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ORIGINAL ARTICLE

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; Dautremepuits *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

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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 get 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 (8km 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 HNO_3 and the volume was reduced to 2–3 ml. This volume was made up to 10 ml with 0.1 N HNO_3 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 ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animal' 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). H_2O_2 level was estimated using the method described by Clairborne (1995). Briefly, 50 µl of the test sample was added to a reacting mixture containing 500 µl of 59 mM H_2O_2 and 950 µl of 50 mM phosphate buffer (pH 7.0). The reaction was carried out at 25 °C and the decrease in absorbance at 570 nm was monitored for 3 min at 150 sec interval. A unit of the enzyme activity is defined as the amount of enzyme catalyzing the decomposition of 1 µmol of H_2O_2 per minute at 25 °C and pH 7.0 under specified condition. SOD activity was determined by measuring the inhibition of autoxidation of epinephrine at pH 10.2 at 30±1 °C according to Misra and Fridovich (1989). Briefly, 0.1ml of the kidney homogenate was diluted in 0.9ml of distilled water to make 1 in 10 dilutions. An aliquot of 0.2ml of the diluted homogenate was added to 2.5 ml of 0.05 M carbonate buffer pH 10.2 to equilibrate in a cuvette and the reaction started by the addition 0.3 ml of 0.3 M of adrenaline. The reference cuvette contained 2.5 ml of carbonate buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of distilled water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds. Protein concentration was determined by the method of Lowry, *et al.* (1951). Briefly, 20 µl of the supernatant was mixed with 1 ml of Biuret reagent (100 mM NaOH, 16 mM Sodium-Potassium-tartrate, 15 mM Potassium iodide and 6 mM $CuSO_4$). Thereafter, the mixture was incubated for 30 min at 25 °C and the absorbance taken at 546 nm. Bovine serum albumin was used as the standard protein and the total protein subsequently calculated

GSH assay

Reduced GSH was determined using the method described by Jollow *et al.* (1974). Briefly, 1 ml of supernatant was treated with 500 µl of Ellman's reagent (19.8 mg of 5,5-dithio-bis-2-nitrobenzoic acid in 100 ml of 0.1% sodium citrate) and 3.0 ml of 0.2 M phosphate buffer (pH 8.0). The absorbance was read at 412 nm in spectrophotometer.

Lipid peroxidation assay

Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method described by Okhawa *et al.* (1979) and expressed as mmol/mg tissue. Briefly, 100 µl of homogenate from rat kidneys was mixed with a reaction mixture containing 30 µl of 0.1 M 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 colour reaction was developed by adding 300 µl of 8.1% SDS (Sodium duodecyl 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 serious 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 $p<0.05$.

Rsults

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)

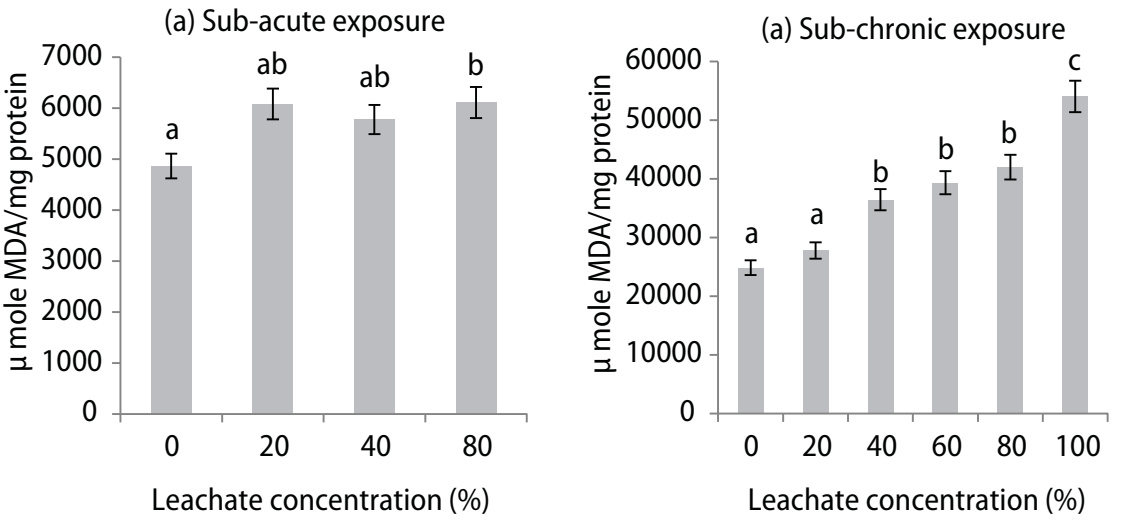

Figure 1. (a & b) Effect of EOMABRIL on lipid peroxidation in a sub- acute (7 days) and sub-chronic (60 days) exposure. Group 1 received 0%, Group 2 received 20%, Group 3 received 40%, Group 4 received 60%, Group 5 received 80% and Group 6 received 100%. Values represent mean ± standard deviation, n=5. Values with different superscript are significantly (p<0.05) different.

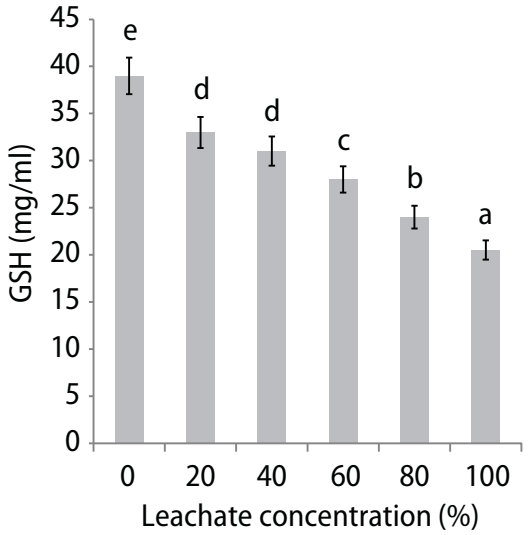

Figure 2. Effect of EOMABRIL on hepatic reduced glutathione (GSH) in a sub chronic (60 days) exposure. Group 1 received 0%, Group 2 received 20%, Group 3 received 40%, Group 4 received 60%, Group 5 received 80% and Group 6 received 100%. Values represent mean ± standard deviation, n=5. Values with different superscript are significantly (p<0.05) different.

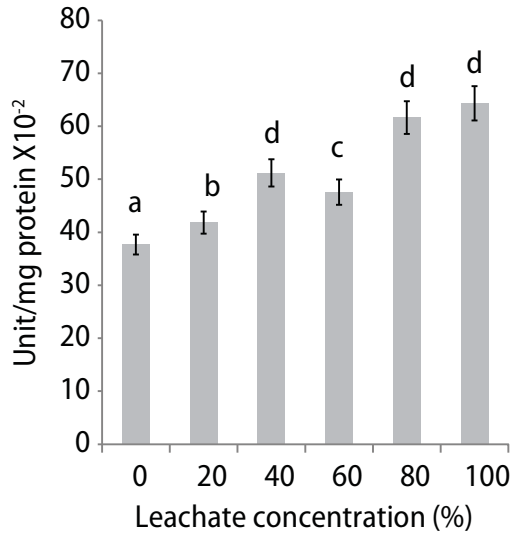

Figure 3. Effect of EOMABRIL on superoxide dismutase activity in a sub-chronic (60 days) exposure. Group 1 received 0%, Group 2 received 20%, Group 3 received 40%, Group 4 received 60%, Group 5 received 80% and Group 6 received 100%. Values represent mean ± standard deviation, n=5. Values with different superscript are significantly (p<0.05) different.

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

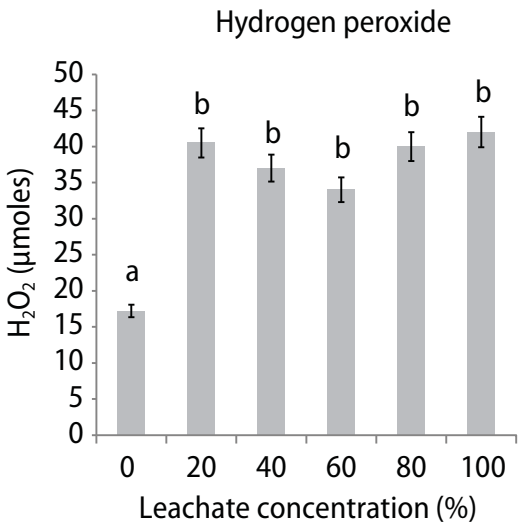

Figure 4. Effect of EOMABRIL on hydrogen peroxide (H_2O_2) level in a sub-chronic (60 days) exposure. Group 1 received 0%, Group 2 received 20%, Group 3 received 40%, Group 4 received 60%, Group 5 received 80% and Group 6 received 100%. Values represent mean ± standard deviation, n=5. Values with different superscript are significantly (p<0.05) different.

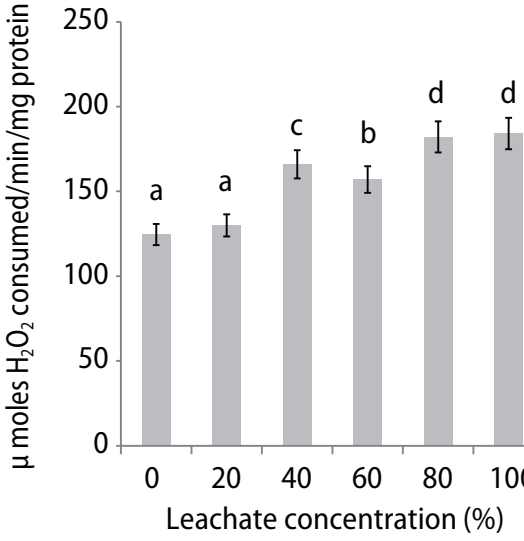

Figure 5. Effect of EOMABRIL on the activity of catalase in a sub-chronic (60 days) exposure). Group 1 received 0%, Group 2 received 20%, Group 3 received 40%, Group 4 received 60%, Group 5 received 80% and Group 6 received 100%. Values represent mean ± standard deviation, n=5. Values with different superscript are significantly (p<0.05) different.

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 give 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.

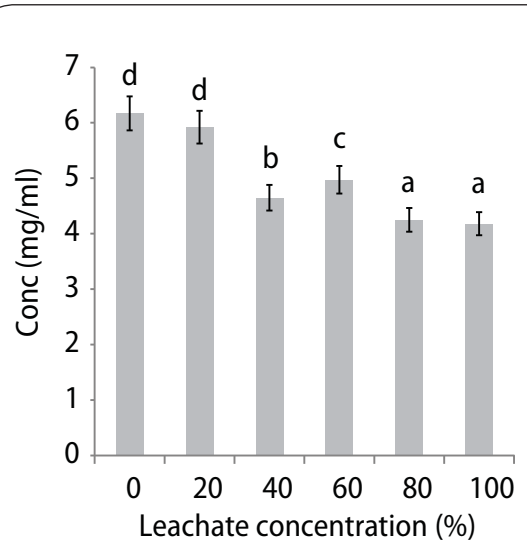

Figure 6. Effect of EOMABRIL on total protein in a sub-chronic (60 days) exposure. Group 1 received 0%, Group 2 received 20%, Group 3 received 40%, Group 4 received 60%, Group 5 received 80% and Group 6 received 100%. Values represent mean ± standard deviation, n=5. Values with different superscript are significantly (p<0.05) different.

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

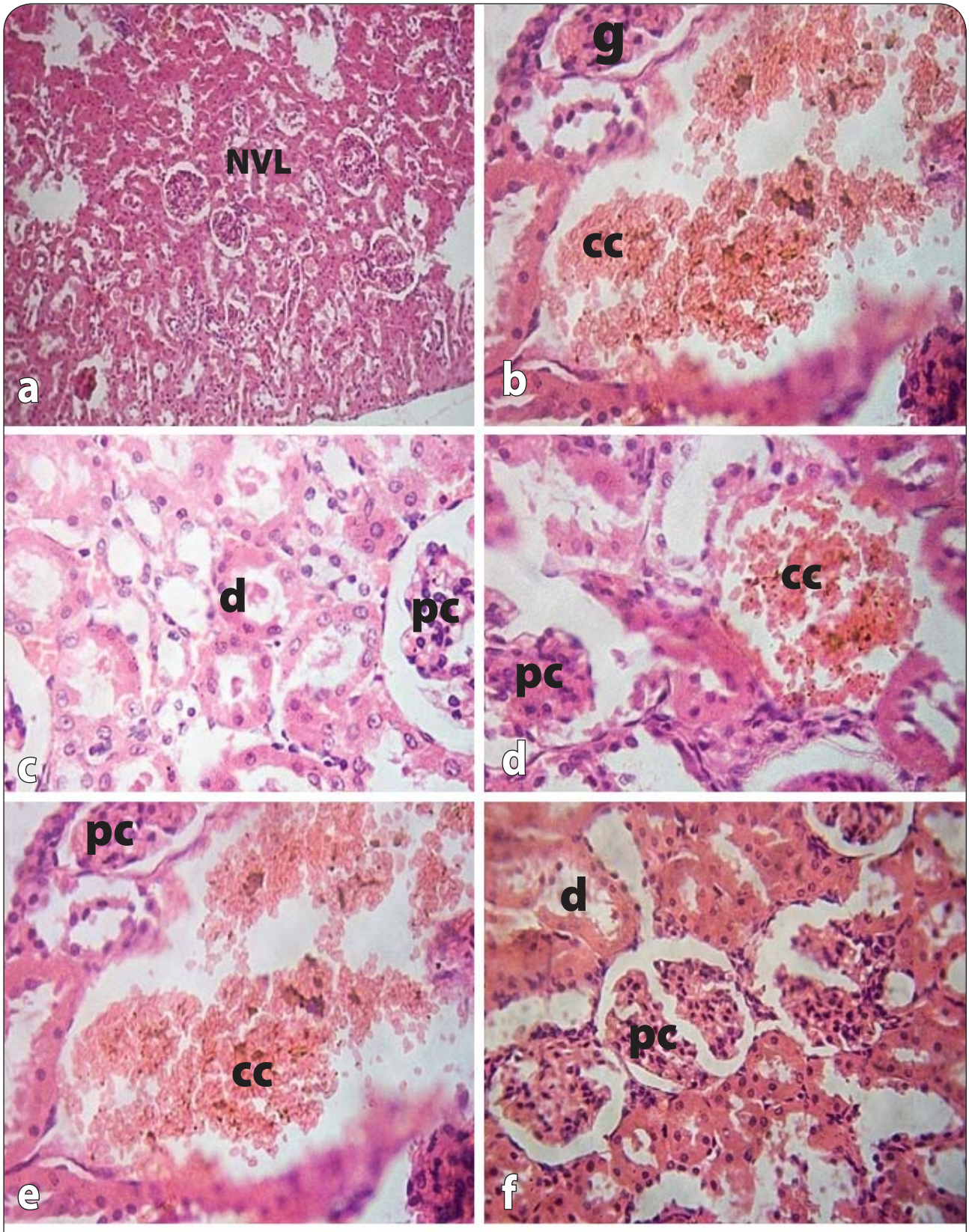

Figure 7. Microscopic findings of kidneys after EOMABRIL administration for 60 days, sub-chronic exposure (× 400). (**Control**) Showed no visible lesions; NVL or the lesion was very mild. (**20%**) EOMABRIL exposed rats showed severe renal cortical congestion; cc and hypertrophy, proliferation and swelling in the lining endothelium of the glomerulus, g. (**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.

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long-term exposure or may be linked to uneven distribution of the mixed-metal (contained in the leahate) in the nephrons of the kidney where about 90% of the total renal blood flow enters the cortex via the bloodstream (Atef, 2011). This is because a relatively high proportion of inorganic substances reach the cortex via the bloodstream than that would enter the medulla (Atef, 2011). This finding supports several observations which reported that experimental animals intoxicated with laboratory Pb, Hg, Cd, Cu and other heavy metals resulted in renal histological alterations (Goran *et al.,* 2008; Al-madani *et al.,* 2009; Sarena *et al.,* 2009; Mission *et al.,* 2011). Also, as observed from the study, EOMABRIL exposed rat showed glomerular tubular degeneration, with protein casts and debris in the lumen of the degenerated proximal tubules. The eosinophilic intranuclear inclusions of debris in proximal tubular cells following the treatment may be traced to the formation of metal-protein complexes (Innocentre *et al.,* 2005)

Additionally, much of the kidney pathology is associated with the decrease in intracellular GSH concentration (Atef, 2011). Hence, GSH concentration is important for survival of the cells. It is also a substrate for glutathione peroxidase. This is one of the most important modulatory mechanisms for free radical scavenging and inhibition of electrophilic xenobiotics attack on cellular macromolecules involves tripeptide glutathione (Cnubben *et al.,* 2001). As revealed by the present investigation, GSH was remarkably declined following EOMABRIL treatment. Reports had shown in several different animal models, as well as in humans, that a decrease in GSH concentration may be associated with nephropathy and pathogenesis of kidney diseases (Yashpal *et al.,* 2011; Palsamy and Subramanian, 2011; Tomino, 2014). Activities of SOD and CAT were markedly increased by EOMABRIL treatment. The increased activity of SOD may be linked to the high level of superoxide anions (0^2) induced by EOMABRIL which results into the accumulation of hydrogen peroxide (H_2O_2) in the renal cells. Similarly, high activity of CAT at extreme dose confirms the precipitation of reacting oxygen species, H_2O_2 in the kidney. The precipitation of hydrogen peroxide (H_2O_2) in nephritic tissue caused hydroxyl radical ($OH^{\bullet}$) generation. This eventually caused damage to renal proteins; bio-membrane and DNA molecule. The rise in the activity of CAT could also be linked to its induction to counter the effect of oxidative stress. Therefore, significant increased in the level of hydrogen peroxide (H_2O_2) indicates oxidative stress and nephritic necrosis. Our observation is consistent with earlier report of Guangke *et al.* (2005). Also, the level of protein was significantly depleted following EOMABRIL exposure. This may be linked to the direct inhibition of protein synthesis or perhaps the protein produced had formed complexes with mixtures of metals in EOMABRIL thereby reduced its levels. Administration of EOMABRIL during sub-acute and sub-chronic exposure resulted in increase in MDA level in treated animals. This result is consistent with previous study on oxidative damage induced in liver, brain, heart, kidney and spleen of animals treated with leachate (Li *et al.,* 2006; Akintunde & Oboh, 2012; Akintunde *et al.,* 2013; Akintunde & Oboh, 2015a; Akintunde & Oboh, 2015b)

As suggested from this study, EOMABRIL hypothesises to inhibit the kidney membrane (Na^{+}-K^{+}) ATPase thereby disrupting the homeostasis of Na^{+} and k^{+} flow. Similarly, other ATPases, including the (Ca^{2+}-Mg^{2+})-ATPase and kidney mitochondrial ATPases may also be the targets of mixed-metal ions (M^{2+}). Hence, in this case, more diverse effects on cellular function might be anticipated. This is in line with previous studies which discovered that, metal (Pb^{2+}, Fe^{2+}, Cd^{2+} etc) binds to the ATPase in renal medullary (Na^{+}-K^{+}) ATPase (Jefferies *et al.,* 2008; Masashi *et al.,* 2010) to inhibits its activity and ATPase-driven transport (Hinton *et al.,* 2009). Binding of metals to the ATPase is reversible and occurs at a site distinct from the oubain binding site (Jefferies *et al.,* 2008). This affects the interaction between the α and β subunits of the ATPase protein complex (Hinton *et al.,* 2009). The binding site occurs at the cytosolic domain of the ATPase

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 EMABRSIL-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 (Osfor *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). 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 polysacchrides 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 (0.02mg/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 & Qjan, 1991). It was reported that chromate is selectively accumulated in the convoluted proximal tubule where necrosis occurs (Wedeen & Qjan, 1991). Also, there was long-term adverse effect of low-dose chromium exposure on the kidneys in chromium workers (Wedeen & Qjan, 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 (Ponnapakkpam *et al.,* 2003). Additionally, tubulointerstitial nephritis with tubular proteineous and glomerulosclerosis was equally observed in animals groups treated with manganese (Ponnapakkpam *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 EOMABRL 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 angiotensis 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 detoxifying enzymes and necrosis of nephritic tubular epithelial cells.

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