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Effects of Municipal Solid Waste Leachate on Leucocyte and Differential Counts in Rats

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Abstract: This study investigated the immunotoxic potentials of raw and simulated leachates from Olushosun municipal solid waste landfill in rat using quantitative leucocyte and differential counts. Blood samples of rats intraperitoneally exposed to 1 – 25 % concentrations of each of the leachate samples for 48 hours were examined post treatment for leukocyte and differential counts. Compared to the negative control, there was concentration dependent and statistical significant decrease in leucocyte ($P < 0.05$) and lymphocyte ($P < 0.001$) count and increase in neutrophil count ($P < 0.001$). There was statistically insignificant decrease in basophil and monocyte count and increase in eosinophil count. The constituents of the tested leachate samples were believed to provoke the observed changes in leucocyte and differential count. This finding suggests that municipal solid waste leachate induced a non-specific immunotoxic effects in rats and may be of public health importance as there is continuous residential and occupational exposure of human population to hazardous substances from solid wastes landfill.

Key words: Differential counts · Immunotoxicity · Leucocyte · Municipal solid wastes · Landfill · leachate · Rat

INTRODUCTION

Nigeria, with an estimated 140 million population [1], generates an annual estimation of 29.78 x 10^9 kg municipal solid waste [2]. This will continue to increase due to population growth, accelerated industrial development and high urbanization rate. The common methods of disposing these wastes are incineration, landfilling and or open dumps at the nearest available space. Wastes decompose in these landfills/dumpsites through a complex and highly variable processes that lead to leachate and landfill gas production. Leachate contains organic and inorganic chemicals and many unidentified toxicants known as non-conventional pollutants (NCPs) [3]. These compounds may pose serious risks to the ecosystems and human health if contamination and exposure occur.

Report from the United Nations Children's Fund showed that about 52 % of the Nigerian population makes use of unprotected underground and surface water for their domestic and commercial activities [4]. This indicates that individuals around landfills/dumpsites who make use of unprotected underground and surface water for domestic and commercial activities may be exposed to chemical substances in leachate through ingestion, inhalation and or dermal contact. Concern about the potential adverse effects of leachates on public health and the environment led to several studies on leachate induced toxicity on microalgae, bacteria, *Daphnia* and fish [5-8], cytotoxicity, mutagenicity and genotoxicity [9-16]. Ecotoxicological studies have resulted in harmful effects been observed in rodent and aquatic species but the extrapolation of these model systems to human population requires multidisciplinary consideration [8, 15,17,18].

Amongst these reports, information on the potential adverse effects of solid waste leachate on the immune system is limited. This is despite that the immune system is one of the most sensitive targets for chemical induced toxicity [19]. The potential for chemicals to cause damage to the immune system is of considerable public health significance and alterations to immune functions may lead to incidence of hypersensitivity disorders, autoimmune and infectious diseases or neoplasia [20]. Quantitative and morphologic investigations of non-specific immune cells, like total leukocyte counts and differentials formed the basic investigations mostly included in immunotoxicity studies [21]. Total leukocyte and

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differential counts have proven useful method in signaling clinically relevant haematologic changes that may result in clinical identifiable autoimmune disorders and various forms of leukemia [22]. In this study, the effect of raw and simulated leachates from Olushosun municipal solid waste (MSW) landfill on total leukocyte and differential counts in rat was investigated.

MATERIALS AND METHODS

Sampling Site, Leachate Sampling and Simulation from Solid Waste: The sampling site is Olushosun landfill at Ojota, located on the Northeastern part of Lagos State (Latitude 6° 34¹N and Longitude 3° 24¹E), Nigeria. Lagos state with a total land mass of about 8,345 square kilometers (about 0.4% of the total land area of Nigeria), accommodates about 6.4% of the total Nigerians [1,23]. The state is characterized with high industrialized activities and all waste generated end up in three state government recognized landfills and many illegal dumpsites [24]. Olushosun landfill, the biggest among the three landfills, covers about 42-hectares of land and with an excavation of about 18m deep into the landfill land. It receives all kinds of wastes from Lagos State, without a leachate collector, landfill liner and landfill cap. It is surrounded by industrial lands to the west, residential quarters to the southeast and northeast and a swamp to the north.

Raw leachate collected from 20 leachate wells (holes in the ground where leachate seeps out) was thoroughly mixed to provide a representative sample for the landfill. Leachate simulation from the solid waste was as previously described [12,14,25]. The raw and simulated leachate samples designated as Olushosun Raw Leachate (ORL) and Olushosun Simulated Leachate (OSL), respectively were filtered to remove debris, the pH was measured and stored at 4°C until use 72 hr after collection/simulation. The samples were analyzed for a number of standard physical and chemical properties according to APHA [26] and USEPA [27] (Table 1) [14].

Animal Exposure and Experimental Technique: Pathogen free male albino rats (6-8 weeks old weighing 132 ± 2 g) obtained from an inbred colony of the animal breeding units of Department of Physiology, University of Ibadan, Nigeria, were used for this study. Five concentrations of 1.0, 2.5, 5.0, 10.0 and 25.0 % (v/v, leachate/distilled water) of each leachate sample were utilized using 5 rats in each group. Each rat in each group was given a single intraperitoneal injection of 1.0 ml of the test sample for 48 hours. The negative and positive control groups of 5 rats/group received same volume of distilled water and cyclophosphamide (20 mg/kg body weight), respectively. At 48 hours post treatment, peripheral blood was collected from the retro-orbital sinus of the rats with heparinized 70 ml

Table 1: Physical and chemical characteristics and heavy metals analyzed in ORL and OSL (Alimba *et al*., 2006)

Parameters	ORL	OSL	FEPA[a]	USEPA[b]
pH	7.30	6.80	6-9	6.5-8.5
BOD[+]	598.00	590.00	50	-
COD[++]	480.00	370.00	-	410
Hardness	540.00	300.00	-	0-75
Total Alkalinity	480.00	540.00	250	20
Total Dissolved Solid	0.32	1.32	2000	500
Ammonia	0.86	78.68	0.01	0.02
Nitrate	3.86	2.46	-	10
Sulphate	68.58	48.20	20	250
Chloride	770.00	240.00	-	250
Copper	0.77	0.44	0.3	1
Lead	1.40	0.69	0.01	0.015
Cadmium	0.58	0.46	0.05	0.05
Iron	1.90	0.83	0.05	0.30
Manganese	0.79	0.46	0.05	0.05
Mercury	0.41	0.23	0.10	-
Arsenate	0.36	0.27	-	-

* All values are in mg/L except pH + Biochemical Oxygen demand ++ Chemical Oxygen demand

[a] Federal Environmental Protection Agency (1991) permissible limits for drinking water

[b](www.epa.gov/safe/mcl.html)

micro-haematocrit capillary tubes into a vial containing 0.5m EDTA. These were analyzed for leukocyte and differential counts at Haematology unit of the University College Hospital, Ibadan, Nigeria. White Blood Cell (WBC) counts were made by the haemocytometer method [28] and the differential WBC counts were made by finding the percentage average of the different types of cells counted in ten fields from Giemsa stained slides [29].

Statistical Analysis: The SPSS 11.0 ® software was used for this analysis. The data were presented as mean±SE for each leachate sample (experimental unit (n) for this analysis was the individual rat). The level of statistical significance of the treated samples compared with the negative control group was determined using student's t- test at the 0.05 and 0.001 level of probability.

RESULTS

Table 2 shows the effect of the different concentrations of raw and simulated leachate samples on leucocyte and differential count in rats after 48 hr exposure. There was a concentration dependent statistically significant (P< 0.05) decrease in the mean leucocyte count observed at the different concentrations of both ORL and OSL compared with the negative control. The decrease was more in OSL treated rats than those treated with ORL. In the analysis of differential count of the leucocytes, there was a statistically significant (P< 0.001) increase in peripheral blood neutrophils at all the tested concentrations of the leachate samples. Likewise, there was marginal increase in eosinophils but this was statistically insignificant. There was a concentration dependent decrease in lymphocyte (statistically significant at P < 0.001), monocyte and basophil counts when compared with the values of the negative control group. In the cyclophosphamide treated rats, there were statistically significant changes in the leukocyte, neutrophils and lymphocyte count. Decrease in monocytes and basophile count and increase in eosinophil count were however, statistically insignificant.

DISCUSSION

Recognition that xenobiotics can impair the function of immune system has led to the development and validation of variety of methods in immunotoxicology. One among the validated methods for investigating the immunotoxic potentials of these chemicals, primarily in rodents is change in cellular components of blood, especially in the leukocyte counts. In this study, rats were exposed to different concentrations of raw and simulated leachate samples from Olushosun MSW landfill and quantitative assessment of total and differential leucocyte count was made. Our results show that the leachate samples caused alterations to leucocytes and differential parameters. Alterations including increase, decrease or shifts in cellular components of blood, especially in leucocyte and differential counts, can be detected in sub chronic studies [30]. A concentration dependent decrease in mean leucocyte count observed is similar to those reported in humans exposed to marijuana smoke [31] and to marijuana and tobacco [32] and in mice orally exposed to 2-ethoxy ethyl acetate for five weeks [33].

Table 2: Changes observed in leukocyte and differential counts in rats exposed to Olusoshun raw (ORL) and simulated (OSL) leachates

Test sample	Concentration (%)	Total Leucocyte (mm^3)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
Negative Control	0	21400±424	54.30±2.55	39.60±3.55	4.00±2.60	1.00±0.35	1.50±0.60
ORL	1.0	14000±339*	56.25±3.45	38.50±2.76	3.30±1.17	1.50±0.38	1.00±0.88
	2.5	13800±326*	59.10±2.36**	37.40±3.86	3.30±2.35	1.50±0.11	0.50±0.78
	5.0	13450±560*	60.15±1.25**	35.32±3.01**	3.00±3.65	2.60±1.30	1.00±1.12
	10.0	10200±424*	61.05±3.68**	31.33±2.56**	2.65±3.10	2.00±1.65	0.86±0.32
	25.0	8750±707*	63.36±2.75**	30.03±3.30**	2.67±2.80	1.89±0.69	0.78±0.46
OSL	1.0	13850±205*	58.52±1.35**	35.50±3.05**	3.50±2.01	1.32±0.15	1.00±0.36
	2.5	10500±290*	5930±3.02**	33.20±2.16**	3.00±1.33	1.54±0.98	1.00±0.23
	5.0	10200±251*	59.95±3.65**	31.43±4.30**	3.00±2.25	1.50±0.86	1.00±0.41
	10.0	8050±559*	61.75±2.85**	31.10±0.68**	2.93±2.30	2.00±1.33	0.34±0.66
	25.0	5800±168*	62.93±4.63**	29.34±2.15**	1.50±1.22	2.15±1.10	0.28±0.36
Cyclophos-phamide	20 mg/kgbw	2.60±320*	64.53±4.65**	31.73±2.11**	2.04±1.67	2.50±1.33	0.10±0.35

* Values significant at P<0.05. ** Values significant at P<0.001.

The results of the differential counts showed neutrophilia. Svobodova *et al*., [34] observed similar results in carp and rainbow trouts exposed to Cu at concentrations close to lethal and albino mice exposed to whole-body gamma irradiation [35]. Lymphocytopenia observed is also similar to a report in Perch dwelling in natural water bodies polluted with a mixture of heavy metals containing Zn, Cu, Pb, Cd and Hg [36] and in humans occupationally exposed to 10 ppm concentration of Benzene [37]. A non significant decrease in monocytes and basophils and a marginal increase in eosinophils observed in this study are in agreement with the report on workers occupationally exposed to cement dust [38].

The physicochemical and heavy metal analysis of the tested leachates showed that they contained some inorganic anions and heavy metals at concentrations above acceptable standards by regulatory authorities [14]. The concentrations of heavy metals such as Cu, Pb, Cd, Fe, Mn and Hg were higher in ORL than in OSL and the concentration of Pb and Fe in ORL were comparatively higher than those of other heavy metals in the two samples [14]. In concert with previous studies [14,34,36,37], the interactions of the organics and inorganic constituents and unidentified toxicants with the blood parameters were responsible for the observed effects. It may be plausible that some of the leachate constituents are capable of suppressing the activities of lymphoid tissues and haematopoietic cells in bone marrow of rat and may inhibit both T and B lymphocytes production in rats. The suppression of peripheral blood leucocytes and differentials in rats may be manifested as decrease resistance to opportunistic microorganisms, viruses and other infectious agents or increase in cancer susceptibility. Apart from the toxic elements, the tested leachates may contain microbes, some of which are opportunistic pathogens. These microbes could produce toxins that may have contributed to the present observations. Indeed, several authors [8, 39, 40] have shown the presence of many pathogens in landfill leachates.

Leachate has been reported to cause liver dysfunction among residents who ingested leachate contaminated water [41] and may be responsible for leukemias and bladder cancers which are the most common cancers among populations living near landfill / dump sites [42]. The present observation corroborates a previous study [14] wherein the tested leachate samples induced various types of chromatid and chromosome abnormalities in rat bone marrow cells. It's also in concert with those of Sang and Li [43] and Tewari *et al*., [44] wherein MSW leachate obtained from China and India respectively, induced chromosome aberration in mouse bone marrow cells. The implication of this is that blood cells may be sensitive to the toxic chemicals present in solid waste leachates.

In conclusion, this investigation implies that municipal solid waste leachates induced a non specific immunotoxic effects in rat. This indicates that human populations residentially and occupationally exposed to sufficient dose of leachate containing chemicals may develop immune related diseases. This calls for a better policy options for solid waste management that will ensure the safety of human population residing and earning their livings around waste disposal sites in Nigeria and worldwide.

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