Nephritic cell damage and antioxidant status in rats exposed to leachate from battery recycling industry

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ABSTRACT
Limited studies have assessed the toxic effect of sub-acute and sub-chronic exposure of leachate (mixture of metals) in mammalian kidney. The sub-acute and sub-chronic exposure of mature male Wistar-strain albino rats (200–220 g) were given by oral administration with leachate from Elewi Odo municipal battery recycling industry (EOMABRIL) for period of 7 and 60 days respectively, at different concentrations (20%, 40%, 60%, 80% and 100%). This was to evaluate its toxic effects on male renal functions using biomarkers of oxidative stress and nephro-cellular damage. Control groups were treated equally, but given distilled water instead of the leachate. All the groups were fed with the same standard food and had free access to drinking water. Following the exposure, results showed that the treatment induced systemic toxicity at the doses tested by causing a significant (p<0.05) alteration in enzymatic antioxidants-catalase (CAT) and superoxide dismutase (SOD) in the kidneys which resulted into elevated levels of malonaldehyde (MDA). Reduced glutathione (GSH) levels were found to be significantly (p<0.05) depleted relative to the control group. Considerable renal cortical congestion and numerous tubules with protein casts were observed in the lumen of EOMABRIL-treated rats. These findings conclude that possible mechanism by which EOMABRIL at the investigated concentrations elicits nephrotoxicity could be linked to the individual, additive, synergistic or antagonistic interactions of this mixture of metals with the renal bio-molecules, alteration of kidney detoxifying enzymes and necrosis of nephritic tubular epithelial cells.

KEY WORDS: EOMABRIL; antioxidant status; sub-acute; sub-chronic; interactions; nephrosis

Introduction
Recently, contamination by toxic substances in the environment has attracted the attention of several researchers both in the developed and developing countries of the world. Many industrial processes especially recycling industries have contributed to the contamination of the lithosphere thereby causing adverse effects on human health (Wang, 2002; Dautrempuis et al., 2004). Heavy metals can accumulate in the soil and as such percolate the water body and aquifer system. This can be of public health concern to both animals and humans if ingested via water drinking or through other means of exposure (Kalay et al., 1999; Ashraf, 2005).

Due to diverse functions and small mass in relation to the resting cardiac output that kidney carries out, it is a target organ both for chemicals that are pharmacologically active and toxic chemicals (Schröder, 2009). The nephrons and its related cells perform multiple physiological functions. It serves as a major mechanism for excretion and homeostasis of water-soluble molecules (Innocentre et al., 2005). This is because it is a metabolically active organ which actively concentrates certain substances. In addition, its cells have the potential to bio-transform chemicals and metabolically activate a variety of compounds (Innocentre et al., 2005). Specific physiological characteristics are localized to specific cell types. This makes them susceptible to, and be the target tissue for toxic chemicals (Innocentre et al., 2005; Schröder, 2009). On the other hand, chemicals may cause severe damage to the cells when exposed. However, renal cells respond to injury by repair and as such the kidney as a whole undergoes cellular lesion. Although there is a substantial capacity within the kidney for repair, there
are also several circumstances where damage may be irreversible. This depends on exposure levels, exposure time, which may vary over a long period of time or is limited to a single event, and it may be due to a single substance or to multiple chemicals (IPCS-UNEP-ILO-WHO, 1991).

Leachate is a liquid, generated during the process of lead-acid battery recycling. It contains mixture of metals. Elewi odo municipal battery recycling industry is located in primordial city of Ibadan, Ibadan North Local Government Area (LGA) of Oyo State, Nigeria. The liquid is leached from heap of auto-battery recycling wastes into nearby water bodies. Also, the components of the leachate may percolate through the soil, polluting these water bodies and gain access to food chains.

Experimental investigations that linked to nephrotoxicity by mixed-chemical and/or metal exposures had been inadequately studied and poorly elucidated. Also, the contribution of mixed-multiple chemicals to the overall incidence of nephropathy and sub-chronic renal failure is not well defined (Schröder, 2009). However, investigation for studying and improving the basic understanding of the mechanisms linked with nephrotoxicity of mixed-metals and pathophysiology of renal injury is highly needed.

Materials and methods

Sampling industry and leachate preparation

The leachate was obtained from Elewi Odo municipal battery recycling industry, located at Ibadan North LGA of Oyo State, Nigeria (latitude 7°25.08’N and 7°25.11’N and longitudes 3°56.45’E and 3°56.42’E). The site is largely used for auto-battery waste recycling activities. It is at the back of a stream in the residential area. It covers about 2 acres of land. A randomized sampling technique (Houk, 1992; Li et al., 2005; Siddique et al., 2005) was employed to collect the first horizon solid soils (0–15 cm deep) from different points on the municipal auto-battery recycling site. Five randomly collected samples from each site were pooled to make a single representative sample. The sample was air-dried, finely ground with a mortar and pestle, and sifted through a 63-μm (pore size) sieve to obtain a homogenous mixture.

Leachate (100%) was prepared from the homogenous mixture according to a standard procedure (ASTM, 1992; Ferrari, 1999). Briefly, 100 g of the sample (homogenous mixture) was added to 100 ml of distilled water (w/v) and shaken for 48 hr at 32°C. After shaking, the sample was allowed to settle for 30 minutes to sediment visible particles, and then the supernatant was filtered with a 2.5 μm filter (Whatman No. 42) to remove the suspended particles. Finally, the sample was stored at 4°C until use. It was designated as Elewi-Odo municipal auto-battery recycling industrial leachate (EOMABRIL). Water samples were collected from nearby stream and wells and designated as STREAM, WELL-A and WELL-B respectively. Also, drinking water was collected at far distance (8 km away) as control and designated as POW.

Heavy metal analysis

The nine metals, namely copper (Cu), lead (Pb), cadmium (Cd), cobalt (Co), chromium (Cr), zinc (Zn), iron (Fe), nickel (Ni) and manganese (Mn) were analyzed in the EOMABRIL, wells and control water sample. Briefly, 100ml each of EOMABRIL and water sample was digested by heating with concentrated HNO3 and the volume was reduced to 2–3 ml. This volume was made up to 10 ml with 0.1 N HNO3 and the concentrations of the metals were estimated using atomic absorption spectrophotometer (AOAC, 2005)

Chemicals and reagents

Epinephrine, Reduced GSH, 5,5-dithio-bis-2-nitrobenzoic acid, hydrogen peroxide and thiobarbituric acid (TBA) were purchased from Sigma (St Louis, MO, USA). Except stated otherwise, all other chemicals and reagents were of analytical grades and were obtained from the British Drug Houses (Poole, Dorset, UK) and the water used was glass distilled.

Experimental design

Sub-acute exposure

Healthy adult male Wistar rats weighing approximately 200–220 g obtained from the Department of Biochemistry, University of Ibadan, Ibadan, Nigeria, were randomly assigned to 4 groups. The rats were acclimatized for a period of 2 weeks. The animals were kept in wire-mesh cages under a controlled light cycle (12 h light/12 h dark), 50% humidity and at 30±2°C and placed on commercially available feed and water administered ad libitum during the period of acclimatization and treatment.

Sub-chronic exposure

A total of 30 healthy adult male Wistar rats weighing approximately 160–220 g were randomly assigned to 5 groups of 5 animals per group. This was chosen because sample size in conventional or typical laboratory experiments involving inbred rodents, the samples size is between 5–7 (Hsieh et al., 1998; Kubota and Wakana, 2011). Five different concentrations (20, 40, 60, 80 and 100%) of EOMABRIL were prepared according to the groups, and the rats in each group were administered 1 ml of EOMABRIL via oral administration for 60 consecutive days. The study period (60 days) was selected because conventional duration for sub-chronic exposure to toxicants ranges between 30–90 days. Also, previous works had made use of 60 days when 500 mg/L of lead (Pb) was exposed to rats via drinking water (Deveci et al., 2011). Corresponding group of animals were administered with the same volume of distilled water via the same route and served as control. All the animals in the various groups had free access to standard laboratory rat pellet and drinking water. Rats were killed by cervical dislocation 24 h after the final treatment, the kidneys were removed and cleared of adhering tissues, washed in ice-cold 1.15% potassium chloride and dried with blotting paper and placed on ice bath.
Animal ethics
All of the animals received humane care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institute of Health (USA). The ethical regulations have been followed in accordance with national and institutional guidelines for the protection of animals’ welfare during experiments (PHS, 1996). The analysis was carried out at the Laboratory, Department of Biochemistry, Bells’ University of Science and Technology, Sango ota, Ogun state, Nigeria.

Three different concentrations (20, 40, and 80%) of EOMABRIL (use as pilot study) were prepared according to the groups, and each rat in each group was administered 1 ml of EOMABRIL per day via oral administration for 7 consecutive days (Brusick, 1980). Corresponding group of animals were administered with the same volume of distilled water via the same route and served as control. Rats were killed by cervical dislocation 24 h after the final treatment. The kidneys were quickly removed, weighed and placed on ice bath.

Biochemical assay
The kidneys were homogenized in 50 mM Tris-HCl buffer (pH 7.4) containing 1.15% KCl and the homogenate was centrifuged at 10,000 g for 15 min at 4 °C. The supernatant was collected for the estimation of CAT activity using hydrogen peroxide as substrate according to the method of Clairborne (1995). 

To determine the SOD activity, the kidneys were homogenized in 50 mM Tris-HCl buffer (pH 7.4). The volume was made up to 300 μl by water before incubation at 37 °C for 2 hours. The color reaction was developed by adding 300 μl of 8.1% SDS (sodium dodecyl sulphate) to the reaction mixture containing the homogenate, followed by the addition of 600 μl of acetic acid/HCl (pH 3.4) and 600 μl of 0.8% thiobarbituric acid (TBA). This mixture was incubated at 100 °C for 1 hour. The absorbance of thiobarbituric acid reactive species (TBARS) produced were measured at 532 nm in UV-Visible spectrophotometer (Model 6305; Jenway, Barloworld Scientific, Dunmow, United Kingdom). MDA (Malondialdehyde) produced was calculated.

Histopathological evaluation
The kidneys were fixed in 10% formalin. They were directly dehydrated in a graded series of ethanol and embedded in paraffin. Thin sections, 5–6 micrometres, were cut by using a microtome, mounted on albumenized glass slides and stained with Eosin and Hematoxylen. Morphological examination of kidney was done by using an ocular micrometer scale under light microscope.

Statistical analysis
The results of the replicates were pooled and expressed as mean ± standard deviation. A one way analysis of variance (ANOVA) was used to analyze the results and Duncan multiple test was used for the post hoc (Zar, 1984). Statistical package for Social Science (SPSS) 17.0 for windows was used for the analysis and the least significance difference (LSD) was accepted at p<0.05.

Results
Antioxidant status in the kidney
The malondialdehyde (MDA) content in kidney homogenates of the treated rats with EOMABRIL were significantly (p<0.05) elevated when compared to their corresponding control rats (Figure 1a) during sub-acute exposure (7-days) by 12.53%, 15.92% and 20.63% respectively. As observed, there was no significant increase (p>0.05) between 20% and 40% doses of the treated rats following sub-acute exposure. As shown in Figure 1b, the rats exposed to EOMABRIL for sixty (60) days (sub-chronic exposure)
had a significant \((p<0.05)\) increase in malondialdehyde (MDA) content when compared to the control group. Effects of EOMABRIL on nephritic antioxidant status are shown in Figures 2–6. Following exposure to EOMABRIL, a dose-dependent significant \((p<0.05)\) decrease in kidney glutathione (GSH) level and increase in activities of SOD and catalase (CAT) were observed in all treated groups.

While 20, 40, 60, 80 and 100% EOMABRIL-treatment resulted in decreased GSH level by 20.0, 22.5, 30.0, 40.0 and 48.75%; SOD activity increased by 13.85, 50.77, 35.38, 30.77 and 55.38%. Hydrogen peroxide levels were markedly elevated in a non dose-dependent manner following EOMABRIL administration by 118.6, 87.2, 65.1, 116.3 and 77.9% respectively when compared with the control.
group. However, CAT activity was increased by 1.53, 30.71, 23.60, 42.48 and 45.04% after dosing the animal with 20, 40, 60, 80 and 100% EOMABRIL, respectively. Lastly, total protein was significantly (p<0.05) depleted in rat exposed to EOMABRIL by 4.05, 24.64, 16.21, 31.12 and 32.25% respectively relative to the control group.

**Nephritic cell damage**

The photomicrographs in Figure 7(a–f) illustrate the different histopathologic changes that were observed in the kidney of animals that were given various doses of EOMABRIL. Administration of EMOABRIL caused severe histopathologic lesions such as renal cortical congestion, medullar damage and abnormal numerous proximal tubules with protein casts and eosinophilic intranuclear inclusions of debris in proximal tubular cells of the lumens.

**Discussion**

Notably, toxic metals are widely generated in the environment and some of them can cause physiological, biochemical and histological disorders. Mammals are exposed to these hazardous substances from innumerable sources, including contaminated air, water, soil and food. However, the physiological effect of chemicals on living subjects is dependent on dose, duration, route of administration and other physiological factors (Roy Chowdhury, 2009). The present work revealed rats that were exposed to leachate from battery recycling industry displayed a pronounced impairment in kidney functions which was confirmed by histopathological alterations. The cortex was suggested to be more damaged than the medulla in EOMABRIL exposed rat. This may be due to
**Figure 7.** Microscopic findings of kidneys after EOMABRIL administration for 60 days, sub-chronic exposure (× 400). *(Control)* showed no visible lesions; NVL or the lesion was very mild. *(20%)* EOMABRIL exposed rats showed severe renal cortical congestion; cc and hypertrophy, proliferation and swelling in the lining endothelium of the glomerulus. *(40%)* EOMABRIL exposed rat showed glomerular tubular degeneration with degeneration in the lining epithelial cells of renal tubules, d with protein casts, pc and debris in the lumen of the degenerated tubules. *(60%)* EOMABRIL exposed rat showed cortical congestion, cc with protein casts, pc in the lumen of the tubules. *(80%)* EOMABRIL exposed rat showed severe renal cortical congestion and numerous tubules with protein casts in their lumens. *(100%)* EOMABRIL exposed rat showed cortical congestion; cc and presence of abnormal numerous tubules with protein casts; pc in their lumens. Generally, all treated rats with EOMABRIL showed necrosis of the glomerular tubules.
Table 1. Concentration of heavy metals detected in EOMABRIL, STREAM, WELL-A, WELL-B and POW (Adapted from Akintunde et al., 2013; Akintunde et al. 2015)

| Parameter | EOMABRIL | STREAM | WELL-A | WELL-B | POW | WHO |
|-----------|----------|--------|--------|--------|-----|-----|
| Cadmium   | 0.006 (100%) | 0.002 | 0.002 | 0.003 | BDL | 0.003 |
| Cobalt    | 0.049 | 0.004 | 0.003 | 0.002 | BDL | 0.05 |
| Chromium  | 0.068 (36%) | 0.011 | 0.015 | 0.014 | BDL | 0.05 |
| Copper    | 0.341 | 0.012 | 0.010 | 0.010 | BDL | 2.00 |
| Iron      | 2.667 (789%) | 1.076 (259%) | 0.011 | 0.030 | 0.050 | 0.30 |
| Manganese | 7.842 (1861%) | 0.223 | 0.239 | 0.239 | BDL | 0.40 |
| Nickel    | 0.050 (150%) | 0.048 (140%) | 0.044 (120%) | 0.049 (145%) | 0.027 | 0.02 |
| Lead      | 0.015 (50%) | 1.548 (15380%) | 0.068 (580%) | 0.306 (2960%) | BDL | 0.01 |
| Zinc      | 0.010 | 0.126 | 0.053 | 0.011 | 0.010 | 3.00 |

EOMABRIL: Elewi Odo municipal battery recycling industrial leachate, POW: Drinking water sample was used as control. All values are in mg/l. The contents of heavy metals detected in EOMABRIL, STREAM and WELLS around the site were higher than the drinking water sample (POW). BDL: Below detection level (Source: WHO, 1988; WHO 2008; Akintunde et al., 2013; Akintunde et al. 2015) Least Observable Effective Concentration (LOEC) set by World Health Organisation (WHO, 1996); values in the brackets: % increase compared with the WHO permissible limits in drinking water.
(Jefferies et al., 2008; Masashi et al., 2010). The (Na\(^+-\)K\(^+-\)) ATPase is the energy-requiring step in the development of the electrochemical gradients that drive solute and water transport in the proximal tubule. More so, inhibition of the (Na\(^+-\)K\(^+-\)) ATPase would not only impair solute and water re-absorption in the proximal tubule but would also impair the transport of substrates for energy metabolism and synthesis in the kidney (e.g., amino acids, citrate, fatty acids, glucose, lactate) (Benard, 2008).

An earlier report in our laboratory revealed dose-dependent decrease in body weights of EMABRIL-treated animals compared with control (Akintunde & Oboh, 2013). This finding supported the discovery of Farombi et al. (2011) who reported a significant reduction in rat body weight following intraperitoneal injection with leachate from landfill. In contrast, our result is inconsistent with earlier reports of Guangke et al. (2005) and Li et al. (2006) who reported increase in body weight of mice treated with municipal landfill leachate. The discrepancy in these results may be linked to leachate composition, which varies with recycling industries or sites and season or species differences.

Moreover, study showed that renal toxicity in rats is a good predictor in human subjects (Rosner et al., 2011). This finding proposed that mixed-metal exposure can cause considerable nephropathies when togetherly exposed than when singly exposed. Nephrotoxic properties of the elements contained in EOMABRIL might be connected to the tubular re-absorption of metal protein complexes, which increase the epithelial burden of elements interaction with organic macromolecules, thus causing a cascade of events leading to cell membrane damage and oxidative stress (Flora et al., 2008). Previous research showed that cadmium (greater than 0.003 mg/L) and chromium caused severe impairment to different nephronic sub-units and subsequently encouraged abnormal excretion of β2-microglobulin following chromium administration and chronic exposure to cadmium (Oxfor et al., 2010). In this study, sub-chronic exposure of rat to EOMABRIL at all concentrations (20, 40, 60, 80 and 100%) significantly mutilated the cell membrane and caused oxidative damage. The toxic response may be that cadmium detected (0.006 mg/L) in the leachate together with other metals additively damaged the kidney membrane integrity and fluidity by increased levels of malondialdehyde (MDA), which was higher than WHO permissible limits (0.003 mg/L) (WHO 2008). Thus, the damage occurred might be at the initial segment of proximal convoluted tubule (S1), while the damaging intermediate metal (lead) of the distal segments (S2–S3) had been documented (Bergamaschi et al., 1993).

The concentration of lead (0.015 mg/L) detected in the leachate of the present investigation was far higher as compared to WHO permissible limits (0.01 mg/L) (Table 1). Earlier findings showed that workers that were exposed to individual lead (Pb) showed a severe damage both in glomerulus and tubules (Cardenas & Roels, 1993). Also, renal biopsy in chronic lead nephropathy with minimal inflammatory response has been documented. Mitochondrial swelling, loss of cristae, and increased lysosomal dense bodies within proximal tubule cells (Kutlubay & Oguz, 2007) were also observed. It was further reported that arteriolar changes were indistinguishable from nephrosclerosis. Experimental studies also showed that Pb acetate at high doses (0.5%) in drinking water for 12 months resulted into early stages of intoxication such as kidney cortex hypertrophy, increase in glomerular filtration rate (GFR) and a comparable increase in tubular antigens excretion (ATSDR, 1988). It has also been reported that exposure to Pb above permissible limits (0.01 mg/L) were characterized mainly by tubule-interstitial changes leading to kidney remodelling and progressive glomerulo-angiosclerosis (ATSDR, 1988; Kutlubay & Oguz, 2007). However, lead concentration (0.015 mg/L) contained in leachate-treated rats of this study caused similar nephrosis by 50% increase when compared with WHO permissible limits (0.01 mg/L). In addition, the glycosaminoglycans (GAGs) and the urinary beta-N-acetylglycosaminidase activity (NAGs) are polysaccharides composed of repetitive disaccharide units (Bastogi, 2008). They are found in the glomeruli and the tubules and their leakage into the urine has been suggested to be a marker of injury to the nephron (Bastogi, 2008). Further study also showed that an increased excretion of GAGs and NAGs are early indicators of damage to the renal papilla, which is rich in GAG (Bastogi, 2008). As revealed from the present study, the rats exposed to the leachate showed psychomotor behavior of increased level of urination compared with the corresponding control. Our observation corroborated the recent study which reported that the presence of high Pb could trigger the increase of urinary excretion of sialic acids, GAGs and NAGs which indicate effect of exposure to lead (Bastogi, 2008) and early index of distal nephrotoxicity.

The absorption of nickel is dependent on its physicochemical form, with water soluble. The metabolism of nickel involves conversion to various chemical forms and binding to various ligands (Daldrup et al., 1983). Most nickel enters the body via food and water consumption, although inhalation exposure in occupational locations is a primary route for nickel-induced kidney toxicity. In large doses (>0.02 mg/L), some forms of nickel may be acutely toxic to humans when taken orally (Sunderman et al., 1988; WHO, 1988). This finding observed that Nickel (0.05 mg/L) detected in EOMABRIL caused renal damage by 60% increase when compared with WHO limits (0.02 mg/L).

Similarly, there were occasional cases of acute tubular necrosis (ATN) following massive absorption of chromium. Chromate-induced ATN has been extensively studied in experimental animals following parenteral administration of large doses of potassium chromate (hexavalent) (15 mg/kg body weight) (Wedeen & Qian, 1991). It was reported that chromate is selectively accumulated in the convoluted proximal tubule where necrosis occurs (Wedeen & Qian, 1991). Also, there was long-term adverse effect of low-dose chromium exposure on the kidneys in chromium workers (Wedeen & Qian, 1991).
However, Chromium from this study caused nephritic cell damage by 36% increase when compared with WHO tolerable limits.

As observed from this study, iron concentration (2.667 mg/L) in the leachate exposed experimental rat model caused kidney dysfunctions by 789% increase in respect to WHO permissible limits (0.3 mg). Similarly, manganese concentration (7.842 mg/L) in the EOMABRIL-treated rats caused kidney dysfunctions by 1861% increase compared to WHO permissible limits (0.4 mg/L). This supports earlier reports which indicated that elevated level of iron is capable of inducing multiple changes in renal tubular epithelial functions. The effect of iron could be related to diminished expression of the beta 1 integrin subunit and impaired proliferation (Sponsel et al., 1996). High level of manganese can bind either to a substrate (such as adenosine triphosphate, ATP), or to a protein directly, thereby causing conformational changes (Huang and Lin, 2004). In addition, reports had shown that high dose of manganese can damage kidneys (inflammation and kidney stone formation) and urinary tract in high fed rats (Ponnapakkam et al., 2003). Additionally, tubulointerstitial nephritis with tubular proteinous and glomerulosclerosis was equally observed in animals groups treated with manganese (Ponnapakkam et al., 2003). In the present findings, Co (0.049 mg/L), Zn (0.01 mg/L) and Cu (0.341 mg/L) detected in EOMABRIL were considerably lower than WHO exposure limits (0.05 mg/L, 3mg/L and 2 mg/L) respectively. The low level or the deficiencies of these metals (Co, Zn and Cu) had been implicated in enhanced expression of certain proteins known as angiotensin II that constrict the blood vessels in kidneys and further aggravate the condition of individuals with obstructive kidney disease (ATSDR, 2004; Naura & Sharma, 2009; Brewer, 2010; ATSDR, 2012). The present finding also supported the result of Bing (2014) which revealed that low levels of these beneficial metals can impair the development and maturation of kidneys in the fetus during pregnancy and at both pre- and post-weaning phases. This in turns increases the risk of renal dysfunction in adult individual (Naura & Sharma, 2009).

Generally, the level of heavy metals in EOMABRIL was higher than STREAM, WELL-A, and WELL-B. Its high levels may be because soil can easily form ligands with metals or likely that it has high capacity to retain heavy metals than inorganic solvents (Akintunde et al., 2015). The considerably higher concentrations of lead (Pb), 1.548 mg/L, 0.068 mg/L and 0.306 mg/L in stream, well-A and well-B respectively, than the leachate (EOMABRIL), 0.015 mg/L of the present study may be linked to the direct discharge of effluent from the factory into the stream. The previous study had implicated that when lead passes through the soil, the complex ligand formation or adhesion capacity with soil and other materials may be weak (Monroe, 2001). In addition, the large concentrations of manganese (7.842 mg/L), iron (2.667 mg/L) in the leachate, and lead (1.548 mg/L) in stream suggest that most of the waste batteries recycled at the industry were made of electrolytes from manganese, iron and lead sulphate. Collectively, the necrosis of renal tubular epithelial cells and injuries induced by EOMABRIL in the present finding could be linked to the individual, additive, synergistic or antagonistic interactions of the metals with the renal bio-molecules (Akintunde et al., 2013; Akintunde & Oboh, 2013; Akintunde & Oboh, 2015).

Conclusion

Following the exposure, EOMABRIL showed that the treatment induced systemic toxicity at the doses tested by causing a significant (p<0.05) alteration in enzymatic antioxidants-catalase (CAT) and superoxide dismutase (SOD) in the kidneys which resulted into elevated levels of malonaldehyde (MDA). Reduced glutathione (GSH) levels were found to be significantly (p<0.05) depleted relative to the control group. Considerable renal cortical congestion and numerous tubules with protein casts were observed in the lumen of EOMABRIL-treated rats. These findings conclude that possible mechanism by which EOMABRIL at the investigated doses elicits nephrotoxicity could be linked to the individual, additive, synergistic or antagonistic interactions of the metals with the renal bio-molecules, alteration of kidney detoxyifying enzymes and necrosis of nephritic tubular epithelial cells.

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REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR) (2012). Toxicological profile for manganese. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Agency for Toxic Substances and Disease Registry (ATSDR) (2004). Toxicological profile for cobalt. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Agency for Toxic Substances and Disease Registry (ATSDR) (1988). Toxicological Profile for Nickel. ATSDR/U.S. Public Health Service, ATSDR/TP-88/19.

Akintunde JK, Oboh G. (2015a). Depletion of cellular adenosine triphosphate and hepatocellular damage in rat after subchronic exposure to leachate from antropogenic recycling site. Hum Exp Toxicol 34(11): 1083–95.

Akintunde JK, Oboh G. (2015b). Sub chronic exposure to leachate activates key markers linked with neurological disorder in wistar male rats. Environ Sci Pollut Res 22: 18541–18553.

Akintunde JK, Oboh G and Akindahunsi AA. (2013). Testicular membrane lipid damage by complex mixture of leachate from municipal battery recycling site as indication of idiopathic male infertility in rat. Interdiscip Toxicol 6(4): 192–197.

Akintunde JK, Oboh G. (2013). Exposure to leachate from municipal battery recycling site: implication as key inhibitor of steroidogenic enzymes and risk factor of prostate damage in rats. Rev Environ Health 28(4): 203–13.
Akinunde JK, Oboh G. (2012). In Vitro Oxidative Damage Induced in Livers, Hearts and Kidneys of Rats Treated with Leachate from Battery Recycling Site: Evidence for Environmental Contamination and Tissue Damage. J Clinical Exp Pathol 2(7): 129.

Akinunde JK, Oboh G. (2013). Municipal Auto-Battery Recycling-Site Leachate Activates Key Enzymes Linked to Non-Insulin Dependent Diabetes Mellitus (NIDDM) and Hypertension. J Diabet Metab 4(1): 235.

Akinunde JK, Oboh G, Akindahunsi AA. (2015). Inhibition of key markers linked with spermatogenesis and cellular ATP by sub-chronic exposure to leachate in a rat model. Arch Environ Contam Toxicol 68(2): 68–79.

Al-Madani W A, Siddiqi NJ, Alhomida AS. (2009). Renal toxicity of mercuric chloride at different time intervals in rats. Biochem Insights 3: 37–45.

American society for testing material (ASTM). (1992). Standard Test Method for Laboratory compaction characteristics of soil using standard effort (12,400ft-lb/ft3 (600KN-m/m3), Annual book of ASTM standards, vol.04.08, D698–791.

AOAC. (2003). Official method of analysis of the (5th edition).

Ashraf W. (2005). Accumulation of heavy metals in kidney and heart tissues of Epinephelus microdon fish from the Arabian Gulf. Environ Monit Assess 101(1): 311–316.

Al-Attar AM. (2011). Antioxidant effect of vitamin E treatment on some heavy metals-induced renal and testicular injuries in male mice. Saudi J Biol Sci 18(1): 63–72.

Bastogi SK. (2008). Renal effects of environmental and occupational lead exposure. Ind J Occup Environ Med 12(3): 103–106.

Bergamaschi E, Mutti A, Alinovi R. (1993). Tubular reabsorption of proteins is a selective process. Contrib Nephrol 101: 92–8.

Bernard A. (2008). Cadmium and its adverse effects on human health. Ind J Med Res 128: 537–564.

Bing L, Wenpeng C, Yi T, Ping L, Qiang C, Chi Z, Lu C. (2014). The genotoxicity of industrial wastes and effluents. Mutation and Environmental Resources 277: 91–138.

Hsieh FY, Daniel AB and Michael DL. (1998). A simple method of sample size calculation for linear and logistic regression. Statist Med 17: 1623–1634.

Huang WH, Lin JL. (2004). Acute renal failure following ingestion of manganees-containing fertilizer. Toxicol Clin Toxicol 42: 305–307.

Innocente F, Rossella A, Enrico B, Antonio M. (2005). Contribution of studies on renal effects of heavy metals and selected organic compounds to our understanding of the progression of chronic nephropathies towards renal failure. Acta Biomed 2: 58–67.

IPCS-UNEP-ILO-WHO.(1991). Principles and methods for the assessment of nephrotoxicity associated with exposure to chemicals. Environmental Health Criteria series, n. 119, World Health Organization, Geneva.

Jagirdar KC, Cipriano DJ, Forfang M. (2008). Function, structure and regulation of the vacuolar (H+)/ATPases, Arch Biochem Biophys 476(1): 33–42.

Jollow DJ, Mitchell JR, Zamplagione N, Gillette JR. (1974). Bromobenzene induced liver necrosis: protective role of glutathione and evidence for 3,4 bromobenzene oxide as the hepatotoxic metabolite. Pharmacol 11: 151–69.

Kalay M, Ay P, Canil M. (1999). Heavy metal concentration in fish tissues from the northeast Mediterranean. Bull Environ Contam Toxicol 63: 673–671.

Kobata K, Wakanaka A. (2011). Sample size formula for Case-cohort Studies. Epidemiology 22(1): 279.

Kutlubay R, Oguz EO. (2007). Histological and ultrastructural evidence for protective effects on aluminium-induced kidney damage by intraperitoneal administration of a-tocopherol. Int J Toxicol 26: 95–101.

Li G, Sang N, Guo D. (2006). Oxidative damage induced in hearts, kidneys and spleens of mice by landfill leachate. Chemosphere 65(6): 1058–1063.

Li Y, Xi G. (2005). The dissolution mechanism of cathodic active materials of spent Zn-Mn batteries in HCl. Journal of Hazardous Material 127: 244–248.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. (1951). Protein measurement with folin phenol reagent. J Biol Chem 193: 265–275.

Masashi T, Regina S, Michael F. (2010). Regulation and Isoform Function of the V-ATPases. Biochemistry 49(23): 4715–4723.

Misra HP, Fridovich I. (1989). The role of superoxide anion in the auto-oxidation of epinephrine and a simple assay of superoxide dismutase. Biochim Biophys Acta 2417: 3170–5.

Missoun F, Slimani M, Aoues A. (2010). Toxic effect of lead on kidney function in rat Wistar. Afr J Biochem Res 4: 21–27.

Monroe M. (2001). Landfill Leachate Treatment: VSEP Officers a Revolution. Available at http://www.vsep.com/company/articles/2.html.

Naura AS, Sharma R. (2009). Toxic effects of hexaammine cobalt (III) chloride on liver and kidney in mice: Implication of oxidative stress. Drug Chem Toxicol 32(3): 293–9.

Ohkawa H, Oishi N, Yagi K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry 95: 351–358.

Osfor MMH, Ibrahim HS, Mohamed YA, Ahmed AM, Abd El Azeem AS, Hegazy MA. (2010). Effect of alpha lipoic acid and vitamin E on heavy metal intoxication in male albino rats. J Anim Sci 6: 56–63.

Palsamy P, Subramanian S. (2011). Resveratrol protects diabetic kidney by attenuating hyperglycemia-mediated oxidative stress and renal inflammatory cytokines via Nrf2–Keap1 signaling. Biochim Biophys Acta 1812(7): 719–731.

Ponnappakam T Iszard M, Henry-Sam G. (2003). Effects of oral administration of manganese on the kidneys and urinary bladder of Sprague-Dawley rats. Int J Toxicol 22(3): 227–32.

Public Health Service (PHS). (1996). Public Health Service Policy on Humane Care and Use of Laboratory Animals, Washington, DC: US Department of Health and Human Services, (PL 99–158. Health Research Extension Act, 1965).

Rosner M, Dolzning H, Schipany K, Mikula M, Brandau O. (2011). Human amniotic fluid stem cells as a model for functional studies of genes involved in human genetic diseases or oncogenesis. Oncotarget 2: 795–12.

Rouach H, Fataccioli V, Gentili M, French SW, Morimoto M, Nordmann R (1997). Effect of chronic ethanolic feeding on lipid peroxidation and protein oxidation in relation to liver pathology. Hepatology 25: 351–355.

Roy Chowdhury A. (2009) Recent advances in heavy metals induced effect on male reproductive function-A retrospective. J Med Sci 2: 37–42.

Saxena PN, Anand S, Saxena N, Bajaj P. (2009). Effect of arsenic trioxide on renal functions and its modulation by Curcuma aromatica leaf extract in albino rat. J Environ Biol 30: 527–531.
Schröder P, Lyubenova L, Huber C. (2009). Do heavy metals and metalloids influence the detoxification of organic xenobiotics in plants? Environ Sci Pollut Res Int. 16(7): 795–804.

Siddique HR, Gupta SC, Dhawan A, Murthy RC, Saxena DK, Chowdhuri DK. (2005). Genotoxicity of industrial solid waste leachates in Drosophila melanogaster. Environ Mol Mutat 46: 189–197.

Sponsel HT, Alfrey AC, Hammond WS, Durr JA, Ray C, Anderson RJ. (1996). Effect of iron on renal tubular epithelial cells. Kidney Int 50(2): 436–44.

Sunderman FW, Jr B, Dingle SM, Hopfer TS. (1988). Acute nickel toxicity in electroplating workers who accidentally ingested a solution of nickel sulfate and nickel chloride. Am J Indus Med 14: 257–266.

Tomino Y. (2014). Pathogenesis and treatment of chronic kidney disease: a review of our recent basic and clinical data. Kidney Blood Press Res 39: 450–489.

Wang WX. (2002). Interaction of trace metals and different marine food chains. Mar Ecol Prog Ser 243: 295–309.

Wedeen RP, Qian LF. (1991). Chromium-induced kidney disease. Environ Health Perspect 92: 71–4.

WHO. (2008). International year of fresh water. General Assembly Resolution A/RES/53/196. Official website: www.wateryear2003.org

World Health Organisation (WHO) (1996). Health criteria and other supporting information. In: (2nd ed.), Guidelines for drinking-water quality 2, WHO, Geneva, 1996, pp. 940–949.

World Health Organization (1988). Chromium. Environmental Health Criteria 61. Geneva, Switzerland.

Yashpal SK, Lin S, Ping X, Fu-you L, Sheldon C. (2011). A glimpse of various pathological mechanisms of diabetic nephropathy. Annu Rev Pathol 6: 395–423.

Zar JH. (1984). Biostatistical Analysis, Prentice-Hall, International, USA, 620.