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Research Article

Biochemical Evaluation of Oxidative Damage Induced by Municipal Landfill Leachate in the Liver and Kidney of Albino Rats

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Abstract

Contamination of ground water by landfill leachate poses potential health hazards for man and his environment. This study investigated the effects of Olusosun Landfill Leachate (OLL) from Ojota, Lagos State, Nigeria on markers of oxidative stress in the liver and kidney of rats after subchronic exposure. Thirty six male rats were allocated into control (tap water) and OLL-treated (10%, 25%, 50%, 75% and 100%) groups with six animals in each group. The animals were exposed to OLL via drinking water for 90 days. Serum was obtained and used to determine markers of liver and kidney functions: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Gamma glutamyl transferase (GGT), Alkaline phosphatase (ALP), bilirubin, creatinine and urea by spectrophotometry. The following markers of oxidative stress were determined in the Post Mitochondrial Fraction (PMF) of liver and kidney; Lipid Peroxidation (LPO), H_2O_2, glutathione and activities of Glutathione-S-Transferase (GST), Glutathione Peroxidase (GPx), catalase and Superoxide Dismutase (SOD). Exposure to OLL resulted in increase in the activities of ALT, AST, ALP, GGT, SOD, GST and catalase and also increased the concentration of bilirubin, LPO and H_2O_2 in the liver of the rats; however there was a decrease in glutathione level and GPx activity. In the kidney, OLL exposure elevated the concentration of urea, creatinine, MDA, H_2O_2 and GSH and also induced GST and SOD activities but depleted catalase and GPx activities. These results show that OLL exposure resulted in hepatic and renal damages in rats and suggest the role of free radicals in its mechanism of toxicity.

Key words: *Olusosun landfill leachate, hepatotoxicity, nephrotoxicity, lipid peroxidation, oxidative stress.*

Introduction

Solid wastes constitute an important and emerging problem. The common methods of disposing wastes in Nigeria are incineration, landfilling and/ or open dumps at the nearest available space (Alimba *et al*, 2006). Wastes decompose in the landfills/dumpsites through a complex and highly variable processes that lead to leachate and landfill gas production. Leachates contain hazardous constituents which may contaminate ground and surface water leading to adverse impact on human health and the environment. The exposure of the environment to landfill leachate may occur in different ways, such as uncontrolled overflow, rainfall, subsidence and infiltration.

There has been a growing concern on the environmental safety of landfill waste products, such as long term build-up of heavy metals in the soil, effects on groundwater and pathogenic effects (Karrasch *et al*, 2006; Cheng *et al*, 2004). Heavy metals have been implicated for various human health problems even at trace levels. Lead has been implicated in various diseases such as anaemia, brain damage, anorexia, mental deficiency, vomiting and even death in human (Bulut and Baysal, 2006; Low *et al*, 2000). Cadmium has been reported to cause activation of cellular protein kinases (protein kinase C) which results in enhanced phosphorylation of transcription factors and consequently lead to the transcriptional activation of target gene expression (Valko *et al*, 2005). Several researchers have found leachate to be quite toxic to rainbow trout and daphnia (Cameron and Koch, 1980; Atwater *et al*, 1983) and to have some toxicological impact on laboratory mice (Raddi *et al*, 1987). Leachate has been shown to be highly toxic to higher plants (Clement and Bouvet, 1993; Clement and Merlin, 1995; Devare and Bahadir, 1994), algae (Baun *et al*, 1999; Cheung *et al*, 1993; Plotkin and Ram, 1984), invertebrates (Baun *et al*, 1999; Earnst *et al*, 1994; Plotkin and Ram, 1984) and fish (Plotkin and Ram, 1984; Wong, 1989). Li *et al* (2006) reported the oxidative damage induced in hearts, kidneys and spleens of mice by landfill leachates. Clement *et al* (1996) found that the toxic effects of landfill leachate varied substantially between test species, landfill location, and type of waste (i.e. domestic, non-hazardous industrial and hazardous industrial).

Lagos State has an estimated population of 9-17 million people and holds approximately 60% of Nigeria's industrial and commercial centers as well as majority of the foreign trade and import centers in the country (MoELS, 2010). Inadequate solid waste management (SWM) is a major environmental problem in Lagos metropolis. There is an absence of any properly designed solid waste disposal facilities in the state therefore posing contamination risk to both ground and surface waters (Longe and Balogun, 2010). All waste generated in Lagos State end up in three

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state government recognized landfills and many illegal dumpsites (LAWMA, 1998). Olushosun landfill is the biggest among the three landfills in Lagos State and covers about 42-hectares of land with an excavation of about 18m deep into the landfill land. It receives deposits of both domestic and industrial wastes from the State. These waste products are dumped in this landfill untreated; posing environmental risks to life in the area and the entire population directly or indirectly (Ogundiran and Afolabi, 2008). The landfill has no leachate collector, landfill liner and landfill cap and hazardous and non-hazardous wastes are dumped in the landfill which could cause pollution of surrounding groundwater. To avoid possible contamination of groundwater, Dolk (1999) reported that groundwater should not be located within 7 km radius to a landfill, but this is not so for Olushosun landfill as residential houses with groundwater (Wells and Boreholes) as only source of water were constructed at about 1.5km radius to the landfill. These groundwater sources are continually being consumed by home owners despite the possible hazardous effect which the landfill leachate can have on the water quality and public health. Reports presented at seminars and conferences also attested to the prevalence of water related diseases in the inhabitants (LAWMA, 2000).

Physicochemical and heavy metal analysis of leachate from Olushosun landfill in Ojota, Lagos State revealed the presence of toxic constituents and heavy metals such as lead, copper, cadmium, chromium, mercury, arsenite, cobalt, nickel, iron and zinc (Farombi *et al*, 2011). These are sources of reactive oxygen species (ROS) which can generate free radicals in exposed tissues (Borek, 2001). Reactive oxygen species are kept in check by endogenous antioxidants which can be enzymes (superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase) and non-enzymes (glutathione, ascorbic acid, ubiquinones, and tocopherol etc). These antioxidants are electron donors and react with the free radicals to form harmless products such as water, but an imbalance between the production and manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage results in oxidative stress which is associated with many disease states including neurological diseases such as Alzheimer's and Parkinson's disease, chronic heart disease, kidney and liver diseases (Coyle and Puttfarcken, 1993).

Liver and kidney are frequent targets of environmental toxicants as the liver is involved in metabolism and the kidney is involved in the excretion and re- absorption of substances. Although, mammalian cells are equipped with both enzymic and non-enzymic antioxidant defenses with different efficacies that protect animals against oxidative abuse caused by a wide range of nephrotoxicants (Karbownik *et al*, 2001) and hepatotoxicants, these defense systems may be overwhelmed by these toxicants.

To assess the toxicity of chemicals after long term exposure, sub chronic and chronic toxicity studies are usually carried out after initial information on toxicity has been obtained by acute testing. Therefore, this study was designed to investigate the hepatotoxic and nephrotoxic effects of Olushosun landfill leachate in rats after long term exposure and the possible role of free radicals in its mechanism of toxicity.

Materials and Methods

Chemicals

Adrenaline, 1-chloro-2, 4-dinitrobenzene (CDNB), 5', 5'-dithiobis-2-nitrobenzoic acid (DTNB), reduced glutathione (GSH), hydrogen peroxide (H_2O_2), Thiobarbituric acid (TBA), were obtained from Sigma Chemical Company, USA. Urea, Creatinine, Alanine aminotransferase (ALT), Aspartate amino transferase (AST), Alkaline phosphatase (ALP), bilirubin and Gammma glutamyl transferase (GGT) assay kits were obtained from Randox Laboratories (Crumlin, U.K.). All other chemicals were of analytical grade.

Study Area and Leachate Sampling

The sampling site/study area is Olusosun landfill site situated in the Northern part of Lagos State, Nigeria (Latitude 6°34¹N and Longitude 3°24¹E). Olusosun landfill is the biggest among the three landfills in Lagos State and started operation in the year 1992. Olusosun landfill is the deposit of household and industrial wastes generated in Lagos State. It is about 42 hectares in size with a life span of 35 years and receives an average of 1.2 million tons of waste annually (Lasisi, 2011). Raw leachate was collected from leachate wells and thoroughly mixed. The leachate sample was then filtered to remove debris and stored until use. The sample was designated Olusosun Landfill Leachate (OLL).

Animal exposure and experimental design

Thirty six albino rats of the Wistar strain weighing between 100g and 150g were obtained from IAMRAT Animal House, University College Hospital, Ibadan. The rats were divided into 6 groups of 6 animals in each group. Group 1 (control) animals drank distilled water for 90 days. Groups II, III, IV, V and VI animals were exposed to 10%, 25%, 50%, 75% and 100% OLL respectively via drinking water for 90 days. The rats were fed with commercially available standard pelleted feed and water *ad libitum* throughout the experimental period. Rats were sacrificed by cervical dislocation 24hours after the last treatment and blood samples were collected from the retro orbital sinus of the rats into centrifuge tubes. Livers and kidneys were immediately removed, washed in ice cold 1.15% potassium chloride solution, blotted and weighed.

Biochemical assays

Blood samples were centrifuged at 3000g for 10minutes to obtain serum for the determination of serum markers of hepatic and renal damage. Serum activities of Aminotransferase (ALT) and Aspartate amino transferase (AST) were determined by the method of Reitmann and Frankel (1957), Alkaline phosphatase (ALP) activity was determined by the method of Wright *et al, (*1972a), bilirubin was determined according to the method described by Tietz *et al*, (1994), Gammma glutamyl transferase activity was determined following the principle described by Szasz (1976) and serum urea nitrogen was

determined by the method of Veniamin and Vakirtzi – Lemonias (1970). Liver and Kidney samples were homogenized in 4 volumes of homogenizing buffer (50mM Tris-HCl mixed with 1.15% potassium chloride solution and pH adjusted to 7.4), using a Teflon homogenizer. The resulting homogenate was centrifuged at 12,500g for 10 minutes in a Beckman L5-50B centrifuge at 4°C to obtain the post mitochondrial supernatant fraction. Glutathione (GSH) was determined in the post mitochondrial fraction of the liver and kidney of the rats according to Jollow *et al* (1974) at 412 nm using 5, 5-dithio-bis-2-nitrobenzoic acid (DTNB), Glutathione peroxidase (GPx) activity was determined according to the method of Rotruck *et al*, (1973), Glutathione *S*-transferase (GST) activity was determined by the method of Habig *et al* (1974) using 1 chloro 2, 4 dinitrobenzene as substrate. The specific activity of glutathione S-transferase is expressed as nanomoles of GSH-CDNB conjugate formed/min/mg protein using an extinction coefficient of $9.6mM^{-1}cm^{-1}$. Superoxide dismutase (SOD) activity was determined by the method described by Misra and Fridovich (1972). Activity of catalase (CAT) was determined according to the method of Sinha (1972). Lipid peroxidation was determined by measuring malondialdehyde (MDA) formation according to the method described by Farombi *et al* (2000).

Statistical analysis

Results were expressed as mean ± standard deviation. Student's t test was used to determine differences between groups. Levels of statistical significance were analysed by analysis of variance (ANOVA), using microcal origin 6.0 software and p-values < 0.05 were considered significant.

Results

Serum biochemical indices

The results on the serum indices of hepatic and renal damage are presented in tables 1 and 4. Serum markers of hepatic damage increased in a dose dependent manner following sub chronic exposure of rats to OLL. Exposure of rats to 10%, 25%, 50%, 75% and100% OLL gave rise to significant increases ($p<0.05$, $p<0.001$) in activities of serum enzymes ALT, AST, GGT, ALP and bilirubin concentration when compared with control. Serum concentrations of urea and creatinine also increased in rats exposed to OLL when compared with control.

Table 1: Effect of Olusosun Landfill Leachate (OLL) on serum markers of hepatic damage in rats after sub chronic exposure.

Treatment Groups	AST Activity (U/I)	ALT Activity (U/I)	GGT Activity (U/L)	ALP Activity (U/I)	Direct Bilirubin (µmole/L)	Total Bilirubin (µmole/L)
I (CONTROL)	19.8±2.8	23.8±1.9	0.61±0.05	45.6±5.9	6.40±0.7	3.89±0.3
II (10% OLL)	25.3±2.7*	26.5±1.9	0.84±0.2	98.0±2.0**	7.01±1.6	3.95±0.2
III (25% OLL)	30.8±3.3**	29.0±5.7	1.51±0.3**	102.1±11.0**	7.63±4.2	5.80±0.9
IV (50% OLL)	32.0±1.7**	29.0±2.0*	2.37±0.4**	142.6±13.0**	7.01±0.5	9.81±1.9*
V (75% OLL)	32.6±7.6*	30.0±2.4*	3.36±0.6**	240.1±28.0*	8.49±0.9*	14.80±0.9**
VI (100% OLL)	36.0±13.4*	30.0±1.7*	3.45±1.0**	275.1±45.9*	8.77±2.1	15.1±3.9*

Values are expressed as Mean±S.D. Significantly different from control,* $p<0.05$;**$p<0.001$.

Table 2: Effect of Olusosun Landfill Leachate (OLL) on the activities of catalase, glutathione peroxidase and superoxide dismutase in the livers of rats after subchronic exposure.

Treatment Groups	Catalase (µmol/mg Protein)	Glutathione Peroxidase (nmoles / mg Protein / Min	Superoxide Dismutase (Units/g Tissue)
I (CONTROL)	15.1±0.9	5.44±1.0	0.3±0.1
II (10% OLL)	16.0±2.3	3.25±0.7	0.8±0.2*
III (25% OLL)	17.6±2.8	3.02±1.1	1.0±0.3*
IV (50% OLL)	18.6±1.2*	2.81±1.7	1.5±0.6*
V (75% OLL)	18.4±1.6	2.71±0.7*	0.9±0.3
VI (100% OLL)	19.4±4.9	2.98±0.3	2.1±0.7*

Values are expressed as Mean±S.D. Significantly different from control,* $p<0.05$.

Table 3: Effect of Olusosun Landfill Leachate (OLL) on the levels of glutathione (GSH), malondialdehyde (MDA), hydrogen peroxide generation (H_2O_2) and glutathione-S-transferase (GST) activity in the livers of rats after subchronic exposure.

TREATMENT GROUPS	GSH (µg/ml)	MDA (nmoles/mg protein)	H_2O_2(µmole H_20_2/min)	GST(µMOLES/min/mg protein)
I (CONTROL)	21.8±0.4	2.6±0.2	20.8±1.9	2.30±0.7
II (10% OLL)	17.0±2.0*	3.3±0.8	21.0±2.0	8.10±1.2*
III (25% OLL)	16.7±3.2	3.7±0.5*	25.8±2.5	13.2±5.0*
IV (50% OLL)	14.7±1.5*	4.1±0.8*	27.7±1.2*	10.1±1.2**
V (75% OLL)	12.1±0.6**	4.6±0.5**	31.7±5.0*	19.3±3.0**
VI (100% OLL)	12.7±0.6**	5.3±0.1**	34.3±7.0*	20.8±2.4**

Values are expressed as Mean±S.D. Significantly different from control,* $p<0.05$;**$p<0.001$.

Table 4:
Effect of Olusosun Landfill Leachate (OLL) on serum levels of urea and creatinine in rats after subchronic exposure.

Treatment Groups	Urea (µmoles/liter)	Creatinine (mg/ml)
I (Control)	4.39±1.0	0.8±0.06
II (10% OLL)	5.1±0.8	1.0±0.04*
III (25% OLL)	5.1±2.9	1.1±0.06*
IV (50% OLL)	7.9±2.8	1.0±0.2
V (75% OLL)	11.3±1.3*	1.1±0.1*
VI (100% OLL)	10.6±0.009*	1.0±0.03*

Values are expressed as Mean±S.D. Significantly different from control,* $p<0.05$

Antioxidant status and lipid peroxidation in the liver

Effects of OLL on hepatic antioxidant status and lipid peroxidation are shown in tables 2 and 3. Significantly high level of lipid peroxidation was associated with subchronic exposure to OLL, as shown by high levels of malondialdehyde in the liver of rats exposed to OLL when compared with control. There was also a dose dependent increase in hydrogen peroxide generation in rat liver following exposure to 10%, 25%, 50%, 75% and 100% OLL respectively when compared with control. Subchronic exposure to OLL led to induction of GST, SOD and CAT activities in rat liver when compared with controls but significantly ($p<0.05$, $p<0.001$) decreased hepatic GSH level and GPX activity.

Antioxidant status and lipid peroxidation in the kidney

Antioxidant status and lipid peroxidation in the kidneys of rats after OLL exposure are shown in tables 5 and 6. Renal hydrogen peroxide generation and malondialdehyde level (an empirical index of lipid peroxidation) increased significantly in a dose dependent manner after subchronic exposure to OLL when compared with control. There was a dose dependent decrease in catalase and GPX activities after OLL exposure compared with control but there was increase in reduced glutathione (GSH) level and activities of SOD and glutathione-S-transferase (GST).

Histology

Photomicrographs of rat liver and kidney sections are shown in Plates 1 and 2. Liver and kidney sections of rats exposed to OLL showed various histopathological damages while sections of liver and kidney from the control group showed no visible lesions

Discussion

The lack of data on waste generation and its effects on health and environment continue to be a major problem, thus hampering proper regulation. Very little is known about the health effects of wastes so that efforts to set exposure levels, to protect humans often prove ineffective. It is pertinent to note that pollution lowers the quality of life in various aspects and affects health and life span (Grover and Kaur, 1999).

TABLE 5:
Effect of Olusosun Landfill Leachate (OLL) on the levels of glutathione (GSH), malondialdehyde(MDA), hydrogen peroxide generation (H_2O_2) and glutathione-S-transferase (GST) activity in the kidneys of rats after subchronic exposure.

Treatment groups	GSH(µg/ml)	MDA(nmoles/mgprotein)	H_2O_2(µmole H_20_2/min)	GST(µMOLES/min/mg protein)
I (CONTROL)	22.0±3.5	5.97±0.86	14.0±0.8	2.0±1.1
II (10% OLL)	26.0±4.9	6.77±0.93	15.6±0.5*	2.0±0.5
III (25% OLL)	40.3±2.9**	8.01±0.97*	18.1±0.8**	7.0±1.2*
IV (50% OLL)	45.3±3.4**	8.34±0.51**	19.6±0.5**	4.0±2.5
V (75% OLL)	46.0±4.4**	8.39±0.17**	19.8±0.3**	17.0±1.6*
VI (100% OLL)	55.7±4.0**	8.46±0.44*	19.3±0.2**	19.0±4.0*

Values are expressed as Mean±S.D. Significantly different from control,* $p<0.05$;**$p<0.001$.

Table 6:
Effect of Olusosun Landfill Leachate (OLL) on the activities of catalase, glutathione peroxidase and superoxide dismutase in the kidneys of rats after subchronic exposure.

Treatment groups	Catalase (µmol/mg Protein)	Glutathione Peroxidase (Nmoles / Mg Protein / Min	Superoxide Dismutase (Units/g Tissue)
I (CONTROL)	28.1±3.6	9.20±1.3	2.3±0.96
II (10% OLL)	19.6±1.3 NS	6.4±0.2*	2.8±0.96
III (25% OLL)	18.6±3.4*	6.1±1.0*	3.5±1.3
IV (50% LL)	17.4±1.8*	5.8±0.6*	5.7±1.2*
V (75% OLL)	16.7±1.0*	5.5±0.4*	9.5±1.7**
VI (100% OLL)	16.2±0.9*	5.3±0.3*	9.6±2.3**

Values are expressed as Mean±S.D. Significantly different from control,* $p<0.05$;**$p<0.001$

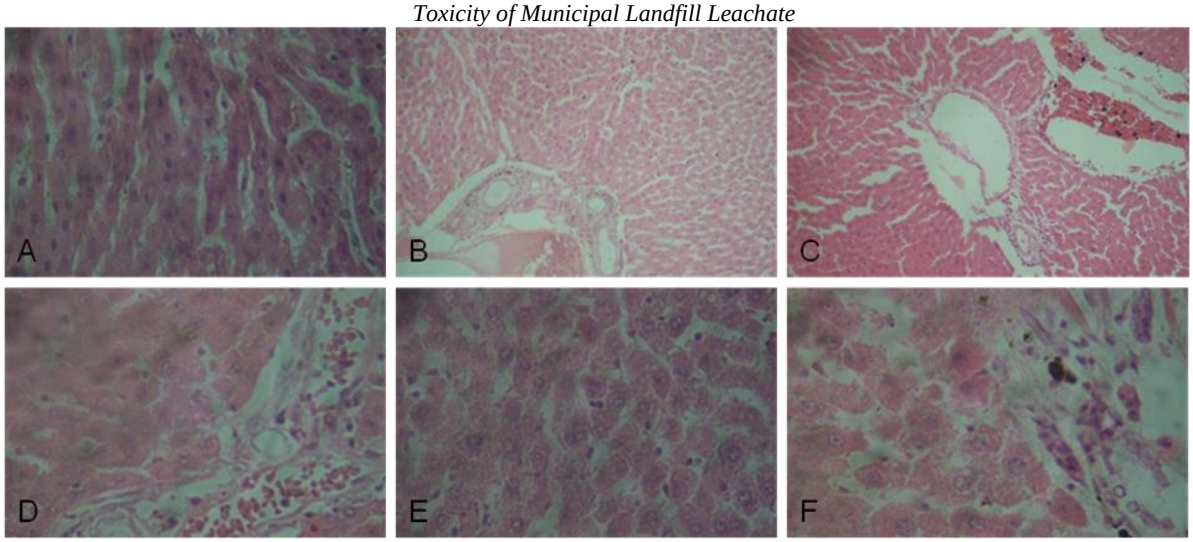

Plate 1

Photomicrographs of the liver sections of rats exposed to various concentrations of OLL. (A) Control; (B) 10% leachate showing moderate portal congestion (C) 25% leachate showing mild periportal cellular infiltration and bile duct hyperplasia (D) 50% leachate showing moderate portal congestion and fibroplasia (D) 75% leachate showing diffuse hydropic degeneration (E) showing mild portal congestion and periportal cellular infiltration

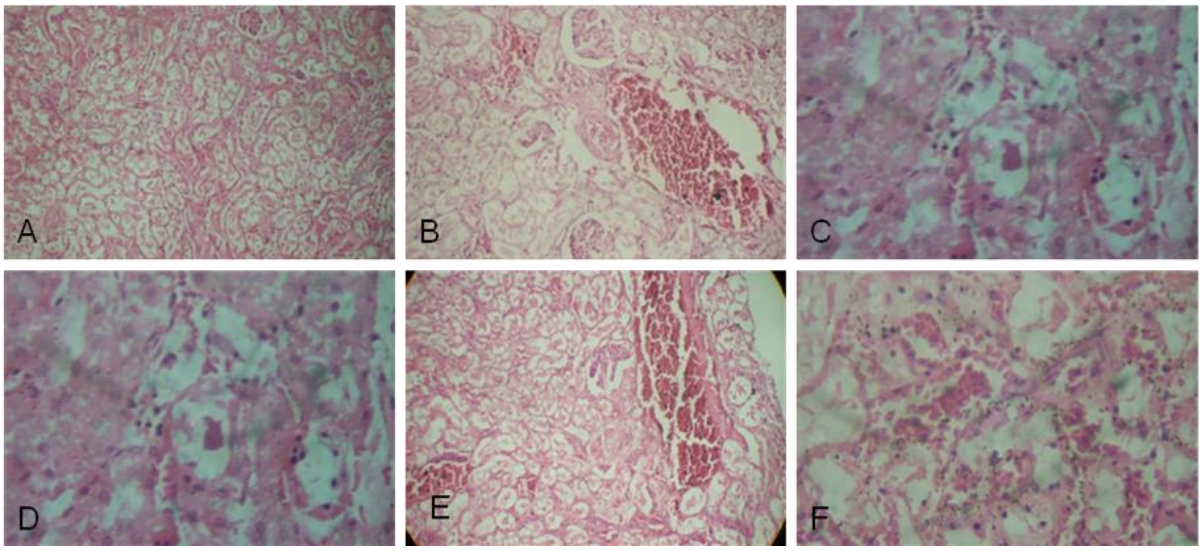

Plate 2

Photomicrographs of the kidney sections of rats exposed to various concentrations of OLL. (A) Control; (B) 10% leachate showing massive renal cortical congestion and interstitial hemorhage (C) 25% leachate showing renal tubular degeneration and protein casts in tubular lumen (D) 50% leachate showing severe periglomerular hemorhage (D) 75% leachate showing severe periglomerular hemorhage (E) showing interstitial hemorhage and cellular infiltration

Besides the direct health effects, the subtle danger of pollutants lies in the fact that they may be mutagenic or toxic and lead to several human afflictions like cancer, atherosclerosis, cardiovascular diseases and premature ageing (Grover and Kaur, 1999). Many pollutants contain a complex mixture of contaminants that may behave independently, or may produce synergistic, antagonistic or additive effects (Reynoldson and Day, 1998). One of the sources of such complex pollutants are municipal landfill sites, where waste is buried in landfill pits. The matrix of contaminants produced by landfills is termed leachate once it has been mixed with rainwater and/or groundwater. Landfill leachate had been implicated in developmental anomalies, birth defects, series of human health disorders and surface and ground water pollution worldwide.

Serum activities of AST, ALT, ALP and GGT were significantly ($p<0.05$, $p<0.001$) increased in rats after subchronic exposure to OLL. Elevation of AST has been reported to be index of hepatocellular injury in rats, while ALT elevation is more associated with necrotic state (Navarro and Senior, 2006). The elevated activities of these serum enzymes could be due to their release from the cytosol into the blood circulation rapidly after rupture of plasma membrane and cellular damage. Also OLL exposure resulted in significant increase in serum urea and creatinine concentrations. The increase in serum concentrations of creatinine and urea observed in this study suggest a decrease in creatinine clearance and urea excretion. It is believed that the capacity for tubular absorption may have been altered in the kidney of rats after

exposure to OLL, thus bringing about functional overload of nephrons with subsequent renal dysfunction.

Malondialdehyde (MDA) was significantly ($p<0.05$, $p<0.001$) elevated in the liver and kidney of rats after subchronic exposure to OLL. This result is consistent with the results of Li *et al* (2006) on oxidative damage induced in heart, kidney and spleen of mice treated with landfill leachate. One of the various markers of oxidative stress is MDA, an end product of lipid peroxidation. Elevated lipid peroxidation observed in the liver and kidney of the rats indicates that reactive oxygen species (ROS) may be associated with the metabolism of OLL leading to peroxidation of membrane lipids of the respective organs. The observed lipid peroxidation resulting possibly from ROS generated by OLL may lead to cell apoptosis. Oxidative stress induced as a result of ROS generation has been shown to be triggers of apoptosis (Shen and Liu, 2006). Exogenous ROS such as H_2O_2 as well as endogenously produced ROS have been shown to induce apoptosis in many cell types (Ueda *et al*, 2002; Chandra *et al*, 2000). The levels of hydrogen peroxide generation were also found to be elevated in the liver and kidney of rats exposed to OLL further confirming the involvement of ROS in the mechanism of toxicity of OLL.

Antioxidant enzymes constitute a mutually supportive team of defence against reactive oxygen species (ROS). The antioxidants protect the organism against oxyradical damage, such as DNA strand breaks, protein oxidation and the induction of lipid peroxidation (Winzer *et al*, 2000). Exposure to OLL led to increase in the activities of SOD and GST in the liver and kidney of rats. This result is consistent with earlier report of Eriyamremu *et al*, (2007). The increased activity of SOD in the liver and kidney of rats exposed to OLL may be due to the enhanced lipid peroxidation in these organs. This would cause an increased accumulation of superoxide radicals, thereby causing the induction of SOD to mop up the radicals. This may be explained as a compensation mechanism against OLL intoxication. The first lines of defence against toxicity are cytosolic and mitochondrial superoxide dismutases (Cu, Zn-SOD and Mn-SOD) which catalyse the dismutation of superoxide anion radical to hydrogen peroxide (Southern and Powis, 1998; Niwa *et al*, 1993). Also the increase in the activity of GST in the liver and kidneyof rats ater exposure to OLL indicates that OLL exposure led to induction of GST in these organs which shows the protective role of the enzyme against lipid peroxidation. Antioxidant enzymes behave as defense mechanisms against oxidizing environment and help for adaptation to new conditions (Uner *et al*, 2001). Glutathione-S-transferase (GST) detoxifies a number of environmental carcinogens, reactive nucleophile, and epoxides intermediates. The increased GST assay was suggested as a useful tool for biomonitoring oxidative stress (Di Giulio *et al*, 1993). A number of studies also reported increase in antioxidant enzyme activities in the presence of excess ROS (Cheung *et al*, 2004; Pellerin-Massicotte, 1997). Glutathione peroxidase activity was considerably depleted in the liver and kidney of rats after OLL administration. The decrease in GPX activity observed in the liver and kidney of the rats is probably due to excessive generation of reactive oxygen species which depleted GPX, thereby affecting functional as well as structural integrity of cell and organelle membranes. This correlates with earlier findings of Regoli *et al*, 2005. There was also a dose-dependent decrease in catalase activity in the kidney but the activity of this enzyme was induced in the liver. The increase in catalase activity in the liver may be due to increase in the generation of H_2O_2 after OLL exposure. The peroxy radical H_2O_2 was trapped by catalase that primarily occurs in peroxisomes. The target function of catalase is to protect the cells from the accumulation of H_2O_2 by dismuting it to form H_2O and O_2 or by using it as an oxidant where it works as a peroxidase. The increase may also be due to increased activity of SOD in the liver, SOD converts superoxide anions to H_2O_2 which is removed by catalase (Aebi, 1984). This result is consistent with earlier report of Guangke *et al* (2005). The decrease in catalase activity in the kidney shows that the activity of the enzyme was overwhelmed in the kidney by the ROS generated by the leachate. It is known that activities of antioxidant enzymes are overwhelmed by excessive generation of ROS. This could also be attributed to super oxide anion generated in the kidney by the leachate; super oxide anion converts catalase to the feroxyl and ferryl states that are inactive forms of the enzyme (Kono and Fridovich, 1982).

Hepatic GSH level was considerably depleted after OLL administration but there was a dose dependent increase in the kidney. The decrease in GSH level in the liver suggests its over-utilization to challenge the prevailing oxidative stress under the influence of ROS generated from OLL. The increase in GSH concentration observed in the kidney may be as a result of an induction mechanism for the neutralization of reactive oxygen species. To neutralize the impact of ROS, both enzymic and non enzymic antioxidants are activated (Filho, 1996). Increased activities of antioxidant enzymes and high GSH pools seem to be important in attenuating oxidative damage to cells.

Histopathological evaluation of target tissues is a suitable biomarker that provides important qualitative and quantitative information about acute or chronic effects of toxic compounds, sometimes not so finely predicted by other parameters (Reynolds *et al*, 2006; Jadhav *et al*, 2007; Thijssen *et al*, 2007). Histopathological examination of the liver and kidney of rats exposed to OLL also provided evidence for the hepatic and renal damages induced in rats after subchronic exposure to the leachate as shown by various histopathological damages in the liver and kidney of rats exposed to OLL when compared with control. Portal congestion, periportal cellular infilteration and bile duct hyperplasia observed in the liver of rats exposed to OLL confirms that OLL is hepatotoxic in rats. Tubular cells are susceptible to toxicants because they are exposed to filtered toxic compounds, therefore renal cortical congestion, renal tubular degeneration and presence of protein casts in the tubular lumen observed in rats after exposure to OLL further provide evidence for the ability of OLL to cause damage to the kidney. The observed histological changes in the liver and kidney of rats exposed to OLL suggest that subchronic exposure to OLL caused irreversible cell death, as well as cellular damage in rats. This could be as a result of toxic effects of heavy metals and other toxic compounds

detected in the leachate sample. Mitsumori *et al*, 1998 reported similar changes in liver and kidney histology and function of rats exposed to cadmium.

In conclusion, OLL induced liver and kidney injury in rats as indicated by the elevation of serum markers of hepatic and renal damage. Also marked induction of lipid peroxidation as well as alterations in antioxidant status in the liver and kidney of rats exposed to OLL suggest the involvement of ROS in the mechanism of toxicity of the leachate. It is therefore recommended that concrete liners or geoliner be provided for Olusosun landfill to prevent leachate from percolating or diffusing into adjoining water bodies and ground water sources in the area should be routinely checked for pollutants due to possible contamination by leachate. There should also be proper monitoring of wastes dumped at Olusosun landfill and other landfills in Nigeria to safe guard the health of inhabitants of Olusosun and people living close to landfill sites.

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