BENI-SUEF UNIVERSITY JOURNAL OF BASIC AND APPLIED SCIENCES 4 (2015) 284–290

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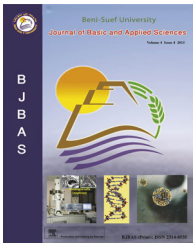

Full Length Article

Brain dysfunctions in Wistar rats exposed to municipal landfill leachates

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ARTICLE INFO

Article history:
Received 24 January 2015
Accepted 19 November 2015
Available online 10 December 2015

Keywords:
Brain damage
Brain weight index
Histopathology
Landfill leachates
Oxidative stress
Rats

ABSTRACT

Brain damage induced by Olusosun and Aba-Eku municipal landfill leachates was investigated in Wistar rats. Male rats were orally exposed to 1–25% concentrations of the leachates for 30 days. Catalase (CAT) and superoxide dismutase (SOD) activities, and malondialdehyde (MDA) concentrations in the brain and serum of rats were evaluated; body and brain weight gain and histopathology were examined. There was significant ($p < 0.05$) decrease in body weight gain and SOD activity but increase in absolute and relative brain weight gain, MDA concentration and CAT activity in both brain and serum of treated rats. The biochemical parameters, which were more altered in the brain than serum, corroborated the neurologic lesions; neurodegeneration of purkinje cells with loss of dendrites, perineural vacuolations of the neuronal cytoplasm (spongiosis) and neuronal necrosis in the brain. The concentrations of Cr, Cu, Pb, As, Cd, Mn, Ni, sulphates, ammonia, chloride and phosphate in the leachate samples were above standard permissible limits. The interactions of the neurotoxic constituents of the leachates induced the observed brain damage in the rats via oxidative damage. This suggests health risk in wildlife and human populations.

1. Introduction

Population growth with subsequent industrial development is the major factor responsible for the inevitable increase in solid waste generation worldwide. Inappropriate disposal of these wastes into unsanitary landfills constitute public health risk and environmental contamination due to landfill gas and leachate production from components of the solid wastes (Alimba, 2013). Leachates contain high concentrations of toxic metals, hazardous organic and inorganic chemicals, radioactive substances, particulate matter and microorganisms; many of which are regarded as emergent environmental contaminants (Efuntoye et al., 2011; Eggen et al., 2010; Øygard and Gjengedal, 2009; Slack et al., 2005). Previously, we observed that mixture of these xenobiotics in leachates from Olusosun and Aba Eku landfills in Nigeria, induced alterations in the liver, kidney, body weight, haematological indices and erythrocyte morphology in rats (Alimba and Bakare, 2012; Alimba et al., 2012). Li et al. (2006, 2010) reported that leachate from Xingou municipal landfill in China induced lipid peroxidation, protein oxidation and disturbed the antioxidant status of liver, spleen, heart, brain and

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http://dx.doi.org/10.1016/j.bjbas.2015.11.003

kidney in exposed mice. It was suggested from these reports that the induction of systemic toxicity by landfill leachates involves free radical generation.

Among mammalian organs, the brain is the most susceptible organ to lipid peroxidation and oxidative injury from xenobiotics due to its membrane rich polyunsaturated fatty acids, low antioxidant status and high iron contents (Jayaraman et al., 2008). Evidence exists that individual chemicals in landfill leachate altered the normal functioning of the nervous system hence increasing neurotoxic diseases among human population (Neal and Guilarte, 2012; Wright et al., 2006). However, there are limited studies on the effects of landfill leachate on the functional and structural integrity of the mammalian brain. Furthermore, neurotoxic assessment is one among the health outcomes suggested by the Agency for Toxic Substances and Disease Registry (ATSDR) to be monitored during exposure to hazardous substances from solid waste disposal sites (Johnson, 1999; Schaumburg et al., 1983). In this study, a 30 day sub-chronic toxicity testing of Olusosun and Aba Eku landfill leachates in Wistar rats was carried out to assess structural alterations in the brain, antioxidant enzyme activities and lipid peroxidation status of the brain and serum, and alterations in brain weight gain. Some physico-chemical parameters and heavy metal compositions of the leachates were also analyzed.

2. Materials and methods

2.1. *Sampling site and leachate collection*

The study sites, Olusosun and Aba-Eku landfills, had been described previously (Alimba, 2013). These landfills were selected due to the high polluting status of the environment through landfill gas and leachate production, which increased public health risk through exposure to landfill chemicals and microorganisms (Alimba, 2013; Efuntoye et al., 2011). Raw leachates were collected from 20 different leachate wells on each of the landfills and thoroughly mixed to produce homogenous samples for each sampling site. The samples were transferred to the laboratory in pre-cleaned 10 litre plastic containers, where they were filtered using glass wool and Whatmann® No. 42 filter paper to remove suspended particles. They were centrifuged at 3000 rpm for 10 minutes and stored at 4 °C. The processed leachates were considered as stock samples (100%) and labelled as Aba-Eku Leachate (AEL) and Olusosun Leachate (OSL).

2.2. *Physical and chemical analysis of the leachate*

Physical and chemical components of the leachates were analysed according to American Public Health Association (APHA) (1998). Nitrate, ammonia, chloride, phosphate, sulphate, total hardness, total alkalinity, biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total solids (TS) were determined. Also iron (Fe), lead (Pb), copper (Cu), manganese (Mn), arsenate (As), cadmium (Cd), chromium (Cr) and nickel (Ni) concentrations were determined according to United States Environmental Protection Agency (USEPA) (1996) and American Public Health Association (APHA) (1998). 100 ml each of the leachate samples was digested by heating with concentrated HNO_3. The resulting mixture was made up to 10 ml with 0.1N HNO_3 and metal concentrations determined using PerkinElmer® A3100 atomic absorption spectrophotometer.

2.3. *Animals and experimental design*

Male Wistar rats (mean ± SD weight; 167.64 ± 4.27 g) were obtained from the animal house unit of College of Medicine, University of Ibadan, Nigeria. They were acclimatized for 2 weeks prior to leachate treatment, and were maintained in laboratory condition of 12 hours dark and light cycle with access to drinking water and standard rodent chow (Ladokun feed Nigeria®) *ad libitum*. Rats in each group (n = 5) was gavaged 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. The leachate concentrations were selected from previous sub-chronic systemic toxicity (Alimba and Bakare, 2012; Alimba et al., 2012), Similar treatment was concurrently given to the negative and positive control groups receiving distilled water and cyclophosphamide (CYP, 40 mg / kg/ bwt) respectively. The animal experiment conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23) (Gad, 2007).

2.4. *Clinical pathology and body and brain weight measurement*

Rats in each of the treatment groups were weighed at the beginning of the experiment using Acculab® USA, Model-vic-303 electronic analytical weighing balance. At the end of exposure, rats were fasted overnight, weighed prior to blood collection and sacrificed. Blood was collected from the orbital plexus using heparinized 70 ml micro-haematocrit capillary tubes into lithium coated serum separator tubes (Sanford, 1954). It was allowed to clot and centrifuged at 3000 rpm for 20 minutes at 4 °C to separate the serum (supernatant). Whole brain from both treated and control rats were surgically removed, rinsed with ice-cold physiological saline and blotted dry to determine the absolute and relative brain weight (brain weight / body weight x 100 g). Brain homogenate was prepared using ice-cold 10 mmol/L phosphate-buffered saline (pH 7.4) containing 0.15 M KCl (10% w/v). The homogenate was centrifuged at 6000 rpm for 30 minutes at 4 °C. Both the brain homogenate and serum (supernatants) were stored at –70 °C prior to biochemical analysis.

2.5. *Biochemical analysis*

The brain homogenate and serum were analysed for antioxidant enzyme activities; superoxide dismutase (SOD; E.C. 1.15.1.1) was assayed according to the method of Nebot et al. (1993), while catalase (CAT; E.C. 1.11.1.6) was in accordance with the method of Johansson and Borg (1988). Lipid peroxidation concentration in the brain homogenate and serum was by malondialdehyde (MDA) determination in accordance with the methods of Esterbauer and Cheeseman (1990). Protein concentration was measured according to the method of Lowry et al. (1951). Analytical grade reagents (Biosystems Laboratories, S.A. Costa Brava, Barcelona, Spain) were used, and the

absorbance of the reactions were measured spectrophotometrically using HAICE®, DR 3000 (Germany).

2.6. Histopathological analysis

Sections of the brain from the treated and control rats were fixed in 10% neutral buffered formalin. The fixed tissues were dehydrated by passing through ascending order of ethyl alcohol–water concentrations, cleared in xylene and embedded in paraffin using rotary microtome. 3 μm thick sections of the tissues were prepared on slides, stained with Haematoxylin–Eosin (H&E) and mounted in neutral DPX medium for microscopic examination at 400×.

2.7. Statistical analysis

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

Data are presented as mean ± SD (n = 5). One-way analysis of variance (ANOVA) was used to determine the differences among various groups. When the corresponding F value (at 95% confidence limit) for the differences in the treated group means was significant pair wise, comparisons between treated groups and the negative control were determined using multiple comparison procedure of the Dunnett post-hoc test, and differences were considered significant ($p < 0.05$).

3. Results

3.1. Physical and chemical analysis of the leachate

Table 1 shows the physico-chemical parameters and heavy metals analyzed in Olusosun and Aba Eku leachates. OSL and AEL were respectively dark brown and brown in colour, with pH values within the range set by National Environmental Standards and Regulations Enforcement Agency (NESREA), (Nigeria) (2009) and United State Environmental Protection Agency (2006). The heavy metals and physico-chemical parameters examined in the leachates were higher than the maximum allowable limits (United State Environmental Protection Agency, 2006; National Environmental Standards and Regulations Enforcement Agency [NESREA], [Nigeria], 2009).

3.2. Body and organ weight gain

Table 2 presents significant ($p < 0.05$) decrease in the percentage body weight gain of exposed rats compared to the negative control. OSL treated rats presented a decrease body weight gain than AEL treated rats. There were significant ($p < 0.05$) increase in means of absolute and relative brain weight gain in OSL and AEL treated rats, although only the 10 and 25% concentrations were significantly different from the negative control (Table 2).

3.3. Biochemical indicators of oxidative stress

Fig. 1(a–c) shows the results of the biochemical tests. SOD activity significantly ($p < 0.05$) reduced in the brain and serum compared to the negative control. This was followed by

Table 1 – Physico-chemical parameters and heavy metals analysed in Olusosun and Aba-Eku landfill leachates.*

Parameters	AEL	OSL	NESREA[a]	USEPA[b]
Colour	Brown	Dark brown	–	–
pH	7.9	6.8	6.0–9.0	6.5–8.5
Nitrate	78.62	87.3	10	10
Ammonia	67.51	83.1	10	0.02
BOD[c]	643.70	602	50	–
COD[d]	712.21	529	90	–
Phosphate	99.15	105.70	2.0	–
Chloride	899.32	991	250	250
Sulphate	124.34	202.36	250	250
Hardness	492	516	–	0–75
Alkalinity	405	421	150	20
TS[e]	1131.24	1140.3	–	–
Cu	1.86	1.92	0.5	1.3
Fe	0.60	0.71	–	0.3
Pb	0.83	0.81	0.05	0.015
Cd	0.41	0.47	0.2	0.05
Mn	0.69	0.58	0.2	0.05
As	0.50	0.60	–	0.01
Ni	0.38	0.51	0.05	–
Cr	0.24	0.43	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.
[b] United State Environmental Protection Agency (2006). www.epa.gov/safewater/mcl.html
[c] Biochemical oxygen demand.
[d] Chemical oxygen demand.
[e] Total solid.

Table 2 – Percentage body weight change, absolute and relative brain weight of rat exposed to Olusosun (OSL) and Aba Eku (AEL) landfill leachates for 30 days.

Leachate conc. (%,v/v)	% Body weight	Absolute brain weight (g)	Relative brain weight (g)
OSL			
DW	9.13	1.29 ± 0.05	0.78 ± 0.02
1.0	7.68	1.43 ± 0.07	0.89 ± 0.09
2.5	5.78	1.47 ± 0.14	0.92 ± 0.04
5.0	2.87	1.52 ± 0.18	0.98 ± 0.07
10.0	0.24	1.60 ± 0.02[b]	0.99 ± 0.05[a]
25.0	–3.07	1.56 ± 0.03[b]	1.00 ± 0.03[a]
CYP	–5.39	1.60 ± 0.03[b]	1.05 ± 0.06[b]
AEL			
DW	9.13	1.29 ± 0.05	0.78 ± 0.02
1.0	7.76	1.34 ± 0.06	0.84 ± 0.04
2.5	5.09	1.37 ± 0.05	0.86 ± 0.03
5.0	1.95	1.44 ± 0.05	0.88 ± 0.08
10.0	1.56	1.56 ± 0.04[a]	0.98 ± 0.04[a]
25.0	–1.74	1.68 ± 0.03[b]	0.98 ± 0.09[a]
CYP	–5.39	1.60 ± 0.03[b]	1.05 ± 0.06[b]

Values are in mean ± SD. Superscripts differ significantly ([a]$p < 0.05$; [b]$p < 0.01$) from Dist water using Dunnett's multiple post hoc test. Dist water (distilled water).CYP (cyclophosphamide; 40 mg/kg/bw).

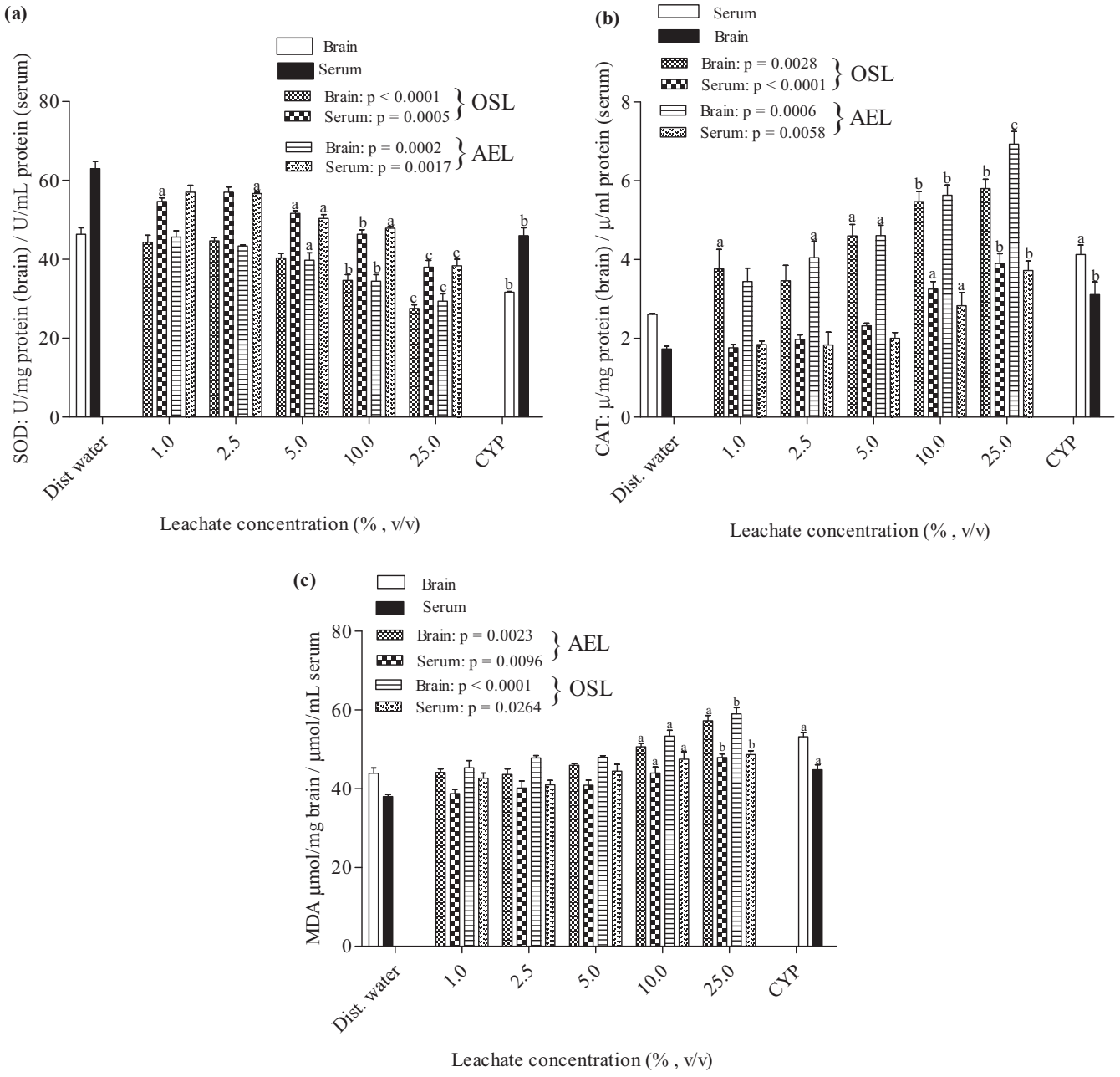

Fig. 1 – (a) Values are in mean ± SD. Superscripts differ significantly ($^{a}p < 0.05$; $^{b}p < 0.01$; $^{c}p < 0.001$) from Dist water using Dunnett's multiple post hoc test. Dist water (distilled water); CYP (cyclophosphamide; 40 mg/kg/bw), SOD (superoxide dismutase), OSL (Olusosun leachate), AEL (Aba Eku leachate). (b) Values are in mean ± SD. Superscripts differ significantly ($^{a}p < 0.05$; $^{b}p < 0.01$; $^{c}p < 0.01$) from Dist water using Dunnett's multiple post hoc test. Dist water (Distilled water); CYP (cyclophosphamide; 40 mg/kg/bw), CAT (catalase), OSL (Olusosun leachate), AEL (Aba Eku leachate). (c) Values are in mean ± SD. Superscripts differ significantly ($^{a}p < 0.05$; $^{b}p < 0.01$) from Dist water using Dunnett's multiple post hoc test. Dist water (distilled water); CYP (cyclophosphamide; 40 mg/kg/bw), MDA (malondialdehyde), OSL (Olusosun leachate), AEL (Aba Eku leachate).

concomitant significant ($p < 0.05$) increase in CAT activity in both brain and serum of leachate treated rats compared to the negative control. SOD and CAT activities in the brain were severely affected by the leachate constitutes than in the serum. MDA concentration in the brain and serum was significantly higher than the negative control. Brain MDA concentration was higher than in the serum.

3.4. *Histopathological assessment of the brain*

Histology of the cerebellum from negative control rats showed relatively normal neurons and evenly distributed purkinje cells (Fig. 2a). Fig. 2(b-f) showed sections of rat brain exposed to the leachates with diffused vacuolations of the neuronal cytoplasm (spongiosis), sub-meningeal spongiosis at the molecular

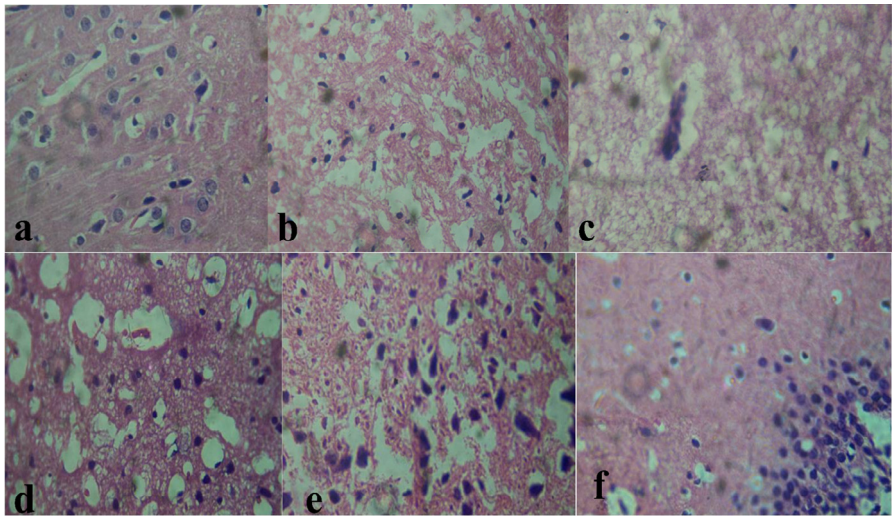

Fig. 2 – (a-f): Sections of the rat brain exposed to Olusosun and Aba-Eku landfill leachates and the negative control (H&E, x 400).

(a) Section of the cerebrum from the negative control showing normal neurons and evenly distributed purkinje cells.
(b) Section of the cerebellum from leachate treated rat showing diffused vacuolation of the neuronal cytoplasm (spongiosis).
(c) Section of the cerebrum from leachate exposed rat showed submeningeal spongiosis at the molecular layer and neuronal necrosis with proliferating astrocytes.
(d) Section of the cerebrum from leachate treated rat showing numerous vacuolation in the neuronal cytoplasm (spongiosis) and inflammation of the neurons.
(e) Section of the cerebrum from leachate treated rat showing vacuolation of the neuronal cytoplasm and swollen endothelial cells.
(f) Section of the cerebellum from leachate treated rat showing neuro-degenerated purkinje cells with loss of dendrites.

layer, neuronal necrosis with proliferating astrocytes, swollen endothelial cells (oedema), inflammation of the neurons and neuro-degenerated purkinje cells with lost dendrites.

4. Discussion

There is convincing evidence that chemicals in landfill leachates are capable of altering the functioning of the nervous system (Schaumburg et al., 1983; Wright et al., 2006). However, the neurotoxic effects of these xenobiotics are yet to be fully characterized since they are enormous and varied. The findings herein showed the involvement of oxidative stress induction in the pathological alterations of Wistar rat brain exposed to landfill leachates. The elevated concentrations of toxic metals and physico-chemical parameters in the tested leachates show the leachability of these parameters from individual wastes in the landfills. They are capable of inducing deleterious effects on biological systems which may be linked to the significant increase in both absolute and relative brain weight gain with concomitant decrease in percentage change in terminal body weight of the treated rats compared to the negative control. Alterations in brain weight gain suggest brain damage due to swollen endothelial cells (oedema) induced by the leachate constituents; mostly the toxic metals (Bailey et al., 2004; Lanning et al., 2002). Li et al. (2010) showed that increase brain weight gain observed in mice exposed to landfill leachates for 7 days was the most obviously affected organ compared to other viscera examined.

Superoxide dismutase activity prevents oxidative damage by scavenging and converting superoxide anions to hydrogen peroxide, while catalase decomposes the hydrogen peroxide to protect tissues from highly reactive hydroxyl radicals (Michiels et al., 1994). Significant alterations in the activities of these enzymes in the brain and serum of the leachate treated rats is linked to the harmful effects of the leachate constituents due to excess undetoxified reactive oxygen species (ROS) formation. Alterations in the antioxidant enzyme activities along with lipid peroxidation induction in leachate treated rats in the brain than in serum support the vulnerability of the brain to leachate induced oxidative damage (Bertoldi et al., 2012; Li et al., 2006). The metallic ions in the leachates are known to permeate brain tissues altering the trans-synaptic movement of neurotransmitters and or receptor associated voltage-sensitive channels and membrane transporters (Leonard et al., 2004; Mejia et al., 1997; Rodriguez et al., 1998). These metallic ions caused loss of functional integrity to the neuronal cell membrane of the leachate treated rats through free radical formation. This assertion may account for the dose-dependent alterations in brain SOD and CAT of mice exposed to leachates (Li et al., 2006) and rats exposed to arsenic (Chaudhuri

et al., 1999). It is also in agreement with Tsarpali and Dailianis (2012) observation that landfill leachates inhibited acetylcholinesterase (enzyme that degrades the neurotransmitter; acetylcholine) activity in mussel. Alterations in enzyme biochemistry and biological molecules involve synergistic and or antagonistic interactions of leachate constituents via free radical production (Bakare et al., 2012; Radetski et al., 2004; Tsarpali and Dailianis, 2012), and this is also considered as a major mechanisms of leachate induced neurotoxicity in mammals (Leonard et al., 2004; Rao and Avani, 2004).

Histopathological examination of tissues is a sensitive end point which detects specific lesions induced directly on tissues or indirectly via free radical generation induced by xenobiotics (Lanning et al., 2002; Travlos et al., 1996). The neurologic lesions in the brain of OSL and AEL treated rats corroborate biochemical observations to suggest neurotoxic oxidative damage. Neuronal necrosis observed in the treated rats is associated with disruption of structural and functional integrities of cell membrane. It is possible that pro-oxidant metals (including As) in the leachates reacted with lipid hydroperoxides of the brain cell membrane to elicit significant increase in malondialdehyde (MDA) formation in the treated rats. This produced MDA interfered with the integrity of the neuronal membrane (Chattopadhyay et al., 2002; Jomova et al., 2010; Knaapen et al., 2004). The occurrence of inflammatory neurons, neuronal necrosis and proliferating astrocytes associated neuro-degeneration in the leachate treated rats suggest reactive oxygen species formation and subsequent disruption of metabolic enzymes. These consequently activated neuronal accidental cell death (necrotic) and or neuronal programmed cell death (apoptotic) pathways (Wright and Baccarelli, 2007; Zheng et al., 1998). Furthermore, inflammatory cells are associated with spongiosis, neuro-degeneration of the purkinje cells and swollen endothelial cells (Park et al., 2009). The presence of swollen endothelial cells (oedema) corroborated increased absolute brain weight gain in the treated rats, and suggests obstruction of cellular fluid flow in the brain cells (Lanning et al., 2002). Neurodegenerations in Purkinje cells may affect impulse/signal conduction (poor signal transmission) from the cerebellum to higher centre that will enhance motor coordination of the body (Gartner and Hiatt, 2001). This may account for the laboured breathing pattern, sluggishness, muscular disorders (muscular stiffness and decreased motor activities), hair loss and ungroomed hair in Wistar rats exposed to OSL and AEL for 30 days (Alimba et al., 2012). Some of these neuro-psychological features were also observed in school-aged children residing near hazardous waste sites, and they correlated with increase As, Mn and Cd concentrations in their hair (Wright et al., 2006).

In conclusion, OSL and AEL induced neurotoxic effects in Wistar rats via oxidative stress provoked by the leachate constituents. This suggests possible health risk to human and wildlife population in close proximity to unprotected landfill facilities due to exposure to hazardous substances via surface and underground water sources.

Acknowledgements

The authors appreciate the contributions of Dr John, Department of Veterinary Pathology, University of Ibadan, Nigeria on the histological analysis and Mr. Samuel Akindele, Department of Biochemistry, Nigerian Institute of Medical Research (NIMR), Yaba, Lagos for the biochemical analysis.

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