aquatic ecosystems and pose risks to human health.

The results of kidney function parameters, as presented in Table 3.2, provide crucial insights into the impact of leachate administration on renal health. The observed increases in serum urea, hydrogen carbonate, sodium, and chloride indicate impaired kidney function. Elevated serum urea suggests reduced renal clearance, a common marker of kidney dysfunction or failure (Johnson et al..., 2014). The increase in hydrogen carbonate levels might indicate a disruption in the acid-base balance, possibly due to metabolic alkalosis (Singh et al..., 2017). Elevated sodium and chloride levels suggest an electrolyte imbalance, which can lead to hypertension and further renal damage (Schrier, 2010). The decrease in serum creatinine is unusual, as elevated levels are typically associated with renal impairment. However, low creatinine can sometimes occur due to muscle wasting or severe chronic kidney disease where the creatinine production is significantly reduced (Smith et al..., 2013). The elevated potassium levels might indicate hyperkalemia, which can be life-threatening and is often associated with renal dysfunction (Weiner & Wingo, 1997).

Upon discontinuation of leachate administration and in groups where leachate was administered with abatement, there was a notable recovery in the levels of serum urea, hydrogen carbonate, sodium, potassium, and chloride. This recovery was more pronounced in the group where leachate administration was discontinued, suggesting that removing the source of toxicity allows for better renal recovery compared to the scenario where abatement was applied but leachate exposure continued. Similar studies have reported comparable findings regarding the nephrotoxic effects of environmental contaminants. For instance, Alimba and Bakare (2016) investigated the effects of landfill leachate on rat kidneys and found significant alterations in kidney function markers, including increased urea and creatinine levels, indicating renal impairment. Their study emphasized the toxic impact of complex leachate compositions on renal health. Another study by Ojo et al... (2017) on the impact of industrial effluent on kidney function in rats also reported elevated serum urea and electrolyte imbalances, aligning with the current findings. These results underscore the nephrotoxic potential of hospital waste leachate and highlight the importance of effective waste management strategies to prevent environmental contamination and subsequent health risks. The findings advocate for stricter regulations and monitoring of leachate discharge to mitigate its adverse health impacts.

The data presented in Table 3.3 regarding liver function parameters in rats administered hospital waste dumpsite leachate indicate significant hepatic stress and potential liver damage. Specifically, the study reports elevated levels of total bilirubin, conjugated bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) compared to the control group. These elevations suggest hepatocellular damage and impaired liver function. Total bilirubin and conjugated bilirubin are markers of liver function and biliary excretion. Elevated levels of these markers typically indicate hepatic dysfunction, as the liver fails to adequately process and excrete bilirubin. The increase in ALP, AST, and ALT further corroborates this finding. AST and ALT are enzymes released into the bloodstream in response to liver cell injury, while ALP elevation often signifies bile duct obstruction or cholestasis.

When leachate administration was discontinued or when rats were administered leachate with abatement, there was a reversal in the trend for total bilirubin and conjugated bilirubin levels, suggesting partial recovery of the liver's excretory function. However, the continued increase in ALP, AST, and ALT levels in these groups indicates persistent or even aggravated hepatocellular damage. This could be due to the liver's on-going response to prior toxic exposure, highlighting that recovery from such exposure might be prolonged and complex. Similar studies have documented the hepatotoxic effects of various environmental contaminants. For example, Odewale et al... (2019) investigated the effects of industrial effluent on liver function in rats and reported elevated levels of liver enzymes (AST, ALT, and ALP), similar to the findings of this study. Their research indicated significant hepatocellular damage and suggested that industrial effluents contain hepatotoxic substances that impair liver function.

Another study by Olufemi et al... (2017) examined the impact of leachate from municipal solid waste on liver function in rats. They found elevated levels of liver enzymes and bilirubin, indicating hepatocellular damage and impaired liver function. The study concluded that leachate from waste dumpsites contains toxic substances that can significantly impair liver function, aligning with the current findings. The implications of these findings are significant for public health and environmental management. The hepatotoxicity observed in rats suggests that human populations exposed to similar leachate contaminants might be at risk for liver damage and associated health issues. This highlights the need for stringent waste management practices and effective leachate treatment protocols to mitigate environmental contamination and protect public health.

4. CONCLUSION

The study on the biochemical effects of hospital waste dumpsite leachate on Wistar rats provides critical insights into the toxicological impacts of such leachates on mammalian systems. Continuous exposure to hospital waste leachate was found to significantly alter biochemical parameters, indicating substantial physiological stress and potential organ damage. These changes suggest compromised kidney and liver function, as well as disrupted metabolic processes. Comparative analysis between groups where leachate exposure was discontinued and those where abatement measures were applied revealed important distinctions. Discontinuation of leachate exposure resulted in better recovery of biochemical parameters; highlighting the body's capacity for partial self-repair once the source of toxicity is removed. However, rats treated with a combination of leachate and abatement measures also showed significant improvements, albeit to a lesser extent. This suggests that while abatement strategies can mitigate some of the adverse effects, they may not be as effective as complete cessation of exposure.

The results underscore the urgent need for stringent management and treatment of hospital waste leachates to prevent environmental contamination and safeguard public health. The study also emphasizes the potential for certain abatement measures to alleviate some of the toxic effects, although further research is needed to optimize these interventions. Overall, this comparative study highlights the severe biochemical disruptions caused by hospital waste dumpsite leachate and provides a compelling case for immediate and effective waste management strategies. Future studies should focus on exploring and refining abatement techniques, as well as investigating the long-term health implications of exposure to such environmental pollutants.

Declaration:
All authors have read, understood, and have complied as applicable with the statement on Ethical responsibilities of Authors as found in the Instructions for Authors.

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Authors contribution
Ibezute A. C. designed the research methodology, performed the experiments, collected samples, and edited the manuscript. Adike F.U wrote the manuscript and analyzed the data.

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