Resources and Environment 2012, 2(4): 150-163
DOI: 10.5923/j.re.20120204.04

Liver and Kidney Dysfunction in Wistar Rats Exposed to Municipal Landfill Leachate

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Abstract There is limited information on the toxicity of landfill leachate on somatic tissues and organs of mammals. The hepatotoxic and nephrotoxic effects of leachates from Olusosun and Aba-Eku landfills in Southwestern Nigeria in Wistar albino rats were investigated. Rats orally exposed to 1 – 25 % concentrations of each of the test leachates were examined for clinical signs of toxicity and body weight gain during exposure. Blood, liver and kidney of surviving rats were examined for serum biochemical parameters, organ weight gain and histopathology. Clinical toxicity signs include ungroomed hair, reduced activities, hair loss, laboured breathing, reduced feed and fluid consumption, abscess and muscular disorder. There was concentration dependent sex related significant ($p<0.05$) decrease in body weight and increase absolute and relative liver and kidney weight gain. The test samples caused increase in aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine but decrease albumin and total proteins. These parameters showed sexual dimorphisms. Histopathological lesions in the liver and kidney include mild to severe multifocal degeneration of the hepatocytes, multiple periportal foci, cellular infiltration, interstitial haemorrhage cortical congestion, degenerative epithelia of renal tubules and necrosis. The concentration of heavy metals and anions in the test samples were above standard permissible limits. These findings show the potentials of landfill leachate to induce liver and kidney dysfunction in rat probably via free radical formation and/or direct chemical disruption of the organs. This may be of health risk in animal and human population expose to chemical substances from waste landfills.

Keywords Kidney, Liver, Landfill Leachate, Rat, Serum Biochemistry

1. Introduction

Solid waste generation by man is inevitable and landfilling is a common method of managing these wastes worldwide. In many developing nations, landfills are without liners and caps and are located near residential quarters. They are also not designated for specific waste type but rather all forms of wastes. Landfills have been reported to release hazardous chemicals through leachate and gases into the environment[1] and these constitute public and environmental health issues in many countries.

The incidence of cancers among residents of Niagara, United States who were exposed to chemicals leaching out of Love canal landfill[2], along with the reports of Meyer[3] and Vianna and Polan[4] on liver dysfunctions and incidence of low birth weight respectively among residents exposed to hazardous chemicals in landfill leachate, increased the awareness about the adverse human health effects that might be associated with landfills. These reports drew the attention of researchers to landfill toxicity studies, with the identification of specific constituents of these chemicals as most commonly used approach to show the hazardousness of chemicals in landfill wastes[5-6]. This method has a major limitation of inability to provide information about all the toxic chemicals present in the waste mixture and the potential synergistic and antagonistic interactions of these chemicals in living organisms. This led to experimental toxicity studies conducted with the aim of determining the toxic effects of landfill leachates and possible mechanisms of toxicity[7-13]. Some of these studies suggest free radical formation in mammalian systems as possible mechanism of landfill leachate induced toxicity and genetic damage [11-12,14 -15]

Systemic toxicity offer a means of understanding the mechanisms of free radical induced organ and or tissue damage. Among the tissues and organs in the mammalian body, liver and kidney seem to be the most sensitive predictor of chemical toxicity. This is due to their involvement in metabolism, detoxification, storage and excretion of xenobiotics and there metabolites, making them important target organs for xenobiotic induced injuries[16]. Apart from

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Published online at http://journal.sapub.org/re

being on the priority list of Agency for Toxic Substances and Disease Registry (ATSDR) as organs mostly affected by chemicals from landfills[17], they also correlate well with histopathological and serum biochemistry and have little inter-animal variability[18]. It is therefore important to evaluate the hepatotoxic and nephrotoxic effects of landfill leachates with the aim of understanding the deleterious effects of complex mixture of chemicals on these organs. In this study, we investigated the toxic effects of two municipal solid wastes (MSW) landfill leachates on the liver and kidney of albino rats via the evaluation of body and organ weight indices, clinical signs of toxicity, serum biochemistry and histopathology. Some physico-chemical parameters and heavy metal constituents of the leachates were also analyzed.

2. Materials and Methods

Sampling site and leachate collection

Two MSW landfills, one each in Lagos and Oyo States, Nigeria, were considered for this study. The sites: Olusosun landfill at Ojota, Lagos state and Aba-Eku landfill at Ona-Ara, Oyo state, have been described previously[10,13,19]. Raw leachate was collected from 20 leachate wells in each of the landfills and thoroughly mixed to provide a homogenous representative sample for each sampling site. This was then transferred to the laboratory in pre-cleaned 10 liter plastic containers, filtered with glass wool and Whatmann® No. 42 filter paper to remove suspended particles; centrifuged at 3000 rpm for 10 minutes and stored at 4^{o}C until use. These were considered as the stock sample (100%) and were labeled Aba-Eku Leachate (AEL) and Olusosun Leachate (OSL).

Physical and chemical analysis of the leachate

Physical and chemical components of the leachates were analyzed according to APHA[20]. Nitrate, ammonia, chloride, phosphate, sulphate, total hardness, total alkalinity, biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total solids (TS) were determined. The concentrations of Iron (Fe), Lead (Pb), Copper (Cu), Manganese (Mn), Arsenate (As), Cadmium (Cd), Chromium (Cr) and Nickel (Ni) were determined according to USEPA[21] and APHA[20]. Briefly, 100 ml of each leachate was digested by heating with concentrated HNO_3. The resulting mixture was made up to 10 ml with 0.1N HNO_3. The concentration of the metals was then estimated using PerkinElmer® A3100 atomic absorption spectrophotometer.

Animals and experimental design

All animal experiments were conducted in accordance with standard guidelines[22] on use of animals for experimental toxicology study. Male and female Wistar rats (6-7 weeks old) were obtained from the animal house of the College of Medicine, University of Ibadan, Nigeria. They were acclimatized for 2 weeks until they were 8-9 weeks (mean ± SD weight of 80.37 ± 2.14g). 5 rats/cage/sex were randomly assigned to a concentration each of the leachates; with cyclophosphamide (CYP, 40 mg/kg/bw) and distilled water as positive and negative controls respectively. They were maintained in laboratory conditions of 12 hours dark and light cycle, temperature of 26 ± 2^{o}C, relative humidity of 70 ± 20% and had access to drinking water and standard rodent chow (Ladokun feed Nigeria®) *ad libitum*. Each animal in a group was gavaged with 0.5 ml of 1, 2.5, 5, 10 and 25 % (leachate diluted with distilled water, v/v) concentrations of each of the leachates for 30 consecutive days. Similar treatment was concurrently given to the negative and positive groups receiving distilled water and CYP respectively.

Clinical observations and body weight measurement

Each rat in each of the treatment group was observed twice daily (before and after exposure) for signs of clinical toxicity in the appearances of the skin and fur, eyes and mucous membrane, behavioural pattern, respiratory system, morbidity and mortality. The body weight of each animal in the treatment and control groups was measured at the beginning of the experiment and once weekly during the exposure period using Acculab® USA, Model-vic-303 electronic analytical weighing balance.

Clinical pathology and organ weight measurement

At the end of exposure period, survivors were fasted overnight, weighed prior to blood collection and then sacrificed by cervical dislocation. Blood was collected from the orbital plexus using heparinized 70 ml micro-haematocrit capillary tubes into lithium coated serum separator tubes, under a light chloroform anesthesia. These were allowed to clot, centrifuged at 3000 rpm for 10 minutes to separate the serum (supernatant) and then stored at -70^{o}C prior to biochemical analysis within 48 hours. The liver and kidneys of the animals were surgically removed, rinsed with ice-cold physiological saline, blotted dry and weighed. The relative organ weight (organ weight/ body weight x 100g) was determined.

Serum biochemistry

Serum biochemical markers: creatinine, urea and total proteins were measured as functional marker for nephrotoxicity; and transaminases and albumins as marker for hepatotoxicity. They were assessed using Randox Laboratory (UK) diagnostic kits. Serum total protein and serum albumin were measured according to Treitz[23] and Doumas et al.[24] respectively. Aspartate aminotransferase (AST) and Alanine aminotranferase (ALT) enzyme activities in the serum were determined according to Reitman and Frankel[25]. Blood urea was determined according to Weatherburn[26], while serum creatinine was measured according to Henry et al.[27]. The absorbances of the tests were measured spectrophotometrically using HAICE®, DR 3000 (Germany).

Histopathological analysis

Slices of the right lobe of the liver and the left kidney from exposed and control animals were fixed in 10% neutral buffered formalin. After 48 hours of fixation, organs were dehydrated by passing through ascending order of ethyl alcohol–water concentrations, cleared in xylene and sequentially embedded in paraffin wax blocks using rotary microtome. Tissue sections of 3–5µm thick were cut, stained

with Haematoxylin–eosin (H–E), then mounted in neutral DPX medium for morphological evaluation before microscopic examination at x 400.

Statistical Analysis

All statistical analyses were conducted with Graphpad prism 5.0® computer programs.

Data are presented as mean ± SE (n=5/sex). One-way ANOVA was used to determine the differences among various groups. When the corresponding F test for differences among the treated group means was significant pair wise, comparisons between treated groups and corresponding negative control were determined using multiple comparison procedure of the Dunnett post-hoc test and differences were considered significant at $p<0.05$, $p<0.01$ and $p<0.001$ levels of significance. Two-way ANOVA was used to determine the significance of the independent effect of leachate and sex and their interactions on toxicity.

3. Results

Clinical signs of toxicity and mortality

During the exposure duration, 3 rats died (a male at the 25% concentration of AEL and one female each at the 5 and 25 % concentrations of OSL) and the survivors showed clinical signs of toxicity such as bluish discolouration of the skin (at the nasal, ear and genital regions) within 24 hours of exposure, laboured breathing pattern and ungroomed hair. Sluggishness and reduced food intake were observed among rats exposed to 5, 10 and 25 % of the leachates within the second and third weeks of exposure. During the fourth week of exposure, frequent sneezing, hair loss (figure 1c), diarrhea, weakness (shown by reduced activities) and reduced food and water intake were observed among the treated and CYP groups. A male rat at the 25% concentration of AEL had its eyeball bulged out of the socket, while a female at the 5% concentration of OSL and a male at the 10% concentration of AEL had abscess in the neck region (figure 1a) and thigh (later became sore, figure 1b) of the left legs respectively. Another female at the 5% concentration of OSL and two males, one each from the 5% concentration of OSL and 25% concentration of AEL were unable to move about freely. The female later died a day to the termination of the experiment. These observations were not sex specific.

Body and organ weight change

Figure 2(a – d) show the weekly and terminal mean body weight gain of exposed rats. There was decrease in weekly body weight gain in treated rats compared to negative control. This was significant ($p<0.05$) only at the 3rd, 4th and termination weeks. Compared to the negative control, the terminal body weight gain of leachate treated rat was lower by 33.35, 32.69, 34.16, 35.64 and 38.13 % (for OSL male), 31.17, 33.26, 32.40, 34.98 and 36.17 % (for OSL female), 32.54, 32.33, 31.42, 33.55 and 38.94 % (for AEL male) and 29.98, 29.22, 32.51, 3.32 and 35.25 % (for AEL female) at the 1.0, 2.5, 5.0, 10.0 and 25.0 % concentrations respectively. Body weight gain of OSL exposed rats was lower than those of AEL and male rats were more affected than corresponding females. Two–way ANOVA showed that only the effects of tested leachate samples (52.51%, $F = 21.15$, $p<0.0001$) was significant in the causation of decrease body weight gain than sex (0.73%, $F = 0.5888$, $p = 0.6236$) and interaction of sex and leachate (0.41%, $F = 0.0544$, $p = 1.0000$). There was a concentration dependent significant ($p<0.05$) increase in both absolute and relative liver and kidney weight gain in treated rats compared to the negative control group (table 1). Organ weight gain was higher in males than females. Only the effect of leachate on the relative organ weight gain was significant {39.51%, $F = 13.10$, $p < 0.0001$ of the total variance for liver weight gain; 29.20%, $F = 7.893$, $p < 0.0001$ of the total variance for kidney weight gain} compared to the effect of sex {2.28%, $F = 1.514$, $p = 0.2147$ of the total variance for liver weight gain; 1.00%, $F = 0.5411$, $p = 0.6552$ of the total variance for kidney weight gain} and the interactions of sex and leachate {1.89%, $F = 0.2093$, $p = 0.9998$ of the total variance for liver weight gain; 0.75%, $F = 0.0678$, $p = 1.000$ of the total variance for kidney weight gain}.

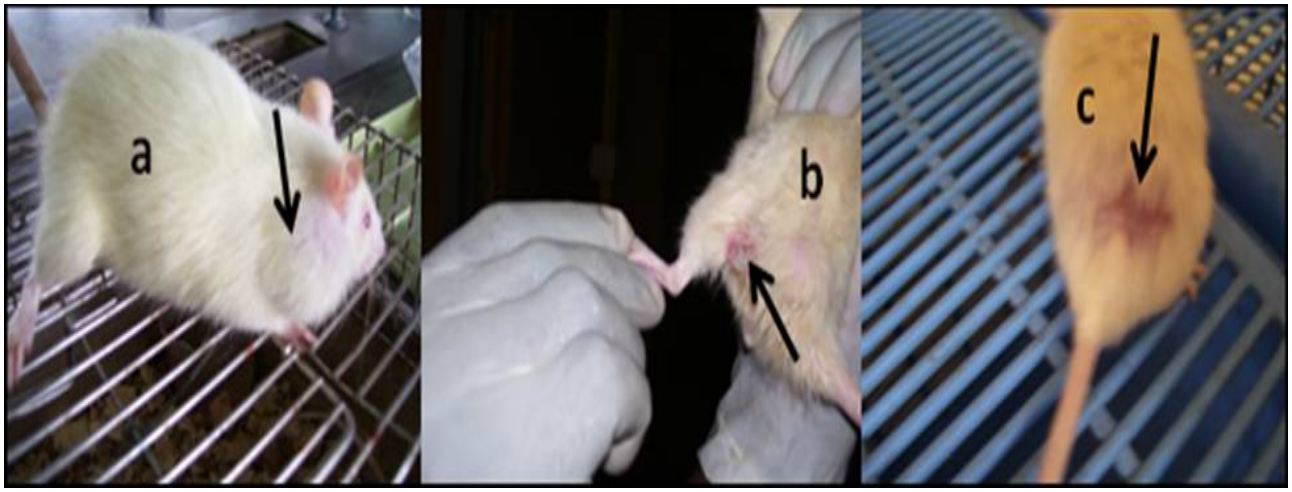

Figure 1. (a-c). Some clinical signs of toxicity observed in Olusosun and Aba-Eku landfill leachates treated rats; (a) Abscess on the neck of a female rat exposed to 5% OSL. (b) Sore on the thigh of the right leg of a male rat exposed to 10% AEL rat. (c) Hair loss in rat treated with 25% AEL

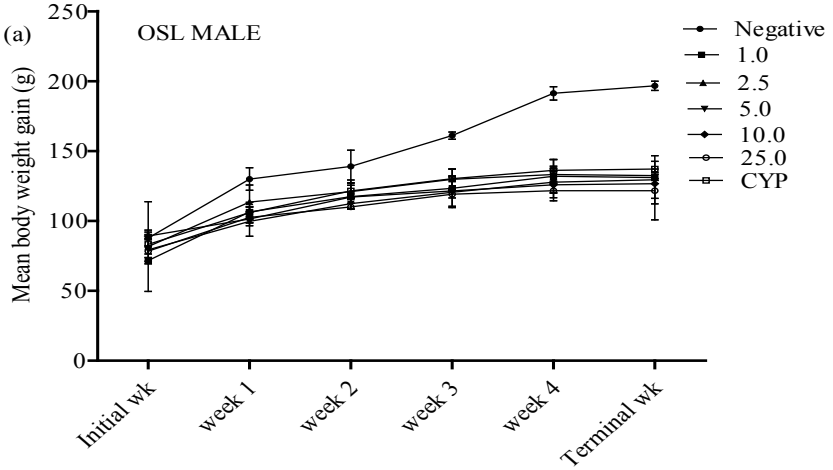

Figure 2a. Mean body weights of male rat orally exposed to different concentrations of Olusosun landfill leachate (OSL) for 30 days. CYP – Cyclophosphamide (40 mg/Kg bwt)

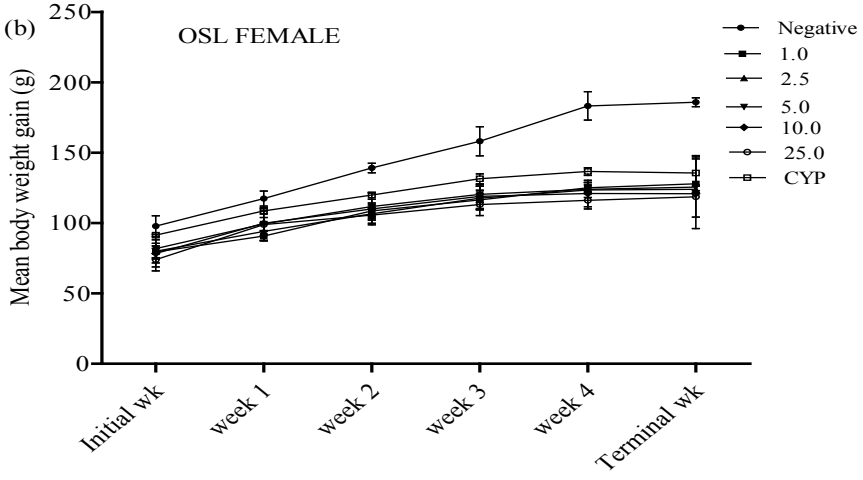

Figure 2b. Mean body weights of female rat orally exposed to different concentrations of Olusosun landfill leachate (OSL) for 30 days. CYP – Cyclophosphamide (40 mg/Kg bwt)

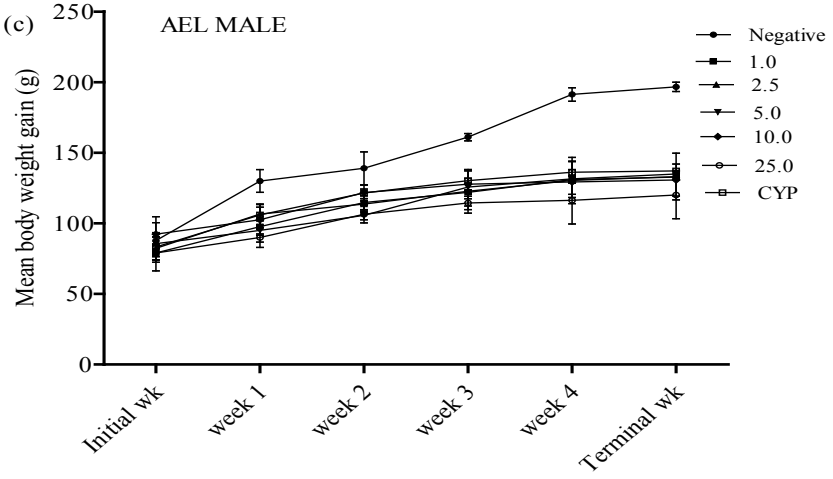

Figure 2c. Mean body weights of male rat orally exposed to different concentrations of Aba-Eku landfill leachate (AEL) for 30 days. CYP – Cyclophosphamide (40 mg/Kg bwt)

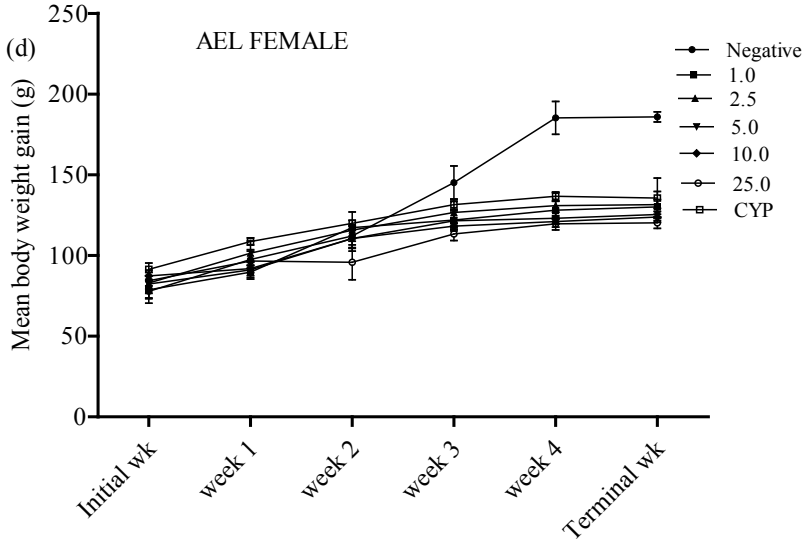

Figure 2d. Mean body weights of female rat orally exposed to different concentrations of Aba-Eku landfill leachate (AEL) for 30 days. CYP – Cyclophosphamide (40 mg/Kg bwt)

Table 1. Absolute and relative liver and kidney weights gain in rats exposed to Olusosun and Aba-Eku landfill leachates

Test sample Organ	OSL				AEL			
weight Sex/ conc. (%)	ALW	RLW	AKW	RKW	ALW	RLW	AKW	RKW
Male								
DW	5.94±0.27	3.02±0.18	0.63± 0.03	0.32±0.02	5.94±0.27	3.02±0.18	0.63±0.03	0.32±0.02
1	5.96±0.07	4.55±0.02[a]	0.78±0.12	0.59±0.01[a]	5.94±0.32	4.46±0.14[a]	0.79±0.01	0.60±0.00[a]
2.5	6.12±0.05	4.62±0.08[a]	0.89±0.04[a]	0.67± 0.01[a]	5.96±0.08	4.48±0.33[a]	0.88±0.00[a]	0.66±0.05[a]
5	6.51±0.06	5.03±0.76[a]	0.92±0.20[a]	0.71±0.11[a]	5.98±0.10	4.48±0.82[a]	0.93±0.00[a]	0.69±0.12[a]
10	6.66±0.12[a]	5.26±0.37[a]	0.98±0.05[a]	0.77± 0.04[a]	6.22±0.05	4.76±0.38[a]	0.94±0.20[a]	0.72±0.05[a]
25	7.13±0.26[a]	5.86±0.80[a]	0.99±0.21[a]	0.81±0.21[a]	6.58±0.13	5.48±0.11[a]	0.95±.0.01[a]	0.79±0.20[a]
CYP	6.61±0.10	4.82±0.11[a]	0.89±0.40[a]	0.65±0.01[a]	6.61±0.10	4.82±0.11[a]	0.89±0.40[a]	0.65±0.01[a]
Female								
DW	5.26±0.18	2.83±0.03	0.52±0.13	0.28±0.02	5.26±0.18	2.83±0.03	0.52±0.13	0.28±0.02
1.0	5.34±0.06	4.18±0.02[a]	0.73±0.00	0.57±0.01[a]	5.39±0.11	4.14±0.42[a]	0.76±0.01[a]	0.58±0.07[a]
2.5	5.37±0.05	4.33±0.07[a]	0.84±0.00[a]	0.68±0.02[a]	5.80±0.06	4.41±0.48[a]	0.78±0.20[a]	0.59±0.06[a]
5.0	5.62±0.04	4.47±1.05[a]	0.89±0.00[a]	0.71±0.14[a]	6.02±0.05	4.80±0.10[a]	0.79±0.12[a]	0.63±0.31[a]
10.0	6.10±0.10[a]	5.05±1.06[a]	0.97±0.01[a]	0.80±0.17[a]	6.14±0.04[a]	5.00±0.04[a]	0.81±0.30[a]	0.65±0.01[a]
25.0	6.87±0.17[a]	5.19±0.12[a]	0.98±0.00[a]	0.83±0.00[a]	6.32±0.06[a]	5.25±0.06[a]	0.86±0.01[a]	0.71±0.21[a]
CYP	5.27±0.08	3.96±0.35[a]	0.80±0.11[a]	0.59±0.06[a]	5.27±0.08	3.96±0.35[a]	0.80±0.11	0.59±0.06[a]

Values are in mean ± SE.
Superscripts differ significantly ($p<0.05$) from corresponding DW using Dunnett's multiple post hoc test.
ALW- Absolute liver weight (g) RLW- Relative liver weight (g).
AKW- Absolute kidney weight (g) RKW- Relative kidney weight (g).
DW- Distilled water.
CYP- Cyclophosphamide (40 mg/kg/bw).

Biochemical indicators of hepatic and renal functions

Figure 3(a–f) shows the results of serum biochemical tests. There is a concentration dependent statistical significant increase in the activities of serum AST and ALT enzymes in leachate exposed rats compared to the negative control. This increase was mostly due to the tested leachate concentrations [60.48% (ALT) and 72.29% (AST) of the total variance] rather than rat sex [0.40% (ALT) and 0.42% (AST), $p<0.0001$] and their interactions [35.38% (ALT) and 26.94% (AST), $p<0.0001$]. The intensities of creatinine and urea in the serum of leachate and CYP exposed rats were significantly ($p<0.001$, $p<0.01$, $p<0.05$) higher than in the negative control except for creatinine where the values at the 1% concentration of both AEL and ORL (male and female) and 5% of ORL (male) were lower than in the negative control. The interactive effects of tested leachates on the sex was more prominent in the intensity of creatinine (48.78%, $p<0.0001$) rather than the effects of leachate concentration and rat sex alone (figure 3c) while leachate concentration affected the intensity of urea most (51.37%, $p<0.0001$) rather than sex and the interactions of leachate and sex (figure 3d). ALT, AST and creatinine were higher in male rats than females

while urea was higher in female than male. The intensities of serum albumin and total proteins of leachate and CYP treated rats showed concentration dependent statistical significant ($p<0.001$, $p<0.01$, $p<0.05$) decrease compared to the negative control. The interactive effects of leachate on the sex was prominent [57.29% (Albumin) and 62.77% (protein), $p<0.0001$] than the effects of leachate [30.85% (albumin) and 31.98% (protein) $p<0.0001$] and sex [1.48% (albumin) and 1.15% (protein), $p<0.0001$] (figure 3e and f) alone. The values of albumin and total protein were higher in females than males.

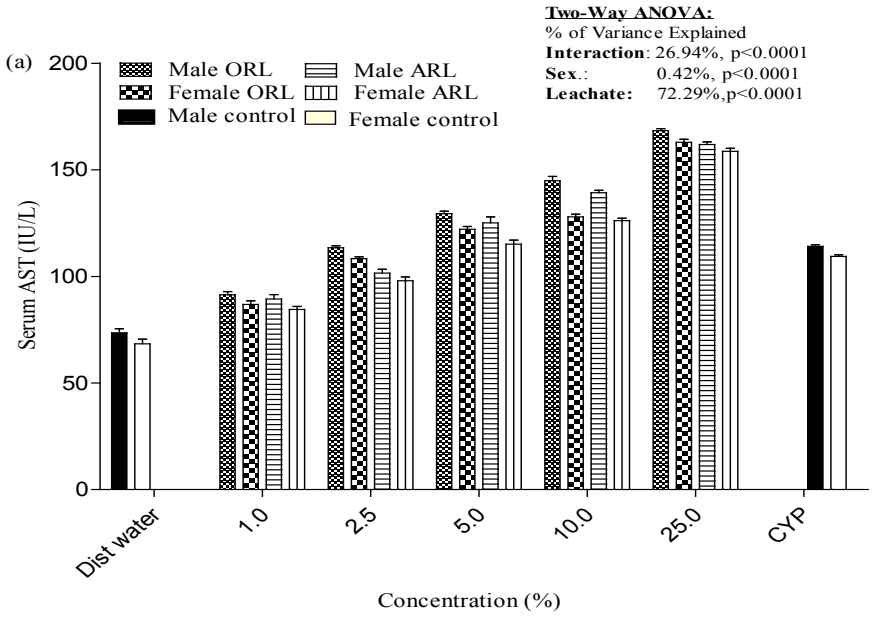

Figure 3a. Effects of Olusosun and Aba-Eku landfill leachates on serum AST activities. End point represents mean ± SE for 5 rats/sex. All values are significantly different ($p<0.001$) compared to negative control. CYP – Cyclophosphamide (40 mg/Kg bwt)

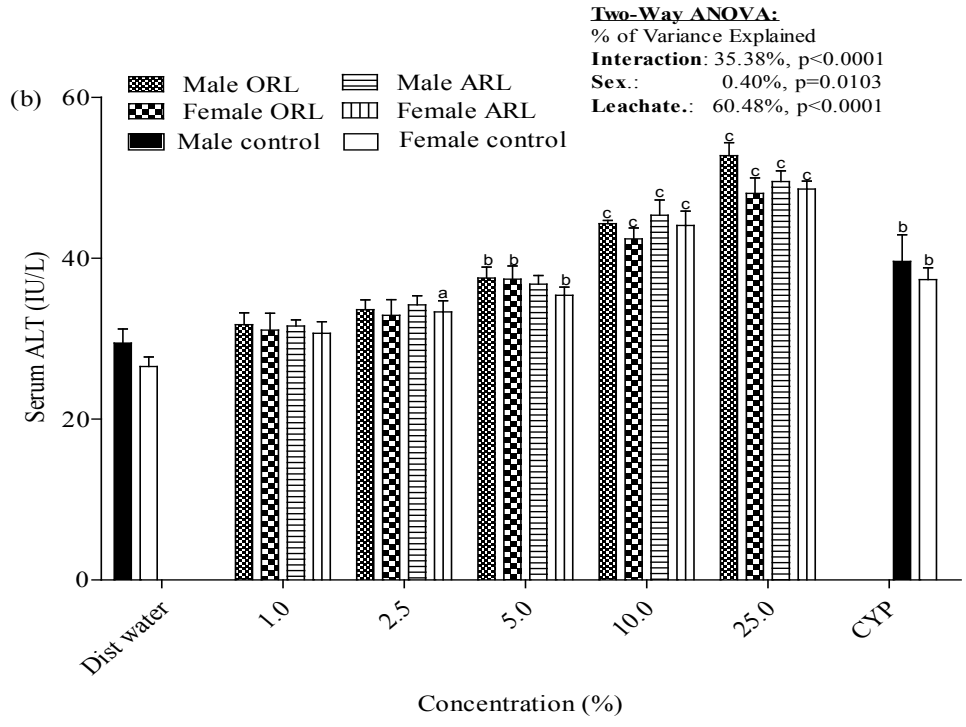

Figure 3b. Effects of Olusosun and Aba-Eku landfill leachates on serum ALT activities. End point represents mean ± SE for 5 rats/sex. Values are significantly different [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ compared to negative control. CYP – Cyclophosphamide (40 mg/Kg bwt)

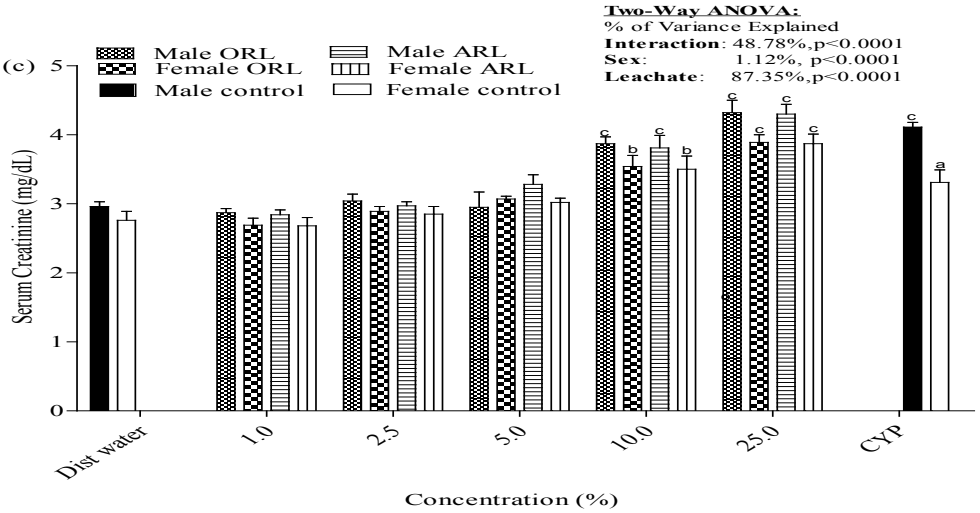

Figure 3c. Effects of Olusosun and Aba-Eku landfill leachates on serum creatinine. End point represents mean ± SE for 5 rats/sex. Value are significantly different [a]p<0.05, [b]p<0.01 and [c]p<0.001 compared to negative control. CYP – Cyclophosphamide (40 mg/Kg bwt)

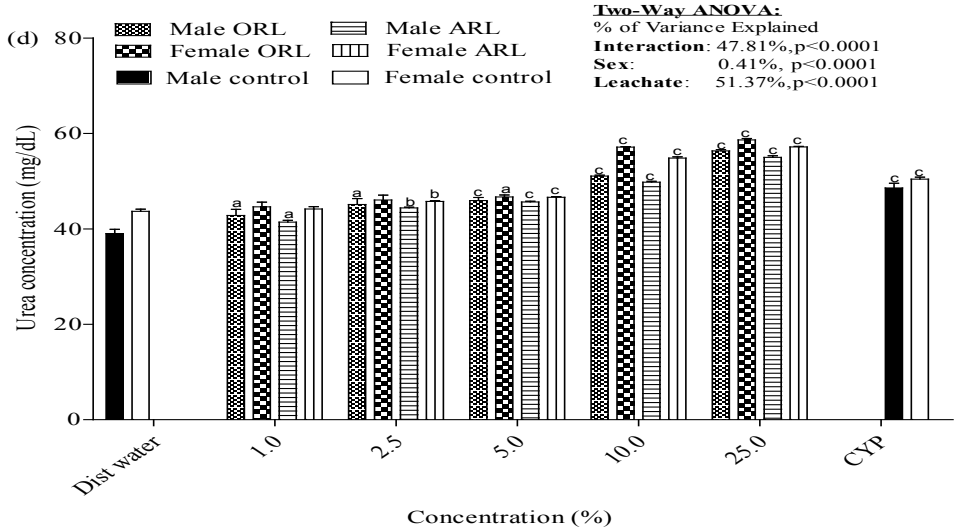

Figure 3d. Effects of Olusosun and Aba-Eku landfill leachates on serum urea. End point represents mean ± SE for 5 rats/sex. Values are significantly different [a]p<0.05, [b]p<0.01 and [c]p<0.001 compared to negative control. CYP – Cyclophosphamide (40 mg/Kg bwt)

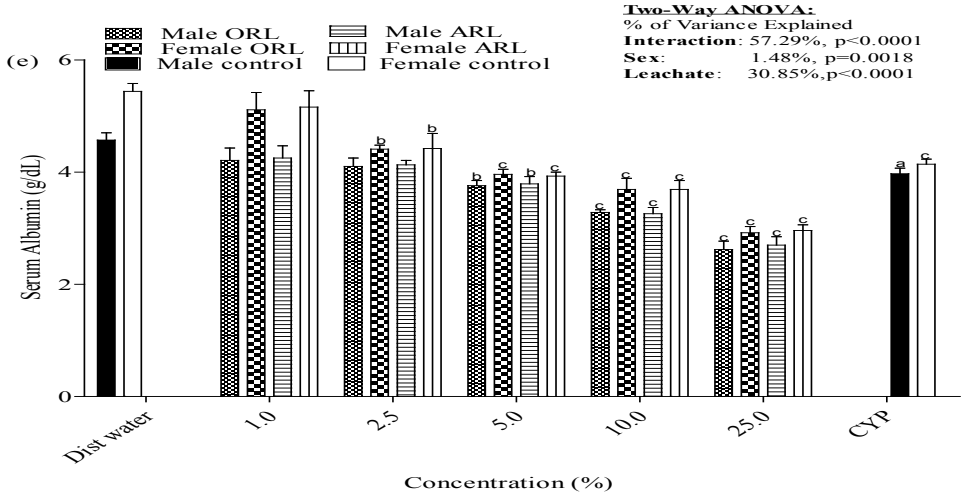

Figure 3e. Effects of Olusosun and Aba-Eku landfill leachates on Serum albumin. End point represents mean ± SE for 5 rats/sex. Values are significantly different [a]p<0.05, [b]p<0.01 and [c]p<0.001 compared to negative control. CYP – Cyclophosphamide (40 mg/Kg bwt)

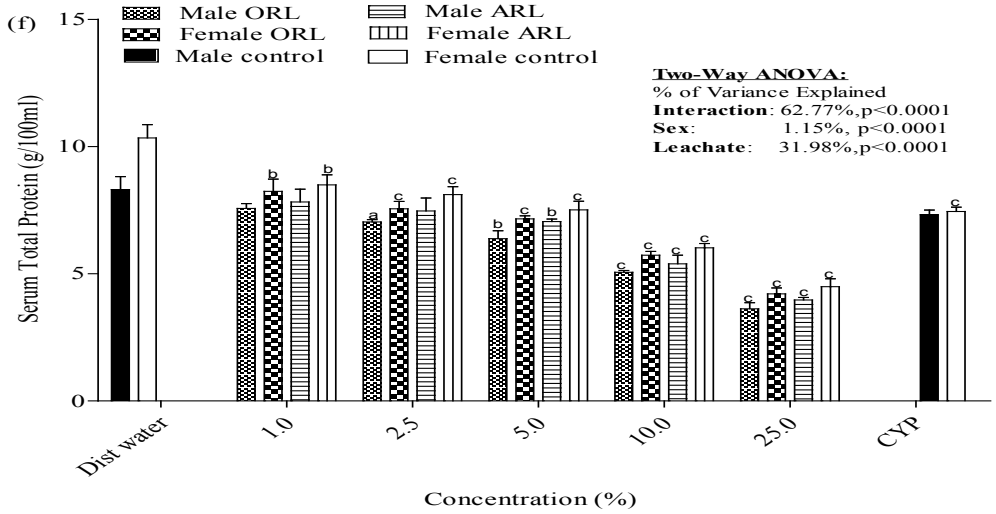

Figure 3f. Effects of Olusosun and Aba-Eku landfill leachates on serum total proteins. End point represents mean ± SE for 5 rats/sex. Values are significantly different $^{a}p<0.05$, $^{b}p<0.01$ and $^{c}p<0.001$ compared to negative control. CYP – Cyclophosphamide (40 mg/Kg bwt)

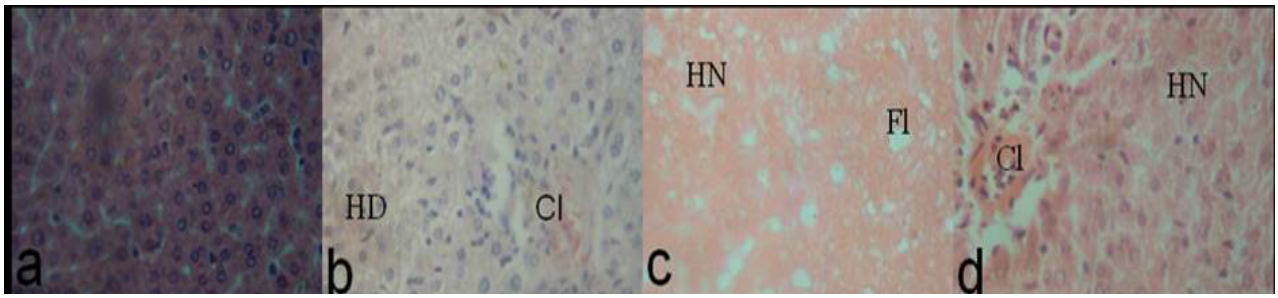

Figure 4. Sections of the liver of rat exposed to Olusosun and Aba-Eku landfill leachates (H&E, x 400). (a) Section of liver from rat in the negative control group hexagonal or pentagonal lobules with central veins. Hepatocytes are arranged in trabecules running radiantly from the central vein. They are regular and contain a large spheroidal nucleus. Some cells have two nuclei each. (b) Diffuse hepatic hydropic degeneration (HD), with mild congestion of the portal vessels. There is also moderate periportal cellular infiltration (CI) by mononuclear cells. These lesions made the trabecular structure to be blurred (c) Diffuse severe hepatic necrosis (HN) and fatty infiltrations (FI). The cytoplasm of most cells is vacuolated. (d) Severe multifocal hepatic necrosis (HN). There is periportal cellular infiltration (CI) by macrophages and neutrophils. Cytoplasm of some cells is empty with vacuole-like space

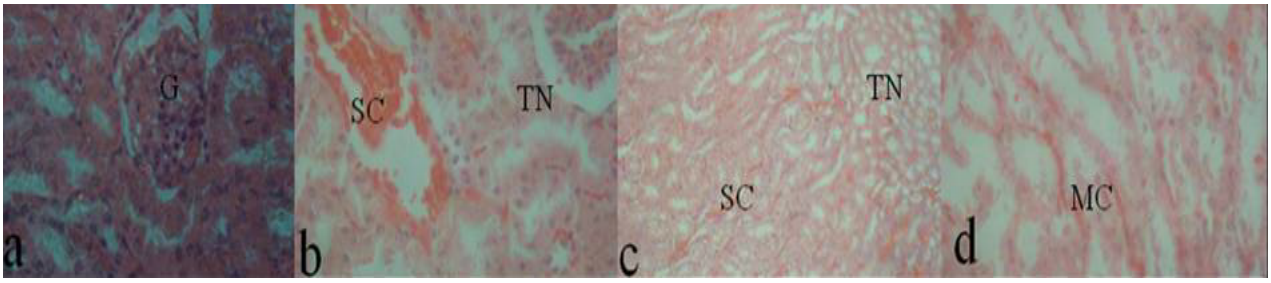

Figure 5. Sections of kidney of rats exposed to Olusosun and Aba-Eku landfill leachates (H&E, x 400). (a) Renal cortex of a control rat. Glomeruli are normal and tightly filing the Bowman's corpuscle (G). Renal tubules are lined with typical thick cubic epithelium (b) Foci of severe congestion (SC), mild interstitial hemorrhage and tubular necrosis (TN) at the renal cortex (c) Mild tubular necrosis (TN) and severe congestion (SC) at the renal cortex (d) Mild congestion of the thin section of Henle's loop in the renal medulla

Histopathological assessment of liver and kidney

The histological presentation of the hepatic sections of negative control rats showed a normal architecture of the hepatocytes. The lesions observed in exposed rats ranged from mild to severe multifocal degenerative and necrotizing hepatitis, which was shown by diffuse hydropic degeneration of hepatocytes and multiple foci of periportal zones. Shrunken hepatocytes and diffused hepatic necrosis with cellular infiltration by macrophages and lymphocytes were also observed in leachate and CYP treated rats (Fig. 4a – d). Normal histology of the cortex and medulla was observed in the kidney of negative control rat while some nephrotoxic lesions were observed in exposed rats. These lesions ranged from mild to severe cortical congestions, degenerative epithelia of renal tubules, interstitial hemorrhage at the cortical region and renal necrotic changes (Fig. 5a – d). The frequency and severity of histopathological alterations in liver and kidney were increased in leachate exposed rats compared to the negative control (table 2) but sex differences were not evident.

Table 2. Frequency and severity of histopathological alterations in liver and kidney of rats exposed to Olusosun and Aba-Eku leachates

Sex	Male						Female					
Conc.(%)	nc	1	2.5	5	10	25	nc	1	2.5	5	10	25
OLUSOSUN LEACHATE (OSL)												
Histological changes												
LIVER												
Necrosis	0	0	1	2	3	4	0	0	1	2	3	4
Cellular infiltration	0	1	1	1	2	3	0	1	1	2	3	4
Congestion	0	0	1	1	3	4	0	0	1	1	2	3
Degeneration	0	0	0	2	3	4	0	0	1	2	3	4
Vacuolation	0	0	0	1	2	3	0	0	0	0	2	4
KIDNEY												
Congestion	0	0	1	2	2	4	0	0	0	1	2	3
Necrosis	0	0	1	2	3	4	0	0	1	1	2	3
Haemorrhage	0	0	0	1	2	3	0	0	0	1	2	2
ABA-EKU LEACHATE (AEL)												
Histological changes												
LIVER												
Necrosis	0	1	1	2	3	4	0	0	1	2	2	3
Cellular infiltration	0	0	1	1	2	3	0	1	1	2	3	4
Congestion	0	1	1	1	3	4	0	0	1	1	2	4
Degeneration	0	0	0	1	2	3	0	0	1	1	2	3
Vacuolation	0	0	1	1	2	3	0	0	1	1	2	4
KIDNEY												
Congestion	0	0	1	2	3	4	0	0	1	2	2	3
Necrosis	0	0	1	1	3	4	0	0	1	2	3	4
Haemorrhage	0	0	1	1	2	3	0	0	0	1	2	3

nc- negative control (distilled water)
Severity of liver and kidney histological changes were assessed using the following scale:
0 = a change that was either absent or sporadic in all animals of a group;
1= a change that was found in a few animals of a group;
2= a change that was rare in all animals of a group;
3 = a change that was relatively common in all animals of a group;
4= a change that was very often found in all animals of a group.

Table 3. Physico-chemical parameters and heavy metals analyzed in Olusosun and Aba-Eku landfill leachates

Parameters	AEL	OSL	NESREA[a]	USEPA[b]
Colour	Dark brown	Dark brown	-	-
pH	7.8	8.1	6.0 – 9.0	6.5 – 8.5
Nitrate	54.4	72.3	10	10
Ammonia	86.4	122.1	10	0.02
BOD[c]	601	594	50	-
COD[d]	512	487	90	-
Phosphate	122.02	215.70	2.0	-
Chloride	1106	1099	250	250
Sulphate	114.34	218.12	250	250
Hardness	532	615	-	0 - 75
Alkalinity	502	623	150	20
TS[e]	3116.67	4100.3	-	-
Cu	2.44	3.86	0.5	1.3
Fe	3.20	4.71	-	0.3
Pb	2.08	2.00	0.05	0.015
Cd	1.44	2.20	0.2	0.05
Mn	2.90	3.10	0.2	0.05
As	1.50	2.60	-	0.01
Ni	1.88	2.51	0.05	-
Cr	2.32	2.43	0.05	0.1

* All values are in mg/L except pH.
[a]National Environmental Standards and Regulations Enforcement Agency (2009) (Nigeria) maximum permissible limits for effluent from wastewater[75].
[b]United State Environmental Protection Agency (2006) (www.epa.gov/safewater/mcl.html).
[c]Biochemical Oxygen Demand.
[d]Chemical Oxygen Demand.
[e]Total Solid.

Physicochemical characteristics

The physico-chemical parameters and heavy metals analyzed in the tested leachate samples are shown in Table 3. The leachates were foul smelling and dark brown coloured with slightly alkaline pH values. The values of COD, BOD, TS, hardness, alkalinity, chlorides, phosphate, sulphate, nitrate, ammonia, Cr, Ni, Mn, Cd, Pb, Fe, As and Cu, were high compared to standard permissible limits. The concentration of these parameters in OSL is higher than in AEL.

4. Discussion

Landfill leachate is a major source of chemical pollutants in terrestrial and aquatic environments, hence a threat to public health. Hepatomegaly, liver and renal dysfunctions have been associated with human exposure to hazardous wastes[3,28-29]. This indicates that the liver and kidney are among the susceptible mammalian organs to hazardous waste chemicals. In this study, we investigated the hepatotoxic and nephrotoxic effects of municipal landfill leachates in rats. Significant decrease in the weekly weight gain of treated rats followed by concomitant increase in both absolute and relative liver and kidney weight gain observed has been implicated with hepatotoxicity and nephrotoxicity[30-31]. It suggests that obstructions of these organs by the leachate constituents, likely the heavy metals, may be responsible for the change in organ weight, due to the role of these organs in sequestering metals immediately following their entry into the body systems[32]. Bakare et al.[33] and Li et al.[12] had previously reported that mice exposed to landfill leachate had reduced body weight gain and increased relative liver and kidney weight gain respectively due to chemical toxicity from the leachates. It is possible that the exposed rats herein bioaccumulated heavy metals from the leachates and this caused increased in organ weight gain. This assumption is supported by the findings of Sanchez-Chardi and Nadal[34] and Sanchez-Chardi et al.[35] that bioaccumulation of heavy metals from landfills in white-toothed shrew (*Crocidura russula*) and wood mouse (*Apodemus sylvaticus*) respectively, is associated with increase relative liver and kidney weight gain.

The clinical signs of toxicity observed in the leachate treated rats further confirm the systemic toxicity of landfill leachates. Anorexia (loss of appetite) is one of the common symptoms of liver injury attributed to the exposure of test chemicals and it is consistent with reduced body weight gain in exposed rats[36]. Labored breathing and sneezing observed at tested concentrations suggest respiratory disorder as a result of toxicosis of the chemicals in the leachates[37]. Hair loss, diarrhea, abscess, muscular disorder (muscular stiffness and decreased motor activities), reduced activities, blue discolouration and ungroomed hair are common symptoms of central nervous and immune systems dysfunction[38]. Mortality observed during exposure was probably due to acute effect of the leachate constituents.

In several organs, cell damage is followed by the release of a number of cytoplasmic enzymes into the circulatory system; this provides the basis for clinical diagnosis. Serum AST and ALT are the most used biochemical markers of hepatocellular necrosis and are considered sensitive indicators of hepatic injury[39-40]. Moreso, they have been used as tools to study cell viability, cell death (apoptosis) and changes in cell membrane permeability[41-42]. The marked treatment related and concomitant increase in the activities of serum ALT and AST observed herein indicate acute hepatocellular injury due to leachate constituents induced necrosis[41,43]. It is possible that heavy metals, other leachate constituents (though not analyzed herein) and their metabolites may induced oxidative tissue damage to the hepatocytes which led to increase in the cell membrane permeability and may eventually lead to hepatocytic cell death[44]. The report of Sanchez-Chardi et al.[35] that increase in heavy metal bioaccumulation increased serum transaminases in wood mice exposed to landfill chemicals is in concert with our findings. Workers and residents with liver dysfunctions due to exposure to chemicals from industrial waste vapours[45] and landfill leachate[3] also showed increased ALT and AST.

Serum creatinine and urea concentrations are biomarkers of renal injury[46-47] and the elevation of these biomarkers is usually associated with impairment of renal function[48]. Increase in serum urea and creatinine in leachate treated rats compared to the negative control may indicate kidney injury due to depression of glomerular filtration rate and renal tubular cell injury by Cd, Cr and As analyzed in the leachates[49]. Heavy metals readily bioaccumulate in the kidney and are responsible for a high number of nephrotoxicity observed in mammals[31,44]. The concentration dependent decrease in serum total proteins and albumin in leachate treated rats may indicate disorders in protein synthesis, metabolism and necrosis as a result of individual actions or interactions of the complex chemicals, such as heavy metals and other unidentified constituents of the tested samples. Metals in their ionic forms, bind to albumin and plasma proteins thereby affecting their metabolic processes and/or transport to the kidney tubules[32,50]. It has been similarly reported that rats exposed to Cd showed reduced serum protein with increased serum urea and creatinine due to functional damage to kidney[51-52] and concomitant decrease in serum albumin and serum total protein has been associated with hepatocellular damage[53]. Based on the percentage variance, it is clear that the impact of the tested leachates in the alteration of serum biochemical parameters is higher than the role played by sex difference. Although high level of steroid hormones like testosterone are known to impair enzymic antioxidant defenses and directly induce oxidative stress[54], this may account for differences observed in some biochemical parameters and body and organ weight gain in exposed male and female rats.

Histopathological examination of tissues is useful in identifying the type of lesions caused by xenobiotics and is acknowledged as the most sensitive end point for detecting organ toxicity[55]. It is also useful in providing information about acute or chronic effects of toxic substances that may

not be detected by other biomarkers[56-57]. Chronic exposure to heavy metals and hydrocarbons, pesticides, phenols, phthalates and inorganic compounds commonly found in leachates[58-61] caused the observed lesions in the kidney and liver. The observed lesions may be due to oxygen deficiency and/or the presence of reactive oxygen species (ROS) induced by metals or other leachate components[44]. Hepatic necrosis and cellular infiltrations with inflammatory cells observed in leachate treated rats have been previously reported in workers exposed to chemicals from industrial wastes due to hepatotoxic substances in the generated wastes[45]. Proximal tubular cells are the most susceptible to toxic xenobiotic since they are the first renal epithelial cells to be exposed to filtered toxic compounds hence, degeneration of epithelia of the renal tubules and renal necrotic changes observed in leachate treated rats indicates damage to the kidney.

It may be deduced from these findings that leachate- induced hepatotoxicity and nephrotoxicity in rat may be associated with free radical formation. This is supported by reports on the activities of heavy metals analysed herein (Cu, Fe, Cd, Pb, Ni, Cr, Mn and As) that they exhibit their damaging effects in the liver and kidney through ROS formation[44,57]. Li et al.[14,62] and Bakare et al.[15] had previously reported that landfill leachate induced damage to kidney and liver of exposed mice is associated with increase thiobarbituric acid reactive substances (TBARS) level. Elevation in the level of TBARS with concomitant increase in serum transaminases had been implicated with hepatocellular injury in preterm infants[63]. Hence, increase in serum transaminases observed herein suggests that the tested leachates caused loss of functional integrity to the hepatocellular membrane through free radical formation. Moreover, histopathological findings of inflammatory responses to tissue damage as shown by infiltration, congestions, necrosis and renal interstitial haemorrhage further support the free radical generation mechanisms. This deduction agrees with an *in vitro* study wherein landfill leachates induced cytotoxic damage on human breast cancer MCF-7 cell by necrosis[64].

Inflammatory cells (macrophages, neutrophils and lymphocytes) function by destroying invading microorganisms and removal of necrotic cells and cellular debris through phagocytosis[65-66]. Phagocytotic process is associated with the formation of intracellular ROS through autoxidation[65,67]. These assertions lend credence to abscess formation in the treated rats. Abscess is associated with inflammatory processes which lead to the production of ~ 100 μM of H_2O_2 (ROS)[68]. Autoxidation is associated with genotoxic damage[69] and cytotoxicity[70]. ROS has been associated with lipid peroxidation, protein and DNA damage, aging and carcinogenicity[15,71-72]. If the affected cellular proteins are those responsible for ion balance, it can lead to disruption of actin filament assembly and ATP production[73-74]. Decrease in serum albumin and total proteins may be due to free radical formation which caused protein damage or protein degradation through oxidative stress induction in rats[12,71-72]. It is also possible that some leachate constituents may undergo metabolic activation to toxic metabolites which induced the hepatotoxic and nephrotoxic effects in rats.

In conclusion, raw leachates obtained from Olusosun and Aba-Eku MSW landfills in Lagos and Ibadan, South-western Nigeria induced liver and kidney dysfunctions in Wistar rats. This indicates that landfill leachate is capable of inducing significant hepatotoxic and nephrotoxic effects in animals and human population if there is sufficient and continuous exposure to chemical substances from the landfill/dumpsites. The implication of this on the present and future generations of exposed animals/human population could be grievous if there is bioaccumulation and biomagnification of hazardous chemicals. This is of great importance in Nigeria and other nations where there is lack of effective policy on solid waste management.

ACKNOWLEDGEMENTS

The authors acknowledge Mrs. Shote of Orthopaedic Hospital, Igbobi Yaba, Lagos state Nigeria for assistance on the biochemical analysis; and Sobule F., Mordi J., Shote A. and Okolie V. of Cell Biology and Genetics Department, University of Lagos, Lagos, Nigeria for technical assistance. This study was partly supported by the vote for Postgraduate studies in the Department of Zoology, University of Ibadan, and University of Lagos Doctoral assistance grant (Ref. No. AD/REG/12240) awarded to CGA.

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