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Impact Of Ikhueniro Dumpsite Leachate On Blood Profile And Blood Film Of Wistar Rats

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ABSTRACT: The challenge of environmental toxicity and the associated unrelenting efforts of man to abate or eradicate its hazards are lifelong efforts. The present study investigated the impact of Ikhueniro dumpsite leachate in Benin City, Nigeria on the hematological blood profile and blood film of Wistar rats. A total of 30 Wistar rats were acclimatized for two weeks and randomly distributed into three groups A to C; group A served as control, while groups B and C were orally administered Leachate only and Leachate + Abatement (*Dialium guineenses* aqueous leaf extract), respectively, once every 48 hours for 30 days. After the exposure period, the surviving rats in group A and half of the rats in group B and C were examined and sacrificed. The experiment continued with the remaining rats in groups B and C. Group B was given only clean water and C only abatement following ealier stated schedule, after which the surviving rats were sacrificed and samples collected. Hematological analysis showed that leachate administration in Wistar rats caused an increase in white blood cells (27.65%), platelet count (13.35%), and platelet crit (45.45%); with a decrease in red blood cell count (7.52%), hemoglobin (5.40%) and hematocrit (6.12%) of Wistar rats when compared to the control. Geimsa-stained blood film revealed the presence of polymorphs and basophils in the blood film of rats administered dumpsite leachate. Nevertheless, when leachate administration was stopped in the group of rats where it was previously administered, there was a slight improvement in some blood hematological indices. However, the administration of *Dialiumguineense* leaf extracts better-improved blood hematological indices. The findings of this study indicate that Ikhueniro dumpsite leachate negatively impacted blood hematological indices in Wistar rats. Dumpsite leachate should be handled and treated carefully to avoid potential health hazards in animals.

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The challenge of environmental toxicity and the associated unrelenting efforts of man to abate or eradicate its hazards are lifelong health and environmental issues that must be addressed decade after decade (Aganbi *et al*., 2015). Different chemical substances prevalent in the air, land, and water bodies have been implicated in ecotoxicology (Ghosh *et al*., 2017). Effluents from gas flaring, untreated sewage and leachate are typical examples of environmental toxicants (Alimba *et al*., 2012). Landfill leachate is a complex mixture of chemicals capable of posing toxicity risk to aquatic wildlife. Several researchers have assessed leachate toxicity using bacteria, algae, plants, invertebrates, fish, and genotoxicity (Thomas *et al*., 2009; Sisinno *et al*., 2000). In some of such studies, life forms have been reported to have suffered from a wide range of toxicities due to leachate assimilation or contamination. Several chemical compounds present in most leachates have been reported to be the cause of adverse responses from the

test organisms; these include, but are not limited to, ammonia, heavy metals, alkalinity, and recalcitrant organics and their concentrations in the leachates investigated in such studies have been found to depend on the types of waste landfilled (Alimba et al., 2012). Due to the plenitude of chemical constituents in land leachates, several mechanisms are believed to be involved in its toxicity to living organisms such as the generation of free radicals leading to cellular oxidative damage, inflammation, and alterations in hematopoietic protein[2]. Many species of plants and fungi have been used in recent years for the amelioration of leachate-induced toxicity as well as other forms of cellular toxicities in several studies (Ghosh et al., 2017; Singh et al., 2017; Kumar et al., 2018). It is a known fact that various parts of *Dialium guineense* (velvet tamarind), a medicinal plant that belongs to the family of Leguminosae, are used traditionally in the treatment of a variety of diseases (Onoagbe and Abu, 2017). Hence, in this study, an attempt was made to investigate the impact of Ikhueniro dumpsite leachate on the hematological blood profile and blood film of Wistar rats and its possible abatement with aqueous leaf extract of *D.guineense.*

MATERIAL AND METHODS

Collection of Dumpsite Leachate: Raw leachate was collected from five different leachate collection points around the dumpsite and thoroughly mixed to provide a homogenous representative sample for each sampling site. This was transferred to the laboratory in pre-cleaned 4-liter plastic containers, filtered with a muslin cloth to remove suspended particles, and stored at 4^{o}C until use. This was considered as the stock sample (100%) and was labeled Ikhueniro dumpsite leachate in Benin City, Nigeria. The sample was analyzed for several standard physical and chemical properties according to procedures outlined in the Standard Methods for the Examination of Water and Wastewater (APHA, 1998; USEPA, 1996).

Collection, Identification, and Classification of plants: Fresh leaves of *Dialium guineenses* (black velvet) were collected from Upper Sakponba, Benin city; the taxonomic identity of the plant was confirmed at the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin City, Edo State, Nigeria. Phytochemical analysis (such as carbohydrate, alkaloids, tannin, saponin, cardiac glycoside, anthraquinone, phlorotannins, steroids, and flavonoids) of the crude powder of the leaves collected was carried out according to the standard procedures to identify the constituents as described by Sofowora (1993), Trease and Evans (1989), Kokate *et al*. (2008) and Harborne (1998).

Preparation of Abatements: The abatement was the aqueous leaf extract of black velvet tree (*Dialiumguineenses*). The leaves collected were air-dried to crispiness in the laboratory (prevailing room temperature of 30 ± 2°C) for two weeks. The dried materials were reduced to coarse form using a pestle and mortar and further pulverized to very fine particles using Viking Exclusive Joncod pulverizing machine (Model: YLH2M2 - 4). 28g of the powdered leaves were subjected to infusion extraction and exhaustively extracted with 0.5 L of warm water for four hours. The extracts were filtered and stored at a temperature of about 4°C in a clean container before use.

Collection and Acclimatization of Experimental Rats: Thirty (30) male and female Wistar rats (6-7 weeks old) weighing within the range of 100 – 150 g were obtained from the Anatomy Department, University of Benin, Nigeria; and housed in wooden cages with wire mesh covers. The rats were acclimatized for 2 weeks until they were 8-9 weeks and their weights were taken. The animals were fed with standard rodent chow (Bendel Livestock Feeds Limited, Ewu, Edo State, Nigeria) and given distilled water *ad libitum.*

Experimental Setup: The rats were distributed randomly into three groups of ten animals (5 males and 5 females) each. The rats were administered different treatment protocols as stated below.
Group A – Control (C)
Group B - Leachate (L)
Group C - Leachate + Abatement A (LA)
This was allowed for 30 days. Half of the rats were sacrificed while the remaining rats in groups B and C were given the protocol below for another 30 days.
Group B_N – Clean water with no leachate (NL)
Group C_N – Abatement A with no leachate (NLA)

The rats were maintained in laboratory conditions; and had access to drinking water and standard rodent chow (Bendel Livestock Feeds, Ewu, Edo State, Nigeria®) *ad libitum.* Each animal in a group was gavage 2ml of the different protocol as described above for 30 consecutive days (once every 48 hours). At the end of the exposure period, survivors were fasted overnight, weighed (using Acculab® USA, Model-vic-303 electronic analytical weighing balance), and sacrificed under slight Anesthesia. Blood and liver samples were collected.

Collection and Preparation of Samples: Blood was collected from the inferior vena cava of the rats with a plain 5ml sterilized syringe into a vial containing 0.5

ml EDTA for hematological analysis under light anesthesia. The blood in the bottle containing anticoagulants was immediately transferred to the laboratory for analysis.

Laboratory Analysis: Hematological analysis was carried out using Sysmx KX-21N automated machine (Sysmx corporation Kobe, Japan) following the manufacturer's instructions. Briefly, the sample was mixed and placed in contact with the sample probe for aspiration when the buzzer sounds twice "beep, beep" and when the LCD screen displays ANALYZING, the sample was removed. Following this, the unit executed automatic analysis, and the result was displayed on the LCD screen and printed out. A drop of blood previously stored in an EDTA bottle was placed on a slide. The blood was spread using the coverslip and left to dry at room temperature. The dried film was stained using Geimsa stain and left for 30 minutes. The film was rinsed with water and dehydrated using ascending grades of alcohol(starting from 70%, 90%, 96%, and absolute); and cleared in xylene for 5 minutes. The section was mounted using shandom's mount (Distrene Dibutyl Phthalate xylene), covered with a coverslip, and allowed to dry. The slides were examined using a Leica CME light microscope (Model – 1349522X).

Data Analysis: All statistical analyses were conducted with Statistical Package for Social Scientists (SPSS) and Microsoft Excel computer software. Data were expressed as mean ± standard error (SE). One-way Analysis of Variance (ANOVA) was used to determine the differences among various groups and observed differences in mean were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The result of the hematological analysis is shown in Tables 1 and 2. Leachate administration caused an increase in white blood cells ($16.25\pm1.89 \times 10^9$ cells/L), platelet count ($422.75\pm97.32 \times 10^9$ cells/L), and platelet crit (0.32±0.08%); with a decrease in red blood cell count ($6.27\pm0.70 \times 10^{12}$ cells/L), hemoglobin (16.13±1.56 g/dL) and hematocrit (45.93±3.05%) of Wistar rats when compared to the control. The co-administration of leachate and *Dalium guineensis* aqueous leaf extract further increased white blood cells ($16.03\pm3.22 \times 10^9$ cells/L), platelet count ($681.50\pm57.64 \times 10^9$ cells/L), and platelet crit (0.54±0.04%); with a further decrease in red blood cell count ($6.27\pm0.70 \times 10^{12}$ cells/L) of Wistar rats when compared to the control.

Table 1: Changes in blood hematological profile of Wistar rats in response to treatment during exposure to Ikhueniro dumpsite leachate.

		C	L	LA	P-Value
1.	WBC($\times 10^9$ cells/L)	12.73±0.74	16.25±1.89	16.03±3.22	P > 0.05
2.	LYM (%)	68.48±4.94	67.88±2.06	57.48±4.45	P > 0.05
3.	MID (%)	8.48±0.68	9.78±0.23	10.75±0.65	P > 0.05
4.	GRAN (%)	23.05±4.98	22.35±1.96	31.78±5.04	P > 0.05
5.	LYM ($\times 10^9$ cells/L)	8.75±0.91	10.95±1.01	8.80±0.90	P > 0.05
6.	MID ($\times 10^9$ cells/L)	1.10±0.07	1.60±0.23	1.68±0.21	P > 0.05
7.	GRAN ($\times 10^9$ cells/L)	2.88±0.55	3.70±0.70	5.55±2.15	P > 0.05
8.	RBC($\times 10^{12}$ cells/L)	6.78±0.38	6.27±0.70	6.24±0.36	P > 0.05
9.	HGB (g/dL)	17.05±0.85	16.13±1.56	16.30±0.65	P > 0.05
10.	HCT (%)	48.93±2.40	45.93±3.05	46.20±1.63	P > 0.05
11.	PLT ($\times 10^9$ cells/L)	373.00±101.43	422.75±97.32	681.50±57.64	P > 0.05
12.	PCT (%)	0.22±0.06	0.32±0.08	0.54±0.04	P > 0.05

NB: All values are expressed as Mean±SE. P<0.05 indicates a significant difference; P>0.05 indicates a non-significant difference.

Table 2: Changes in blood hematological profile of Wistar rats in response to treatment after exposure to the toxicant.

		L	NL	NLA	P-Value
1.	WBC($\times 10^9$ cells/L)	16.25±1.89	11.53±1.59	11.30±2.06	P > 0.05
2.	LYM (%)	67.88 ± 2.06^a	85.05 ± 2.10^b	84.00 ± 2.91^b	P < 0.05
3.	MID (%)	9.78 ± 0.23^a	5.73 ± 0.63^b	4.93 ± 0.79^b	P < 0.05
4.	GRAN (%)	22.35±1.96	9.23±1.67	11.08±2.17	P > 0.05
5.	LYM ($\times 10^9$ cells/L)	10.95±1.01	9.78±1.28	9.40±1.61	P > 0.05
6.	MID ($\times 10^9$ cells/L)	1.60±0.23	0.68±0.15	0.60±0.16	P > 0.05
7.	GRAN ($\times 10^9$ cells/L)	3.70±0.70	1.08±0.24	1.30±0.35	P > 0.05
8.	RBC($\times 10^{12}$ cells/L)	6.27±0.70	6.34±0.22	7.46±0.61	P > 0.05
9.	HGB (g/dL)	16.13±1.56	15.43±0.58	16.03±0.68	P > 0.05
10.	HCT (%)	45.93±3.05	51.45±2.58	56.60±1.11	P > 0.05
11.	PLT ($\times 10^9$ cells/L)	422.75±97.32	623.00±74.03	669.25±113.46	P > 0.05
12.	PCT (%)	0.32±0.08	0.49±0.05	0.55±0.10	P > 0.05

NB: All values are expressed as Mean±SE. P<0.05 indicates significant difference; P>0.05 indicates Non-Significant difference
[abc]Different superscripts within a row indicate significantly different; similar superscript within a row indicates no significant difference.

Nevertheless, when leachate administration was stopped in the group of rats where it was previously administered, there was a reduction in the white blood cell count ($11.53 \pm 1.59 \times 10^9$ cells/L) with an increase in red blood cell count ($6.34 \pm 0.22 \times 10^{12}$ cells/L) and hematocrit (51.45±0.58%) when compared to leachate administered groups of rats. When leachate administration was stopped in the group of rats where leachate was previously co-administered with *Daliumguineensis* aqueous leaf extract, there was a further reduction in white blood cell count ($11.30 \pm 2.06 \times 10^9$ cells/L); with a further increase in red blood cell count ($7.46 \pm 0.61 \times 10^{12}$ cells/L) and hematocrit (56.60±2.58%) when compared to leachate administered groups of rats.

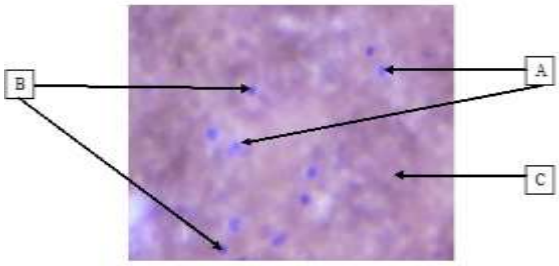

Plate 1: Blood film of control male rat (Giemsa x 100)

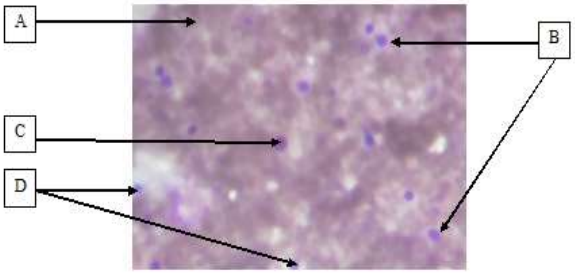

Plate 2: Blood film of male rat exposed to leachate only (Giemsa x 100)

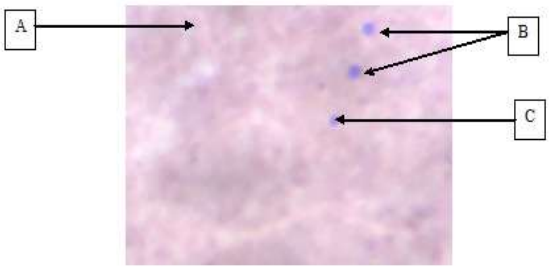

Plate 3: Blood film of male rat exposed to leachate + *D. guineensis* (Giemsa x 100)

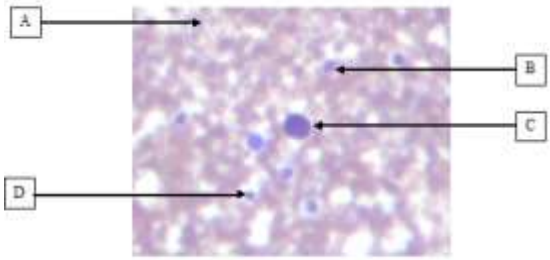

Plate 4: Blood film of male rat with leachate exposure discontinued (Giemsa x 100)

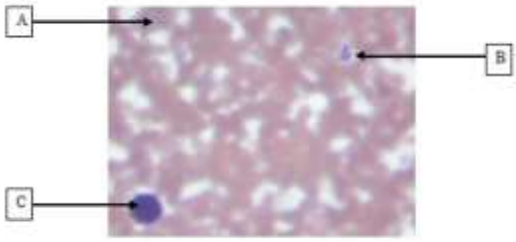

Plate 5: Blood film of male rat with leachate exposure discontinued, but *D. guineensis* exposure continued (Giemsa x 100)

Histological findings in blood tissues of Wistar rats exposed to Ikhueniro dumpsite leachate and other protocols are shown in Plates 1 to 5; while the summary of the findings is shown in Table 3. Geimsa-stained blood film revealed that the blood tissue was well lined up in order, and was well-distributed in the control group (Plate 1). The presence of polymorphs and basophils in the blood film of rats administered dumpsite leachate (Plate 2) differentiated it from the control. The presence of polymorphs was also observed in the blood film of Wistar rats co-administered leachate with *Dalium guineensis***,** but in a slightly reduced number when compared to the leachate group. When leachate administration was stopped in groups of rats where it was previously administered only and in groups of rats where it was co-administered with *Dalium guineensis* aqueous leaf extract, a slight reduction in the number of polymorphs and basophils present was also observed (Plates 4 and 5, respectively); although the later groups of rats had fewer polymorphs.

Table 3: Summary of histopathological findings in the blood of male Wistar rats exposed to Ikhueniro dumpsite leachate and other abatements

		C	L	LA	NL	NLA
1	Neutrophils	+	-	-	-	-
2	Lymphocytes	+	+	+	+	-
3	Monocytes	-	-	-	-	-
4	Polymorphs	-	+	+	+	+
5	Basophils	-	+	-	+	+
6	Eosinophils	-	-	-	-	-

Key: + indicates presence; - indicates absent

Investigation of hematological parameters represents a useful process in the diagnosis of many diseases as well as the investigation of the extent of damage to the blood (Onyeyili *et al*., 1991). This is relevant since blood constituents change with the physiological conditions of the animals. Hematological studies are important because blood is the major transport system of the body, and evaluation of the hematological profile usually furnishes vital information on the body's response to injury of all forms, including toxic injury (Schalm *et al*., 1975; Ihedioha *et al*., 2004). Hematological constituents reflect the physiological responsiveness of the animal to its internal and external environments which include feed and feeding (Esonu *et al*., 2001) as well as drugs (Iheukwumere *et al*., 2007). The white blood cell and the differential count are usually carried out to provide information on the proportion of the different white cells present in circulating blood (Cheesbrough, 2002). In this study the leachate induced lymphocytosis, granulocytosis, and monocytosis. Similar findings have been observed by Ita and Udofia (2011). According to them the increased white blood cell and differentials were a result of crude oil administration in rats. Of all the white blood cell populations counted, the lymphocytes were the mostly proliferated cells, followed by the granulocyte and monocytes. This contradicts the findings of Alimba *et al*. (2006) who reported a decrease in leucocyte and lymphocyte, basophil, and monocyte count, with an increase in neutrophil and eosinophil count as a result of Olusosun and Aba-Eku landfills leachate in South-western Nigeria. It is also possible that the membranes of these lymphocytes were oxidized as the rats were subjected to Ikhueniro waste dumpsite leachate; as a high concentration of lymphocytes in the blood is suggestive of a high degree of infection after trauma leading to high antibody production (Abbas and Lichtman, 2003). Increased granulocyte levels suggest high cellular damage/inflammation and depressed immunity; while increased monocyte count might be a sign of a chronic infection, an autoimmune disorder, or a blood disorder (Shugaba *et al*., 2012). One of the major functions of lymphocytes is their response to antigens (foreign bodies) by forming antibodies that circulate in the blood or the development of cellular immunity (Frandson, 2003). Granulocytes such as neutrophils are the first responders to inflammation and cell damage, while eosinophils are primarily associated with parasitic infections and an increase in their number may indicate such (Alberts, 2005). Eosinophils along with basophils and mast cells are important mediators of allergic responses and associative pathogenesis in the development of asthma (Rothenberg and Hogan, 2006). Ingestion of Ikhueniro waste dumpsite leachate may have induced an increase in the metabolic rate, with the resultant increase in the generation of free radical**s** with the attendant cellular damage. The immune system responds to these damages by the production of oxidants during stressful conditions. During such responses, free radical**s** are produced by the neutrophils the first responders to inflammatory cells to remove damaged cells. Being highly mobile, neutrophils quickly congregate at a focus of infection, attracted by cytokines expressed by activated endothelium, mast cells, and macrophages (Ear and McDonald, 2008). Monocytes help the immune system fight infection. These white blood cells can turn into dendritic cells and macrophages when the immune system detects a foreign substance. Dendritic cells identify foreign substances and communicate with B and T cells to help the body build immunity to a particular substance. Macrophages destroy parasites, bacteria, and other organisms (Ugochukwu *et al*., 2003). The reduction in hematocrit, hemoglobin, and red blood cell counts is suggestive of altered peripheral blood composition which is a reflection of disrupted hematopoietic process and interference with different stages of red cell synthesis and mature red blood cells. Heavy metals are known to cause inhibition of aminolevulinic acid dehydratase activity, thereby altering the heme synthesis pathway. Similar findings have also been reported by Hounkpatin *et al*. (2013) as a result of the toxicity of cadmium, mercury, and their combination; the study revealed a significant decrease in red blood cells (RBC), hemoglobin concentration (HGB) in Wistar rats. Mannem (2014) investigated the toxicity of lead acetate on various hematological parameters in male albino Wistar rats; and reported a significant reduction in the mean RBC values. Again, Nikolic *et al*. (2013) showed that Pb, Cd, and Cu intoxication significantly decreased values of erythrocytes, hemoglobin, and hematocrit in treated Wistar rats. The primary function of the erythrocytes is to carry oxygen bound to hemoglobin from the lungs to the tissue. Furthermore, hemoglobin in erythrocytes is an excellent acid-base buffer, thus erythrocytes are the most responsible for the buffer capacity of whole blood (Ersley and Gabuzba, 1985). Hematocrit is used to measure blood-carrying capacity and is directly associated with percentage hemoglobin (HB%) declination in this study. A decrease in hematocrit may be due to suppressed bone marrow hematopoietic system, causing iron deficiency in the synthesis of haem protein of hemoglobin (Klauder and Petering, 1977; Guyton and Hall, 1996). Low hematocrit indicates anemia or oligospermia (Wepener *et al*. 1992). The changes in the blood HCT value have often been shown to be a good indicator of heavy metal toxicity. Platelets are cytoplasmatic fragments of bone

marrow megakaryocytes (Laki, 1972). They are dynamic blood particles whose primary function, along with the coagulation factors, is hemostasis, or the prevention of bleeding. Platelets interact with each other, as well as with leukocyte and endothelial cells, searching the vascular bed for sites of injury, where they become activated (Machlus *et al*., 2014). In addition to their important role in hemostasis and thrombosis, accumulating evidence demonstrates that platelets contribute to the inflammatory process, microbial host defense, wound healing, angiogenesis, and remodeling (Jain, 1975).

Increased platelet count and platelet crit in rats administered Ikhueniro dumpsite leachate was similar to the findings of Hounkpatin *et. al*. (2013). According to them, the increase in platelet count was a result of chronic doses of cadmium, mercury, and combined cadmium and mercury in Wistar rats. Again, Barman *et al*. (2014) reported that the levels of platelet count (PLT), platelet crit (PCT), and mean platelet mass (MPM) were significantly decreased, and platelet distribution width (PDW), platelet large cell ratio (P-LCR) and mean platelet volume were increased in workers involved in a lead-acid battery manufacturing plant. The increase may be a result of the presence of a substance in the leachate which may have resulted in the increased production of thrombocytes. Too many platelets can lead to certain conditions, including stroke, heart attack, or a clot in the blood vessels. Thrombocytosis may be caused by anemia due to iron deficiency, inflammation, or infection.

Conclusion: The findings of the present study have shown that Ikhueniro dumpsite leachate negatively impacted blood hematological indices in Wistar rats. Dumpsite leachate must be handled and treated carefully to avoid potential health hazards to animals. This study discovers that the administration of *Dialium guineense* leaf extracts better-improved blood hematological indices which can be subsequently employed for the amelioration of leachate-induced toxicity as well as other forms of cellular toxicities.

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