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Research Article

Clinicopathological Studies on Rats Exposed to Municipal Landfill Gaseous and Particulate Emissions

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ABSTRACT

The study assessed the Clinicopathological changes of Wistar rats exposed to municipal landfill gaseous particulate emissions. Four groups of Wistar rats were exposed to the different sites of landfill emission with use of filters for filtering landfill gaseous emission while the control group was not exposed. Performance of the animals was evaluated weekly, blood and lung, liver, kidney and heart tissues were taken for haematological and histopatholgical examinations. At the end of six weeks exposure, the peak weight was recorded as 108.96±0.45 of Group B as compared to 126.352±0.88 of the Control Group, as well as the decrease in the body weight of Group A-D compared to the Control Group. There was leucocytosis, lymphocytosis and neutrophilia in the rats that were exposed to landfill emission. There were pulmonary, hepatic, and renal lesions while the heart was spared. Changes were observed on the use of filters for filtering landfill gaseous emission. The 3M 6001 CN Gas Mask Carnister Cartridge which filters 50% of landfill emission together with black polythene nylon restricting external air into the filtered cage was not sufficient to reduce the effect of landfill gaseous emission at the end of the exposure period. The screening with PM2.5 material which filters 100% of PM2.5 emission was only able to screen PM2.5 emission but could not screen other landfill gaseous emission. This indicates that landfill gaseous emission is capable of inducing respiratory problems and tissue damage on dumpsite workers if there is sufficient and continuous exposure to landfill gaseous emissions. To prevent the hazard that may arose from dumpsite activities; gas nose mask respirator is recommended for an individual who works as scavengers and landfill workers before entering the dumpsite environment..

Keywords: *Dumpsite, Respirators, Clinical assessment, Landfill Gaseous Emission*

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INTRODUCTION

Air pollution is one of the environmental problems confronting growing cities and is currently the challenge being faced by many developed and developing countries; its effects on human lives are enormous as it causes disease and can result in chronic illness. Apart from the health risk, it contributes to the changing climatic conditions, which are potential sources of threats to local and international communities (Komolafe et al., 2014, Nwafor et al., 2019).

Solid waste, a major source of environmental pollution is any solid, semisolid liquid or contained gaseous materials discarded from industrial, commercial, mining or agricultural operations and from community activities (Resource Conservation and Recovery Act RCRA 2000). Solid waste includes garbage, construction debris, commercial refuse and sludge from water or waste treatment plants or air pollution. In Nigeria, the state is responsible for the protection of the environment as enshrined in the Nigerian 1999 constitution (section 20) "The state shall protect and improve the environment and safeguard the water, air and land, forest and wild life of Nigeria,". This responsibility falls on the Local Government, the third tier of government.

The occurrence of air pollution in any giving environment is a function of both natural and anthropogenic factors. However, anthropogenic factors are presently considered as the largest sources of indoor and outdoor air pollution. Air pollution is spatial-temporal. Thus, Ubuoh and Akhionbare (2011), Agwu et al (2013) posit that the quality of ambient air is determined by the extent of pollution in that environment.

Solid waste management in Ibadan has been a challenging problem for more than half a century. This has necessitated formulation and implementation of many policies towards minimizing the effects of environmental pollution. Akinremi (2006), observed a relationship between environmental pollution, life expectancy and mortality consequent on location of Nekede dump site in Imo State, Nigeria. According to the World Health Organization Report (2005), more than two million premature deaths that occur each year can be attributed to the effects of indoor and outdoor air pollution. Thus, air pollution is a significant risk factor for a number of diseases. Sulphur dioxide and nitrogen dioxide have been reported to cause respiratory and cardiovascular diseases in children and adult (Brook et al., 2010 and WHO 2011). The study evaluated the clinicopathological changes of rats exposed to municipal landfill gaseous particulate emissions

MATERIALS AND METHODS

Study Area: The Awotan dumpsite situated in Ibadan under the management of Oyo State Waste Management Authority (OYOWMA) was used for the study. It has been operational since 1997 and has an area of about 20.59 hectares divided into 9 platforms comprising of both old and new dumpsites. About 25 m radius of this dumpsite is residential and commercial areas. Biodegradation of the wastes occur aerobically and facilitated by mixing with the use of Bulldozer.

The non-biodegradables (metal, glass wares, cans, plastics and rubbers) are normally removed by the registered scavengers who were about forty-two in number. During dry season, the wastes are usually set on fire with attendant release of noxious gases that further escalate the degree of air pollution.

This dumpsite comprises of residential and commercial area within 25m radius. It has a size of approximately 20.59 Hectares which is divided into 9 platforms for waste dumping (fresh waste dumps and old waste dumps) and biodegradation will occur aerobically. Bulldozer mixes the waste on each platform to help in further biodegradation.

Experimental Design and Procedure: Twenty-five four-week-old male Wistar rats weighing between 50 and 55 gram were used for the study. They were preconditioned under natural photo period of alternating 12-hour of light and darkness for two weeks and fed liberally with pelletized feed and water. They were marked with non-toxic dye (picric acid) for grouping and identification and also weighed weekly.
The animals were randomly allotted to five groups of 5 each namely; Control, Group A, Group B, Group C and Group D and exposure pattern as follows:

- Control group exposed to environment free from dumpsite area and landfill gas emission
- Group A: Platform 5
- Group B: Platform 6
- Group C: Platform 6, 9
- Group D: Platform 5, 9

Platform 5 being old dump site, Platform 6 new dump site and Platform 9 had the Scavengers' gatherings. All the groups had 10 hours (8:00 till 18:00) of exposure to the respective environment daily for six weeks; totaling 420 hours for the duration of the study.

During the non-exposure periods (6:00 pm – 8:00 am), the respective habitat was as described as follows: The control group animals were housed in wire gauze cage at room temperature and humidity. Groups A, B and C were housed in wire gauze cages with ambient temperature and humidity from the dumpsite area. Also, the Group D was housed in wire gauze cage filtered with 3M 6001 CN gas mask canister cartridge with 50% filtration area and polythene nylon at ambient temperature and humidity (Plates A-C).

Sample collection and laboratory processing: Blood samples were taken for haematological evaluation using method by Schalm *et al*. 1975, Jain, 1986, Zinkl, 1986 and Kelly, 1984. On Day 21 and 42, two rat(s) was selected from each group for euthanasia. The liver, kidney, heart and lungs were examined and tissues preserved in Bouin's fluid and formalin for histopathological examination (Clayden 1962).

Statistical Analysis
Quantitative data from the growth performance and blood parameters were expressed as means ± standard deviation. The groups were compared using two-tailed student t-test, ANOVA and Bonferroni's multiple comparisons at α=0.05.

Plate 1
A: Wistar rat Housing: No filter. **B**: Wistar rat Housing: Filtered with Pm 2.5 material of 100% filter area. **C**: Wistar rat Housing: Filtered with gas mask canister cartridge of 50% filter area.

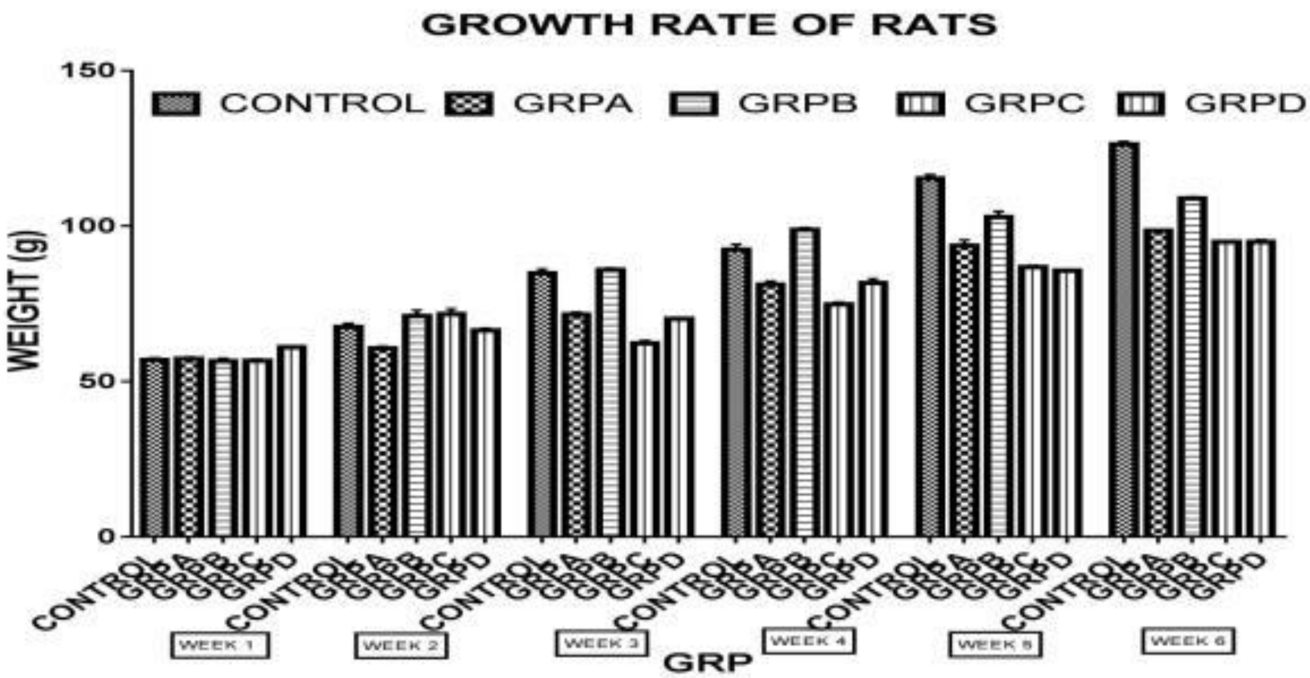


Figure 1: Growth rate of rats exposed and unexposed to dumpsite for six (6) weeks
*: Significant as compared with control; $p < 0.05$
Legends:
GRP A: Group A (Free exposure to gaseous emission)
GRP B: Group B (Free exposure to particulate matter emission)
GRP C: Group C (Exposure to particulate matter with PM $_{2.5}$ material of 100% filter area)
GRP D: Group D (Exposure to gaseous emission with gas mask canister cartridge of 50% filter area)

RESULTS

Body weight changes:

The was increase and decrease in weights of Group A, B, C and Group D (Figure 1) Wistar rats as compared to the Control Group (p<0.05). The peak weight was recorded as 108.96±0.45 of Group B as compared to 126.352±0.88 of the Control Group at the end of six (6) weeks exposure.

Haematological profile

Heamatological parameters following 21 days of exposure to landfill gaseous and particulate matter emissions revealed significant leucopenia, thrombocytopenia, lymphopenia, neutropenia and monocytopenia (Table 1). These were observed in the rats that were exposed to the platform of old dumps with no filter when compared with the control. At the end of the exposure period of 42days, there was a significant leucocytosis, lymphocytosis and neutrophilia but eosinopaenia when compared to the leucocyte values of the rats that are not exposed to landfill emission (p<0.05). After 21 days exposure, the rats that were exposed to the platform of new dumps with no filter showed significant leucopaenia and thrombocytopaenia. Aftert 42days exposure period there was significant leucocytosis, lymphocytosis and neutrophilia when compared to the leucocyte values of the rats that were not exposed to landfill emission. The rats that were exposed to the platform of new dumps and scavenger gathering filtered with PM 2.5 materials (100% filter area) at the end of exposure period showed leucocytosis when compared to the rats that were not exposed to landfill emission.

Furthermore, the rats that were exposed to the platform of old dumps and scavenger gathering filtered with gas mask canister cartridge (50% filter area) showed significant decrease in leucocyte, Platelet, lymphocyte, neutrophil and monocytes values at 21days exposure but there was significant increase in packed cell volume (PCV) and red blood cell (RBC) count values at the end of the exposure period when compared to the leucocyte, platelet, lymphocyte, neutrophil, monocyte, packed cell volume and red blood cell count values of the rats that were not exposed to landfill emission.

Histological observations

No lesion was seen in the histology of the organs harvested from the control group (Table 2; Plates 2-5). The histology of the heart in all the experimental groups (A, B, C and D) was normal following 21 and 42-day exposure. Inflammatory features such as congested glomerular capillaries and interstitial cellular infiltration were observed in kidney sections of groups A, C and D after 42 days of exposure (Table 2; Figures 3-5). Histology of the liver and lung specimens from all the experimental groups showed features of chronic inflammatory reactions after both 21 and 42 day exposure but more severe in the latter duration (Table 2; Figures 3-6).

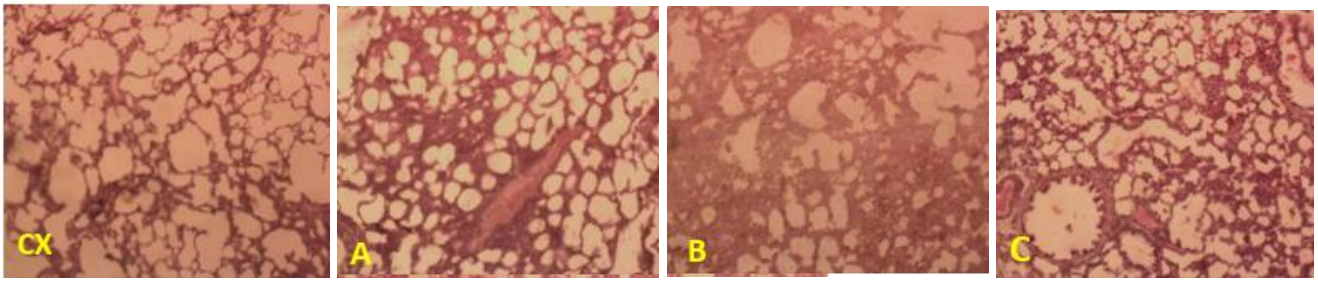
Table 1:
Haematology parameters for 21 and 42 day- exposure period in rats to municipal dumpsite

Group	PCV (%)		H.B (g/dl)		RBCx10^{3}µl		WBCx10^{3}µl		Platelet x10^{3}µl		LYMx10^{3}µl		NEUTx10^{3}µl		MONx10^{3}µl		EOSx10^{3}µl	
Days	21	42	21	42	21	42	21	42	21	42	21	42	21	42	21	42	21	42
Cont	36±1.4^{a}	33±1.4^{a}	12.2±0.3^{a}	10.6±1.4^{a}	6.16±0.1^{a}	5.2±0.1^{a}	8.9±0.1^{a}	4.5±0.1^{a}	201±0.3^{a}	81±0.1^{a}	5.7±0.1^{a}	2.8±0.2^{a}	3±0.2^{a}	2±0.0^{a}	0.2±0.0^{a}	0.1±0.0^{a}	0±0^{a}	0.±0.0^{a}
A	34±5.7^{a}	31±1.4^{a}	11.5±0.3^{a}	10.2±1.4^{a}	5.24±1.5^{a}	5.3±0.1^{a}	7.1±0.1^{b}	10.5±0.1^{b}	187±1.4^{b}	134±1.2^{a}	4.9±0.2^{b}	5.9±0.2^{b}	2.0±0.1^{b}	4±0.4^{b}	0.1±0.0^{b}	0.2±0.1^{a}	0.2±0.0^{a}	0±0^{b}
B	36±1.4^{a}	28±1.4^{a}	11.8±1.1^{a}	8.9±0.8^{a}	6.13±0.1^{a}	4.4±0.1^{a}	7.8±0.1^{b}	7.5±0.1^{b}	147±1.4^{b}	110±7.1^{a}	5.1±0.0^{a}	4.6±0.3^{b}	3.0±0.2^{a}	3±0.3^{b}	0.1±0.0^{a}	0.1±0.0^{a}	0.1±0.0^{a}	0.2±0.0^{a}
C	37±1.4^{a}	31.5±2.1^{a}	12.7±0.3^{a}	9.95±0.9^{a}	6.24±0.0^{a}	5±0.7^{a}	8.9±0.0^{a}	5.9±0.3^{b}	202±0.4^{a}	74±33.1^{a}	5.8±0.3^{a}	3.5±0.5^{a}	3.0±0.1^{a}	2±0.2^{a}	0.2±0.0^{a}	0.1±0.0^{a}	0.1±0.0^{a}	0.1±0.1^{a}
D	35±1.4^{a}	44±1.4^{b}	11.± 0.3^{a}	14.6±0.3^{a}	5.27±0.6^{a}	7.3±0.0^{b}	5.9±0.1^{b}	5.1±0.4^{a}	132±2.8^{b}	88±20^{a}	3.8±0.1^{b}	3.2±0.0^{a}	2.0±0.2^{b}	2±0.3^{a}	0.0±0.0^{b}	0.1±0.0^{a}	0.2±0.0^{a}	0.1±0.1^{a}

* values with different superscript are significant across column at 5%.

Table 2:
Histopathological findings of some major organs post 21 and 42-day exposure

GROUP	HEART		KIDNEY		LIVER		LUNGS	
	21DAYS	42 DAYS	21 DAYS	42 DAYS	21DAYS	42 DAYS	21DAYS	42 DAYS
CONT	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
A	No visible lesion seen	No visible lesion seen	No visible lesions seen.	There was a mild to moderate congestion of glomerular capillaries, at the cortex	There was a mild diffuse vacuolar degeneration of hepatocytes	There was a very marked centrilobular atrophy and vacuolar degeneration of the hepatocytes	There was a severe congestion and haemorrhage of the pulmonary interstitium	The interstitium was thickened by proliferation of septal cells and inflammatory infiltrates
B	No visible lesion seen	No visible lesion seen	No visible lesions seen.	No visible lesions seen	There was a moderate portal congestion with mild periportal cellular infiltration	There was a moderate diffuse vacuolar degeneration and necrosis of hepatocytes	There was a moderate to severe proliferation of septal cells	There was a moderate to severe congestion and thickening of interstitium
C	No visible lesion seen	No visible lesion seen	There was a severe interstitial congestion	There was a severe interstitial congestion and oedema	There was a mild periportalvacuolar degeneration of hepatocytes	There was a severe diffuse vacuolar degeneration of hepatocytes	No visible lesions seen	There was a severe proliferation of septal cells
D	No visible lesion seen	No visible lesion seen	No visible lesions seen.	There was capillary congestion and a few cellular infiltrates in interstitial spaces	No visible lesions seen	There was a swelling and degeneration of hepatocytes.	The alveolar wall was moderately thickened by inflammatory cells	There was a severe proliferation of septal cells, cellular infiltrates and attenuation of alveoli



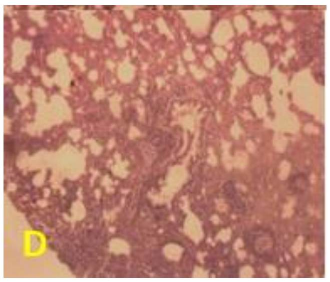
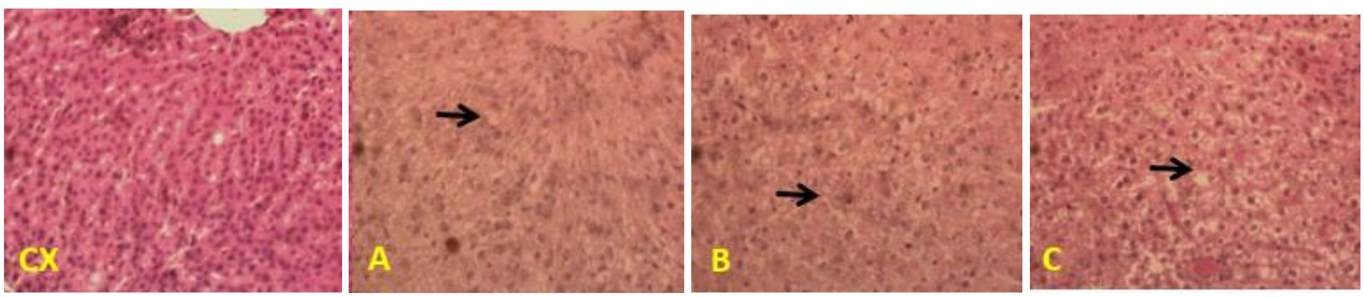


Plate 2:
Photomicrograph of the lung post 42day exposure: CX (control) – Normal. Group A – Interstitial thickening by proliferation of septal cells and inflammatory infiltrates. Group B – There was moderate to severe congestion and thickening of the interstitium. Group C - Massive proliferation of septal cells noted. D – Extensive proliferation of septal cells, cellular infiltrates and attenuation of alveoli were observed. HE x100



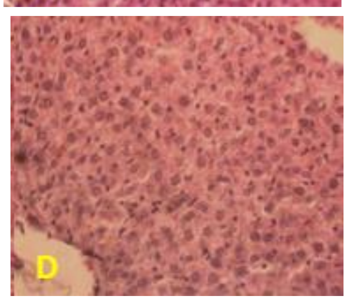
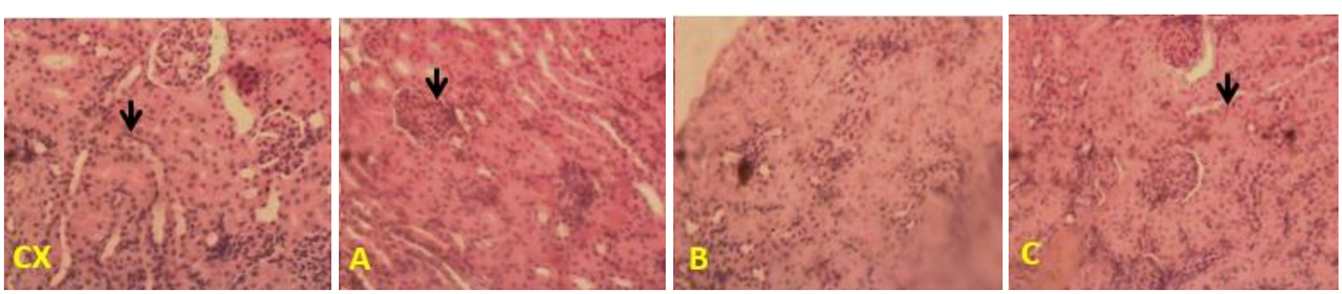


Plate 3:
Photomicrograph of the liver post 42day exposure: CX (control) – Normal. A - Marked centrilobular atrophy and vacuolar degeneration of the hepatocytes (arrow). B – Moderate diffuse vacuolar degeneration and necrosis of hepatocytes (arrow). C - Severe diffuse vacuolar degeneration of hepatocytes (arrow). D – There is a swelling and degeneration of hepatocytes. HE x400



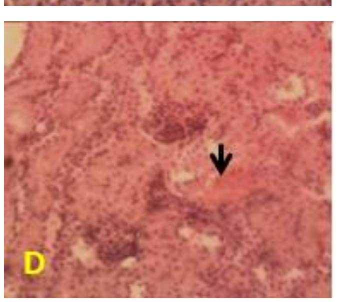
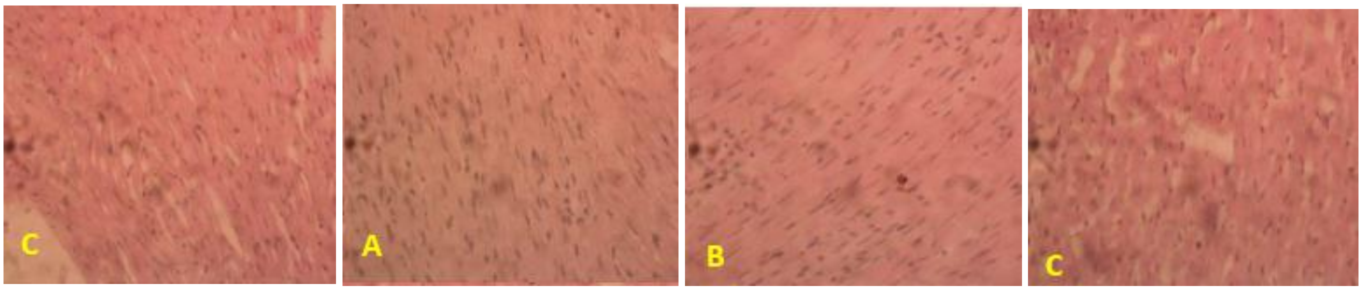


Plate 4:
Photomicrograph of the kidney post 42day exposure: CX (control) – normal. A – Moderate congestion of glomerular capillaries (arrow), at the cortex. B- No visible lesions seen. C - Severe interstitial congestion and oedema (arrow). D - Capillary congestion and a few cellular infiltrates in interstitial spaces (arrows). HE x400



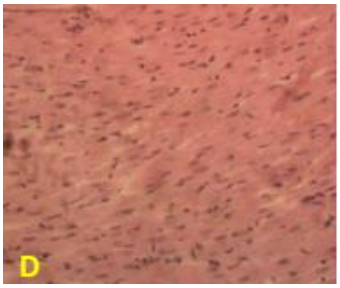


Plate 5:
Photomicrograph of the heart muscle post 42day exposure. No lesion observed in any of the groups HE x400

DISCUSSION

The changes in weight gain may be due to change in the ecological environment and reaction to gradual exposure of landfill gases and particulate matter resulting from activities on the dumpsite. The leucocytic changes in the exposed rats suggest stress and possible immunosuppression in the animals.

Chronic exposure to landfill gaseous emission and particulate matter emission interfered significantly with tissue oxygen supply causing hypoxia and thus provoking adaptive responses like increase and decrease in leucocyte, platelets, lymphocyte and neutrophil values, decrease in monocyte and increase in packed cell volume and red blood cell count values after 42 days exposure period. The high white blood cell count (leucocytosis) or values indicates that there could be an infection and that the immune system is working to destroy it. It may also be a sign that specimen is experiencing physical or emotional stress. Thrombocytosis (elevated platelet count) may precipitate intravascular clotting of blood (thrombosis); when a thrombose gets dislodged its likely to result in pulmonary embolism if it is to the lung or cerebrovascular accident (stroke) if to the brain or acute renal failure if the kidneys are involved. All these pathologies are of significant morbidity and mortality. Thus the people residing in the vicinity of the dumpsite are at risk of serious health hazard.
Histopathological examination of tissues is useful in identifying the type of lesions caused by xenobiotics and is acknowledged as the most reliable end point for detecting organ toxicity (Lanning et al., 2007). It is also useful in providing information about acute or chronic effects of toxic substances that may not be detected by other biomarkers (Amacher et al., 2006 and Jadhav et al., 2007).

The histopathological findings in the lungs and liver were not un anticipated considering the fact the environmental pollutants in the study areas were largely inhalational. Thus they precipitated the inflammatory responses observed in the lung specimens. The observed lesions may be due to oxygen deficiency and high Concentrations of CO2, CH4 and other landfill gas emissions that may have precipitated tissue hypoxiaa. The liver is the major metabolic organ for ingested matter thus the observed hepatocyte degeneration and fatty changes seen in the liver specimens were consequent upon what the rats fed on during the daily period of exposure. During humid weather conditions as obtained in dry season and harmattan, the inhalational pollutants will be dispersed by wind and invariably inhaled by people residing or transacting business on regular basis within the vicinity of the Awotan dumpsite. This may precipitate pneumonia, chronic air way diseases such bronchitis, asthma and bronchiectasis. At the advent of the rainy season, the toxic particulate materials are washed off into drains and streams and rivers, the polluted runoff water may invariably find its way into the deep wells that serve as water sources for the people living in the adjoining areas. When this is consumed either as water in drink or in food, similar pathological processes seen in the liver specimens of the experimental rats may be triggered off in the residents of the affected areas. This portends a grievous health hazard. In a related study by Alimba, et al. (2012), in which wistar rats were exposed to municipal landfill leachate; mild to severe multifocal degeneration of the hepatocytes, multiple periportal foci of cellular infiltration and interstitial haemorrhage cortical congestion, degenerative epithelia of renal tubules and necrosis were observed in the liver and kidney of the rats.

The haematological and histopathological parameters evaluated in this study showed that the severity of parameter alteration and organ damage increased with duration of exposure to land fill gaseous emission. The filters, both gas mask canister cartridge and black polythene nylon were unable to reduce the severity extent. Considering the fact that most tissue and organ pathologies whether benign or malignant usually have an initial inflammatory phase; if this study had lasted further, more sinister pathological changes might have been observed. Thus continuous and protracted exposure of people to landfill gaseous emission and particulate matters with precipitate multi system organ pathologies of varying types and degrees. Thus there is the need to find alternative solid waste disposal systems that will not be inimical to the health of the population.

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