ORIGINAL ARTICLE

Eur. J. Anat. 23 (1): 65-76 (2019)

Heavy metals obtained from waterways induced neurodegeneration in the prefrontal cortex of Wistar rats

Temidayo D. Adeniyi[1], Peter U. Achukwu[2], Abdulazeez A. Abubakar[3], Adedayo D. Adekomi[4]

[1]Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Pure and Applied Sciences, Kwara State University, Malete, Kwara state, Nigeria, [2]Department of Medical Laboratory Science, Faculty of Health Science and Technology, College of Medicine, University of Nigeria, Enugu Campus, Enugu state, Nigeria, [3]Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Medical Sciences, Ondo city, Ondo State, Nigeria, [4]Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Osun State University, Osogbo, Osun State, Nigeria

SUMMARY

The level of heavy metals in Nigeria waterways is grossly influenced by the irrepressible disposal and recycling of electronic waste. The impact of heavy metals obtained from waterways on the prefrontal cortex of experimental rats was investigated in this study. Thirty (30) adult male Wistar rats weighing about 150-180 g were used in this study. Ten rats apiece were assigned randomly into three groups. Pooled sampled water and water containing the highest average concentration of combined heavy metals recorded in the waterways was given to the Wistar rats within the treatment groups *ad libitum* for 65 days. Blood sera were obtained for analysis of oxidative stress markers. The prefrontal cortex was processed for paraffin embedding, and sections stained for histological, histochemical and immunochemical evaluations. $P < 0.05$ was regarded as significant for data using one-way analysis of variance. Oxidative damage was observed in animals from the treatment groups when compared to the control. The analysed levels of oxidative stress markers showed statistically significant differences, except between groups given pooled sampled water and combined metals. Neurodegeneration was attested from the histological and histochemical evaluations, and the immunohistochemical evaluation revealed marked astrocytosis with induced oxidative stress while comparing the experimental groups.

Keywords: Electronic-waste – Heavy metals – Neurodegeneration – Waterways

INTRODUCTION

About one billion individuals are figured to suffer from some forms of disability in the world while neurological impairments account for one-fourth of the twenty top causes of disabilities globally (WHO, 2011). Exposure to environment-derived heavy metals is principally implicated in causing cognitive and neurological deficits (Neal and Guilarte, 2012). Heavy metals are known as environmental pollutants with the attendant toxicity of increasing concern (Nagajyoti et al., 2010; Karri et al., 2016). It was reported that developing countries including Nigeria have high levels of heavy metals contamination (Huang et al., 2014; Izah et al., 2016). Poor disposal and recycling of electronic waste (e-waste) is a significant source of heavy metals contamination in developing countries, mainly Asian and African nations (Azuka, 2009, Shamim et al., 2015). Most rivers were reported to have well above the recommended permissible levels for heavy metals due to these irrepressible

Corresponding author: Temidayo D. Adeniyi. Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Pure and Applied Sciences, Kwara State University, Malete, Kwara State, Nigeria. Phone: +234 803 061 2404. E-mail: temidayo.adeniyi@kwasu.edu.ng

Submitted: 6 November, 2018. *Accepted:* 12 December, 2018.

practices (Huang et al. 2014; Izah et al. 2016). In 2013, United Nations University projected the global e-waste generation by 2017 to reach about 65.4 million metric tons from the previous 20-50 million metric tons with the annual growth rate of 33% (UNU, 2013; Shamim et al., 2015). Unfortunately, about 75 % to 80 % was reported to be dumped as second-hand devices for recycling and disposal in developing countries, particularly Western Africa (Shamim et al., 2015; Ibrahim, 2017).

Perhaps, the primary concern now is the public health hazard heavy metals generated from e-waste to man. In spite of dearth research findings, the public health significance of heavy metal contact from the disposal and recycling of e-waste has been heightening (Shamim et al., 2015). The usual hazardous, toxic and nonbiodegradable e-waste generated heavy metals include cadmium, arsenic, lead, nickel, chromium, copper, manganese, zinc, mercury, iron and aluminium (Jinhui et al., 2011; Shamim et al., 2015; Zeng et al., 2016). Of particular interest, neurotoxicants are lead, mercury, cadmium and chromium, reported by Chen et al. (2011) to induce neurodegeneration. These metals adversely impact the waterways and agricultural practices due to their uptake in edible crops; thus human beings are contaminated by food chain (Puschenreiter et al., 2005). They are subsequently transformed into toxic compounds resulting in various health risks straddling from mild eye injury to severe cellular alteration (Akinseye, 2013). The toxicity of these heavy metals remains to be determined, despite the fact that several of them are suspected to have neurotoxicity in vulnerable populations (Chen et al. 2011).

Wright and Baccarelli (2007) established from their findings that oxidative stress is stimulated by heavy metals. Oxidative stress was observed as a rise in reactive oxygen species (ROS) production, with depleting of the set aside antioxidant (Wright and Baccarelli, 2007; Valko et al. 2016). Heavy metal ion metabolic balance breakdown forms ROS, which results in oxidative damage in macromolecules (Valko et al., 2016). Neurons are incapacitated to detoxicate ROS, and are thereby endangered by oxidative stress (Chen et al., 2011). Vulnerability to lead was reported to increase astrocyte filaments, damage cellular organelles and increase oxidative stress activities in the central nervous system (CNS) (Struzyñska et al., 2001; Chiurchiù et al. 2016). Lead may interact with membrane lipids and proteins, whereby the integrity of the cell membrane is compromised (Sanders et al., 2009). Cadmium was reported to prompt oxidative damage in humans and animals (Joseph, 2009). It also suppresses gene expression, and inhibits DNA damage repair and apoptosis (Bishak et al., 2015). Johansson et al. (2007) and Petroni et al. (2012) reported that mercury results in ROS upregulation, which adversely impairs the function of the mitochondria electron transport system. Oxidative stress was also evident in rats' cerebra exposed to chromium (Nudler et al., 2009).

This study, therefore, proposed to investigate neurodegeneration induced by heavy metals from waterways in Kwara State, Nigeria using Wistar rats. This study has been carried out in Kwara State, Nigeria in 2017.

MATERIALS AND METHODS

Procurement of animals

Thirty (30) first filial (F1) generation inbred adult male Wistar rats weighing about 150-180 g were procured from the animal facility of the Institute for Advance Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, and employed in this study. They were acclimatised for 14 days and were fed pelletized rat feed and water *ad libitum* throughout acclimatisation before use. The rats were housed in plastic cages and kept in standard laboratory conditions under natural light-dark cycle at room temperature.

Chemicals Procurement

Analytical chemicals procured from Sigma-Aldrich, USA for this study include: Mercury thiocyanate ($Hg(SCN)_2$), Cadmium acetate dihydrate ($Cd(CH_3CO_2).2H_2O$), Chromium oxide (Cr_2O_3), and Lead (II) acetate trihydrate ($Pb(CH_3CO_2).3H_2O$).

Preparation of solutions

The solutions were prepared in line with the empirical findings from heavy metals obtained in the waterways carried out in same study area by Adeniyi et al. (2017) 0.009 g of $Pb(CH_3CO_2).3H_2O$, 0.001 g of $Hg(SCN)_2$, 0.045 g of $Cd(CH_3CO_2).2H_2O$ and 0.318 g of Cr_2O_3 were weighed using Meltzer sensitive weighing balance. It was then dissolved in 1 liter of distilled water to form the final concentration of 0.009 mg of $Pb(CH_3CO_2).3H_2O$, 0.001 mg of $Hg(SCN)_2$, 0.045 mg of $Cd(CH_3CO_2).2H_2O$ and 0.318 mg of Cr_2O_3 per milliliters solutions.

Experimental design and treatment of animals

The thirty (30) rats were separated into three groups of ten animals each selected by simple randomisation (Table 1). The duration of treatment lasted 65 days, standard long-term treatment for rats (Adeyemi et al., 2007).

The procedures used in this experimental study were approved by the Health Research and Ethics Committee of the University of Nigeria Teaching Hospital, Enugu State. The approval is in line with the National Institute of Health (NIH) in the "Guide to the Care and Use of Animals in Research and Teaching" (NAS, 2011). The approval is with protocol number 025/02/2017.

Biochemical assays

The animals were sacrificed by cervical dislocation. Their blood samples were obtained by cardiac puncture, transferred into separate plain bottles under aseptic conditions and centrifuged with a bench centrifuge at 4000 revolutions per minute for 5 minutes to get the sera. The sera were pipetted

into serum vials and preserved at -20°C until when the biochemical analysis was carried out. The preserved sera were assayed for Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Hydrogen peroxide (H_2O_2) and Malondialdehyde (MDA) employing the method of Mistral and Fridovich (1972); Paglia and Valentine (1967); Wolff (1994) and Deiana et al. (1999) and Stocks and Domandy (1971) respectively.

Tissue preparation and neurohistological evaluations

Their skull was opened with the aid of bone forceps leaving the brain tissue intact (Van Zutphen et al. 1993). The prefrontal cortex was excised from the anterior cerebral cortex. The brain tissues were fixed in 10% buffered formal saline, grossed and processed for paraffin tissue embedding (Drury and Wellington, 1980). The prepared sections were stained for histological evaluation, histochemical assessment and immunohistochemical evaluation utilising the method of Bancroft and Gamble (2008), Marsland et al. (1954), Hoehn et al. (2003) and Delcambre et al. (2016) respectively.

Statistical analysis

Data obtained were analysed using a GraphPad Prism statistical package soft version 6 (California, USA). Values of biochemical parameters within groups of experimental animals were compared by one-way ANOVA, followed by Uncorrected Fisher's LSD test for multiple comparisons. Data taking at P value less than 0.05 was significant at 95% confident interval.

Photomicrographs

The stained sections were viewed, and photomicrographed with an Olympus U-D03 microscope captured with Olympus DP21 camera. The obtained Photomicrographs were analysed and reported.

RESULTS

Level of serum oxidative stress markers

There was no account of death recorded in the three groups throughout the 65 days of administration. The reactive oxygen species, lipid peroxidation and antioxidant activity, were determined in the serum of experimental animals. Significant differences were observed in the level of H_2O_2 in group 2 (7.49 ± 0.24) and group 3 (7.77 ± 0.04) compared to the control group (3.18 ± 0.30) respectively. The level of H_2O_2 between group 2 and group 3 showed no statistical significance (Fig. 1). The level of serum MDA of group 1 (1.8 ± 0.07) was significantly lower compared to the other groups. Group 2 (2.4 ± 0.09) and group 3 (2.5 ± 0.09) were seen to be significant when compared to the control group respectively. However, there was no significant difference in the level of MDA between group 2 and group 3 (Fig. 2). There were significant differences in the levels of SOD in group 2 (236 ± 9.03) and group 3 (220 ± 4.08) compared to the control group (283 ± 8.58) respectively. No significant difference was observed

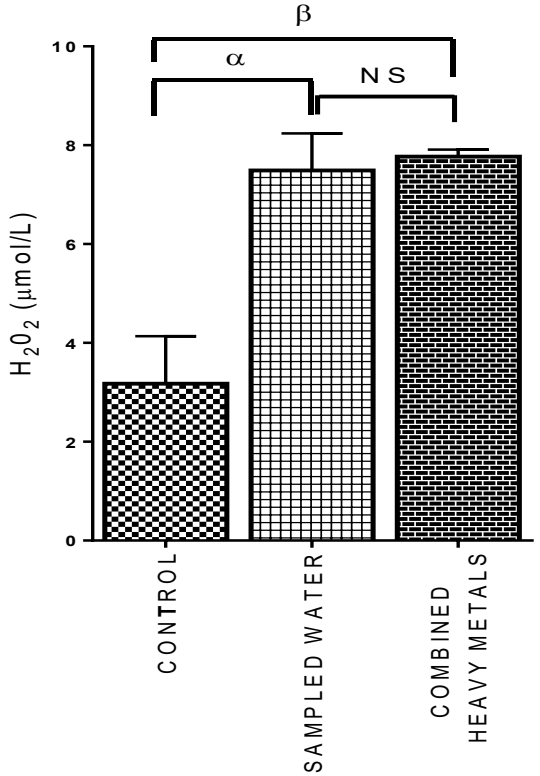

Fig 1. Level of H_2O_2 in experimental groups following 65 days heavy metals exposure $^{\alpha}p < 0.0001$; $^{\beta}p < 0.0001$; NS: No significant.

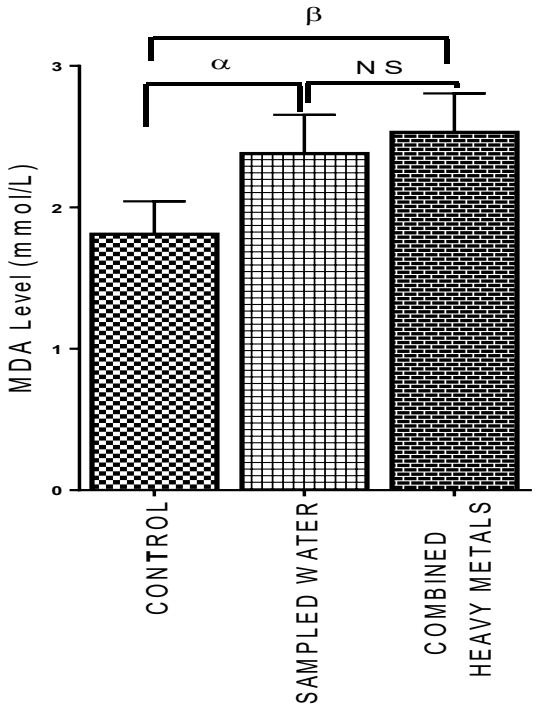

Fig 2. MDA level in the experimental groups following 65 days exposure to heavy metals. $^{\alpha}p < 0.0004$; $^{\beta}p < 0.0002$; NS: No significant

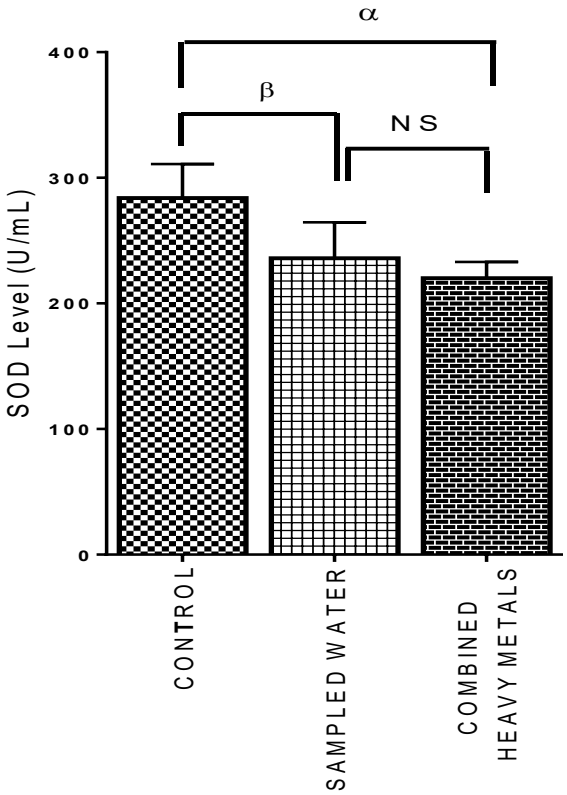

Fig 3. Effects of exposure to heavy metals for 65 days on SOD level in the experimental groups. $^{\alpha}p < 0.0001$; $^{\beta}p < 0.0064$; NS: No significant.

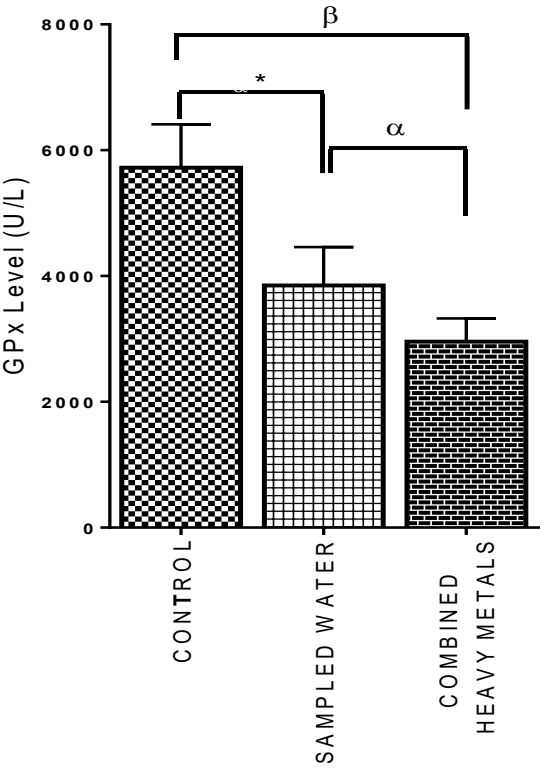

Fig 4. Effects of exposure to heavy metals for 65 days on GPx level in the experimental groups. *p < 0.003; $^{\alpha}p < 0.0018$; $^{\beta}p < 0.0001$

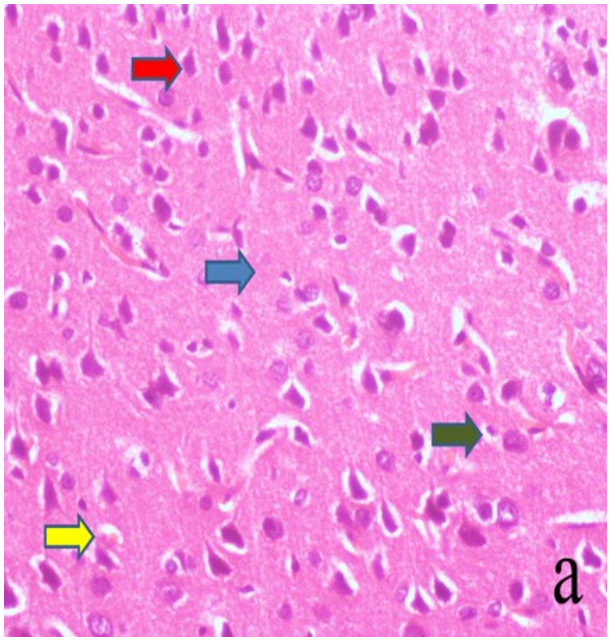

Fig 5. (a) Section from the control group appears normal with normal and intact neurons (red arrow), normal blood vessel (yellow arrow) in the normal neuropil (blue arrow) stained slightly eosinophilic and normal glial cells interspersed within the neuropil (green arrow).

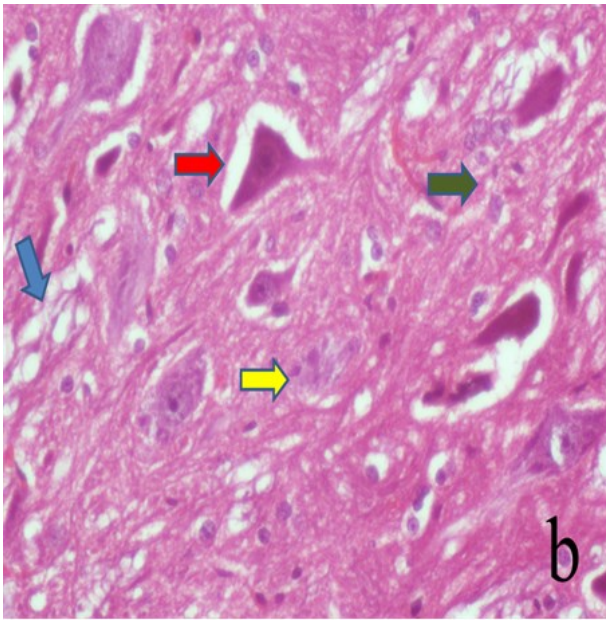

Fig 5. (b) The section from rats following pooled water administration shows features of neurodegeneration: swollen or ballooned neurons some with karyorrhexis (yellow arrow), neurofibrillary tangle (red arrow), neuropil vacuolation (blue arrow) and reactive astrocytic gliosis (green arrow).

in SOD level between group 2 and group 3 (Fig. 3). The level of GPx in the serum of group 2 (3849 ± 217.50) and group 3 (2955 ± 117.24) was significantly lower when compared to the control group (5724 ± 217.50) (Fig. 4).

Neuronal morphology

The general neuronal morphology was demonstrated using haematoxylin and eosin stain (Fig.

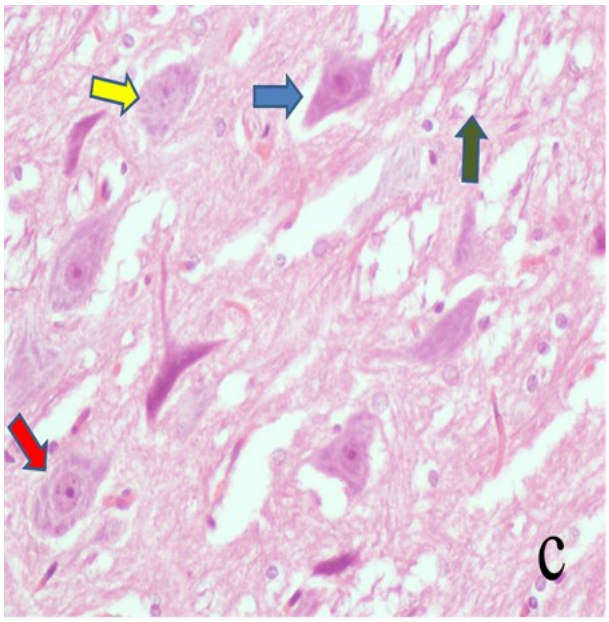

Fig 5. (c) The section from the combined heavy metals group shows features of neurodegeneration: swollen or ballooned neurons (red, yellow and blue arrows) some with karyorrhexis (yellow arrow), reactive astrocytic gliosis with neuropil vacuolation degeneration (green arrow) (x 400, haematoxylin and eosin stain).

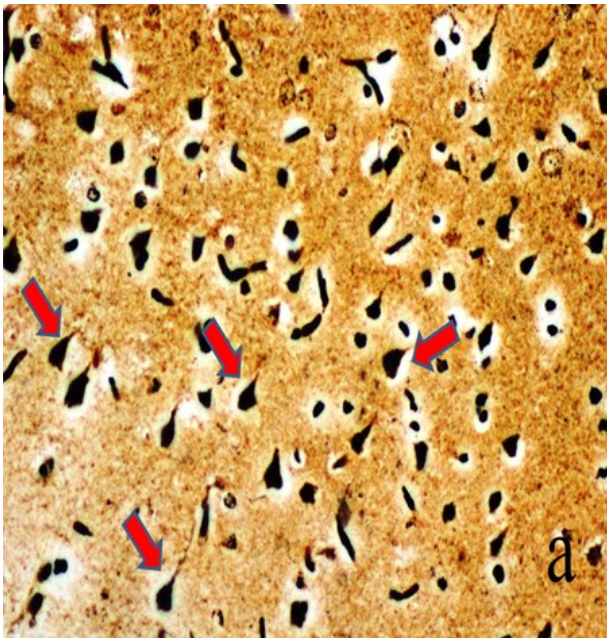

Fig 6. (a) Section from the control group appeared normal with normal and intact neurons (red arrows).

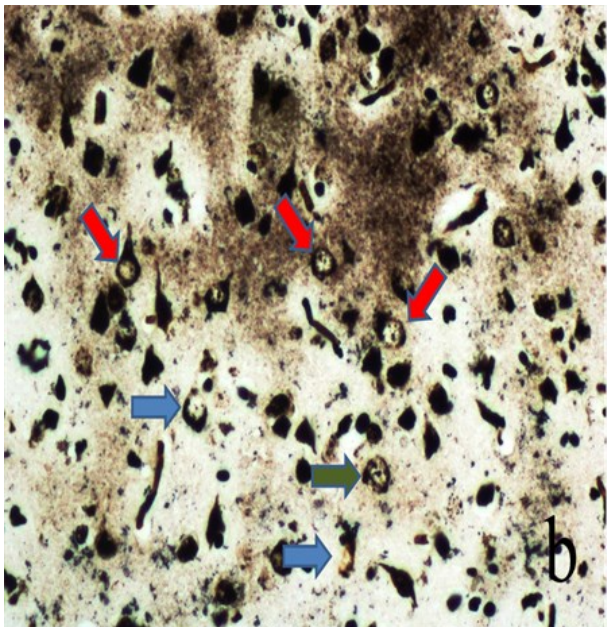

Fig 6. (b) The section from rats administered with pooled sample water shows features of neurodegeneration in the cortex characterised by the presence of degenerate neurons with swollen nuclei (red arrows) and some with no nuclei (blue arrows) and cytoplasm (green arrow).

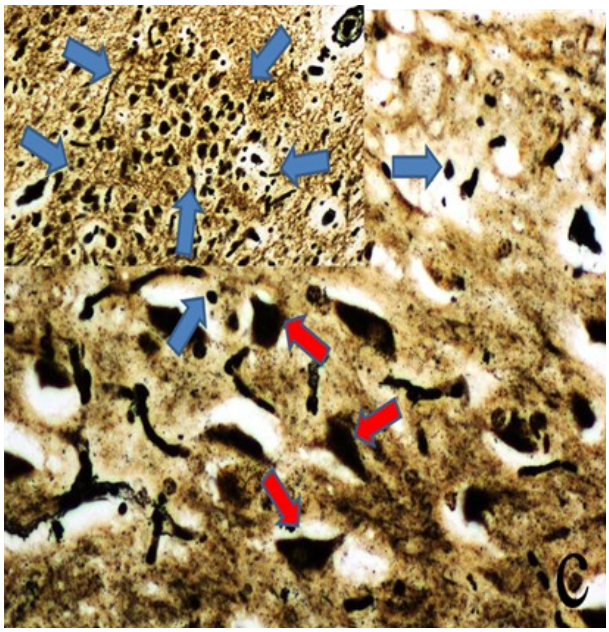

Fig 6. (c) Neurohistochemical evaluation from combined heavy metals is characterised with the presence of degenerate swollen neurons with pyknotic nuclei (red arrows) and marked astrocytic gliosis (blue arrows) (x 400, Bielschowsky's silver impregnation stain)

5). The section from the control group (Fig. 5a) depicts normal neuronal structures, whereas the neurohistological features from the treatment groups reveal neurons with distorted morphology, with different features of neurodegeneration as a result of neurotoxicity from the administered neurotoxicants (Figs. 5b and 5c). The neuronal membrane and presence of neurodegenerative features were further demonstrated using Bielschowsky's silver impregnation stain (Fig. 6). The section from the control group revealed normal neurons with a well-outlined neuronal membrane (Fig. 6a). The treatment group shows neurons with different degenerative features (Figs. 6b and 6c). Reactive glial immunoreactivity was demonstrated using glial fibrillary acidic protein (GFAP) immunostaining (Fig. 7). The treatment groups show strong astrocytic immunoreactivity (Figs. 7b and 7c) compared with the control group (Fig. 7a). The demonstration of oxidative stress was also carried out

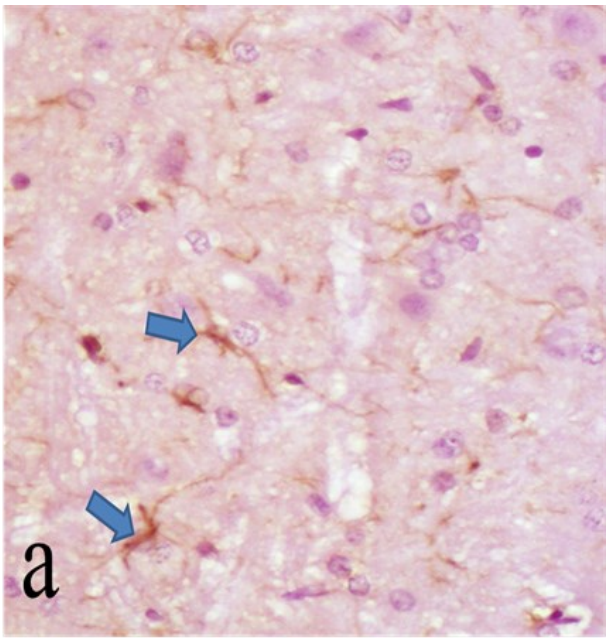

Fig 7. (a) Section from the control group shows mild astrocytic immunoreactivity with specific and uniform staining for glial fibrillary acidic protein (blue arrows)

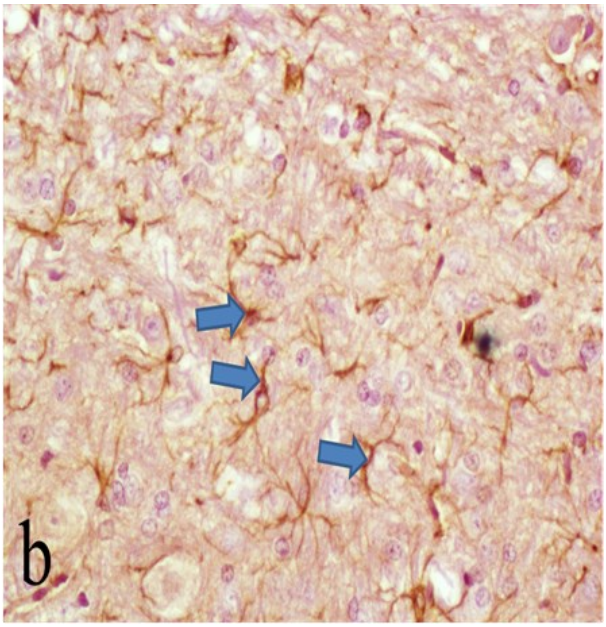

Fig 7. (b) The section from rats following pooled sampled water administration depicts strong immunoreactivity with numerous intensely stained astrocytes (blue arrows).

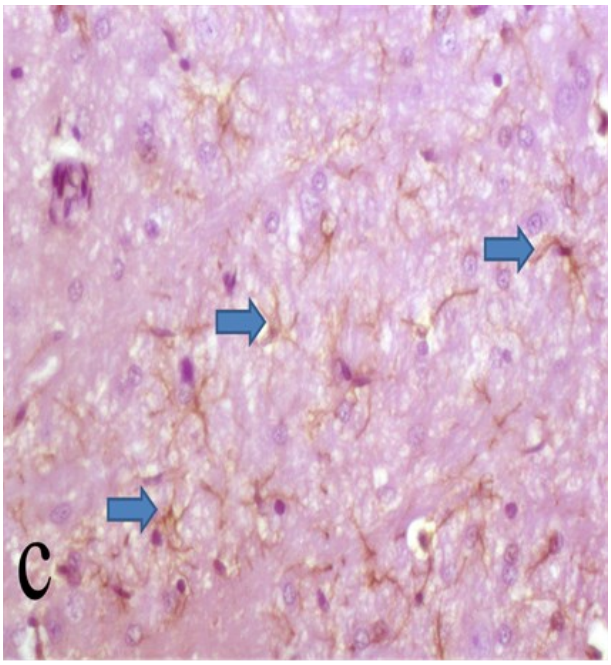

Fig 7. (c) Immunohistochemical evaluation from combined heavy metals group shows strong immunoreactivity with intensely stained astrocytes (blue arrows) (x 400, Glial fibrillary acidic protein immunostain).

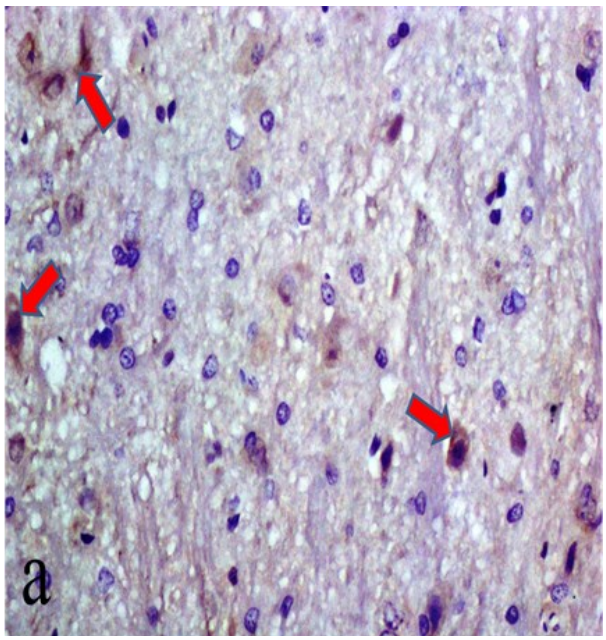

Fig 8. (a) Immunohistochemical evaluation from the control group shows low immunoreactivity with mild expression (red arrows).

using inducible nitric oxide synthase (iNOS) immunostaining (Fig. 8). iNOS reactivity was intensely expressed in the treatment groups (Figs. 8b and 8c) when compared with the control group (Fig. 8a). This revealed heavy metals induced oxidative stress through inordinate nitric oxide synthesis.

DISCUSSION

Heavy metals effect on ROS

This study revealed an increase in hydrogen peroxide level in the treatment groups when compared to the control group (Fig. 1). The significant increase observed in the H_2O_2 level agrees with findings from previous works (Houston, 2011; Oyagbemi et al., 2015). Hydrogen peroxide can oxidise cellular components; thus, hydrogen peroxide inside the cells increases after the tissue damage (Foyer and Noctor, 2005). However, no significant difference in H_2O_2 level from rats administered with pooled sample water (group 2) and combined heavy metals (group 3) was observed ($P_{cal} = 0.2144$, $P > 0.05$). The finding suggests hy-

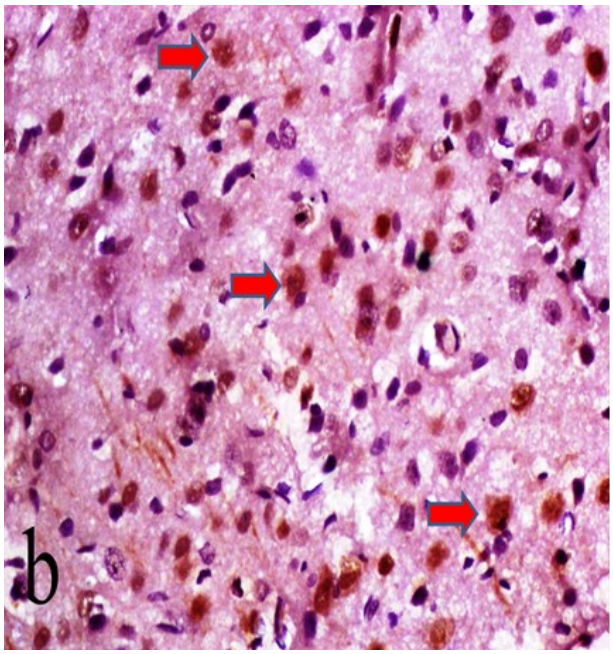

Fig 8. (b) The section from the group administered with pooled sample water depicts strong immunoreactivity with intense expression (red arrows).

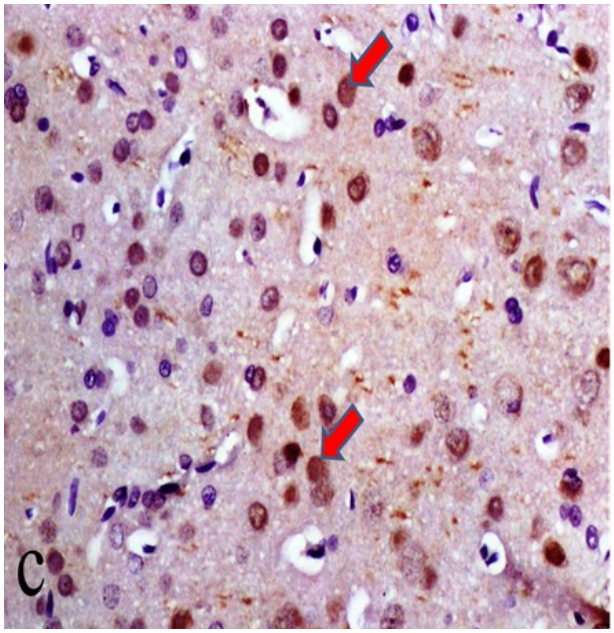

Fig 8. (c) The section from the combined heavy metal group indicates strong immunoreactivity which is intensely expressed (red arrows) (x 400 magnification, inducible nitric oxide synthase immunostain).

drogen peroxide expression is similar in the two groups (Fig. 1).

Acute or chronic heavy metal toxicity is mediated primarily through the injurious tissue ROS production (Faix et al., 2005). Cell membrane components and DNA are particularly reactive to ROS damage, which can result in neurodegeneration (Schulz et al., 2000; Ibrahim et al., 2012; Padurariu et al., 2013). Padurariu et al. (2013) reported that the resultant effects of such damage, especially to fatty acids of the biological membrane, are formed from lipid peroxidation reactions.

Heavy metals effect on lipid peroxidation

MDA finding in this study shows greatly enhanced lipid peroxidation in treated rats compared to the control (Fig. 2). No significant was however observed while comparing the MDA level between groups administered with pooled sampled water (group 2) and combined heavy metals (group 3) (P cal = 0.0811, P > 0.05) (Figure 2). This suggests that MDA expression is equal in the two groups. The significant increase observed in this result agrees with previous reports (Soudani et al., 2012; Apaydin et al., 2014; Oyagbemi et al., 2015; Reckziegel et al., 2016).

Lipid peroxidation is indicated by an increase in the MDA level (Demir et al., 2011). MDA peroxidised polyunsaturated lipids molecules which is diagnostic for oxidative damage (Ferreiro et al., 2012; Bas and Kalender, 2016). Increase in lipid peroxidation level ensues in pathological damage of organs like brain, kidney, and liver (Soudani et al., 2012; Oyagbemi et al., 2015; Reckziegel et al., 2016).

Heavy metals effect on antioxidant enzyme activities

SOD and GPx activities are frequently used as oxidative stress markers in tissue and blood (Pathak and Khandelwal, 2006). They are endogenous antioxidant enzymes that protect the body against oxidative damage (Gilgun-Sherki et al., 2001; Padurariu et al., 2013). This role is achieved as they speed up the reduction of ROS reaction through different mechanism thereby decreasing their power to cause oxidative damage (Gilgun-Sherki et al., 2001; Padurariu et al., 2013).

SOD level following exposure

The substantial reduction in SOD level observed following heavy metal exposure agrees with previous works (Pardeep and Bimla, 2004; Flora, 2009; Reckziegel et al., 2016). However, there was no significant observable difference while comparing SOD activity between the group administered with pooled sample water (group 2) and combined heavy metals (group 3) (P cal = 0.1889, P > 0.05) (Fig. 3). This suggests SOD activity is equal in the two groups.

SOD catalyses the conversion of superoxide anions to H_2O_2 and oxygen (Kalender et al., 2015). It is known to be the number one defence against ROS and decreases in its expression indicates oxidative damage (Antonio-García and Massó-Gonzalez, 2008; Jambunathan, 2010). In the studies of superoxide anions and their role in neuronal degeneration, SOD is a significant defence of the neuronal cells against increased ROS and oxygen radical level (Jambunathan, 2010).

GPx level following exposure

The considerable decrease recorded in the activity of GPx (Fig. 4) agrees with the work of Pardeep and Bimla (2004), Oyagbemi et al. (2015) and Reckziegel et al. (2016). GPx catalyses the reduc-

Table 1. Grouping and Treatment of animals.

Group	Treatment
Group 1	Control group had access to food and distilled water *ad libitum*
Group 2	Treatment group 1, had access to food and pooled sampled water from the study area *ad libitum*
Group 3	Treatment group 2, had access to food and dissolved the mixture of 0.009/L mg of Pb $(CH_3CO_2).3H_2O$, 0.001 mg/L of $Hg(SCN)_2$, 0.045 mg/L of $Cd(CH_3CO_2).2H_2O$ and 0.318 mg/L of Cr_2O_3 water *ad libitum*

ing of H_2O_2 to H_2O and oxygen thereby protecting the cell from oxidative damage induced by H_2O_2 (Messarah et al., 2012; Bas et al., 2014). It influences the donation of reducing equivalent by GSH/GSSH system to ROS while making ROS stable. It also detoxifies mitochondrial and cytosolic peroxides (Schulz et al., 2000).

Effect of heavy metals on prefrontal cortex of experimental animals

Histological and histochemical findings

Several parameters were employed in this study to observe the morphology of the neurons following the effects of treatment in the prefrontal cortex of rats. Based on the higher functions associated with prefrontal cortex of the brain coupled with regional variation in neuronal plasticity, the morphology of the neurons were investigated to reflect the difference in their response to the effect of treatment on the prefrontal cortex following exposure.

This study employed one histological stain, Haematoxylin and Eosin, and one histochemical stain, Bielschowsky's silver impregnation. These two complementary and independent methods demonstrated different features of neurodegeneration (Figs. 5 and 6). The degeneration pattern of the neurons was necrotic. Necrosis in this context is seen as neuronal swelling, with a gradual decline of plasma membrane integrity and nuclear swelling similar to that described by Barrett et al. (2001).

Fig. 5b and 5c which is representative of prefrontal cortex from the pooled sampled water and the combined group respectively, share similar morphologic appearance. These characteristic appearances eventually result in neuronal death. Neuronal death is fundamental in the neuropathology of various degenerative disorders of the CNS (Cordeiro et al., 2010). The present finding is in line with previous works in which different neurotoxicants investigated resulted in neuronal damage and death (Pardeep and Bimla, 2004; Soudani et al., 2012; Ranjan et al., 2015; Makanjuola et al., 2016; Mahmoud et al., 2017).

It has also been shown that, in neurons, heavy metals produce an increase in ROS with resultant membrane damage, defective synaptic connection, and increased neuronal death (Antonio et al., 2003). This correlated with the ROS findings (Fig. 1). The brain has some characters that make it susceptible to these ROS mediated injuries. The brain lipids possess a significant amount of polyunsaturated fatty acids, which increases the susceptibility of the neuronal membrane to lipid peroxidation. This is also correlated with the MDA findings (Fig. 2) and the histological appearance in this study. The histological appearance is as shown in the structures of the membrane observed in the treatment groups (Figs. 5b, 5c, 6b, 6c) when compared with the control groups (Figs. 5a, 6a). Singer and Nicolson (1972) described membrane as seabed of the bilipid layer with floating icebergs of protein. Lipid peroxidation accounts for the reduced membrane integrity observed (Valko et al., 2016). Astrocytic increment was obvious in the treatment groups (Figs. 6b, 6c). Structural support and strength to the surrounding neurons in the central nervous system are provided by astrocytes, especially during neuronal assault (Mccall et al., 1996; Eng et al., 2000). The striking reactive gliosis observed in the treatment groups in figure 6 is worth noting since astrocyte is one of the major effector of neuroinflammation (Florianne et al., 2006).

Immunohistochemical findings

Glial fibrillary acidic protein (GFAP) immunostaining expression

GFAP is the marker for mature astrocytes. The prefrontal cortex of treated rats showed strong astrocytic immunoreactivity for GFAP immunostain, resulting in intensely stained astrocytes (Fig. 7). In this study, GFAP immunoreactions establish astrocyte immunological response to the studied neurotoxicants. This expression is observed as dark-brown multi-processed star-shaped cells called astrocytes. Astrocytes are one of the supportive cells of the CNS. They are activated during oxidative damage in response to excitotoxicity (Lui et al., 2011; Adekomi et al., 2017). Reactive astrogliosis, an abnormal astrocyte proliferation, primarily indicates induced neuronal damage (Buffo et al., 2008; Zuchero et al., 2015; Zhang et al., 2010; Olajide et al., 2016; Adekomi et al., 2017). Triolo et al. (2006) reported astrogliosis as a major feature seen in most neurodegeneration disorders as a result of oxidative damage. This agrees with the previous study, where increased expression of GFAP following exposure to heavy metal was acknowledged (Adekomi et al., 2017). This buttresses the histological and histochemical findings in this study, as marked reactive astrocytic gliosis was observed in the prefrontal cortex of experimental rats (Figure 5 and 6). Therefore, it is inferred that the reactive observed astrocytic gliosis in this study is as a result of heavy metals induced oxidative damage.

Induced nitric oxide synthase (iNOS) immunostain expression

The result from this study demonstrates that treatment enhances astrocytic gliosis, which expresses iNOS immunoreactivity (Fig. 8). This as well buttresses the histological, histochemical and GFAP immunostain findings in this study, where marked reactive astrocytic gliosis was observed in

the treatment group. iNOS is seen as a nuclear-cytoplasmic stain from this result. Neuroglia, especially microglia, and astrocyte are activated by inflammatory factors to express iNOS (Saha and Pahan, 2006). These triggered neuroglia produce nitric oxide and superoxide, which results in the formation of peroxynitrite (Pacher et al., 2007; Brown, 2010). Peroxynitrite was reported by Brown (2010) as a potent oxidant causing cell death. Increased iNOS reactivity reveals induced oxidative damage through the synthesis of nitric oxide, which agrees with the work of Olajide et al. (2016). The synthesised nitric oxide produced by iNOS is a defence mechanism seen in neural toxicity while nitric oxide produced by glia mainly by iNOS induction (Bal-Price and Brown, 2001).

Judging by findings in this study, the treatment groups revealed similar results. The heavy- metals -treated water group (group 3) contain mixture of metals with similar relative concentration to that pooled from the waterways in the study area (group 2). Therefore, group 3 was directly designed to mimic the contaminated pooled water sources (group 2) and serve as our positive control. The similarity displayed is made possible by likely interactions between these essential and toxic metals which could be vital metal toxicity modifiers (Barbier et al., 2005; Jadhav et al., 2007). Result from this study is consistent with the findings of Rai et al. (2010; 2013) which indicated that exposures to heavy metals are connected with neurotoxicity.

CONCLUSION

Biochemical, histological, histochemical, and immunochemical evaluations disclosed the vulnerability to four different neurotoxicants found in different waterways in three geographical zones of Kwara State, which affected the functional and structural integrity of the prefrontal cortex of the Wistar rats. It was discovered from findings in this study that the treatment groups displayed similar neurodegenerative defects. Prolonged exposure to a relatively higher contamination may trigger severe neurological damage. This work further stresses the need for intense monitoring and protection from these pollutants in developing countries.

ACKNOWLEDGEMENTS

The authors acknowledge the following personalities who have contributed in different ways. Mr Adekunle Fowotade, the technical head of Pathology Department, University of Ilorin Teaching Hospital (UITH). He granted access to use their laboratory for my work and other staff especially Pastor Oluwumi Olutunde and Mrs Akanbiola for their technical input. Mr Odetunde Abayomi and Mr Okedere Babajide Adedapo, Institute for Advance Medical Research and Training (IAMRAT), University College Hospital, Ibadan; Mr Jonathan Madukwe, Pathology Department, National Hospital, Abuja; Mr Akinyinka, Department of Chemical Pathology, University of Ilorin Teaching Hospital, Ilorin and Dr. Ebenezer Soji Adetona, Consultant Pathologist, University College Hospital, Ibadan.

Dissemination history: Part of the corresponding author's Doctoral thesis submitted to the Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria, Enugu Campus, Enugu State, Nigeria, in partial fulfillment of the requirements for the award of Doctor of Philosophy (Ph.D) degree in Medical Laboratory Science (Histopathology and Neuropathology).

LIST OF ABBREVIATIONS
ROS – Reactive oxygen species
CNS – Central nervous system
SOD – Superoxide dismutase
GPx – Glutathione peroxidise
MDA – Malondialdehyde
GFAP – Glial fibrillay acidic protein
iNOS – Inducible nitric oxide synthase
DNA – Deoxyribonucleic acid
GSH/GSSH - reduced glutathione/ oxidized glutathione

REFERENCES

ADEKOMI DA, ADEWOLE OS, ADEKILEKUN TA, DANIEL AT (2017) Lead induces inflammation and neurodegenerative changes in the rat medial prefrontal cortex. *Anat*, 11(2): 79-86.

ADENIYI TD, ACHUKWU PU, ABUBAKAR AA (2017) Frequency of electronics waste generated heavy metals in urban waterways. *Int J Human Capital Urban Manage*, 2(2): 89-100.

ADEYEMI O, OLOYEDE OB, OLADIJI AT (2007) Physicochemical and microbial characteristics of leachate-contaminated groundwater. *Asian J Biochem*, 2(5): 343-348.

AKINSEYE VO (2013) Electronic waste components in developing countries: harmless substances or potential carcinogen. *Annu Rev Res Biol*, 3(3): 131-147.

ANTONIO MT, CORREDOR L, LERET ML (2003) Study of the activity of several brain enzymes like markers of the neurotoxicity induced by perinatal exposure to lead and/or cadmium. *Toxicol Lett*, 143: 331-340.

ANTONIO-GARCÍA MT, MASSÓ-GONZALEZ EL (2008) Toxic effects of perinatal lead exposure on the brain of rats: involvement of oxidative stress and the beneficial role of antioxidants. *Food Chem Toxicol*, 46: 2089-2095.

APAYDIN FG, KALENDER S, DEMIR F, BAS H (2014) Effects of sodium selenite supplementation on lead nitrate induced oxidative stress in lung tissue of diabetic and non- diabetic rats. *Gazi Univ J Sci*, 27(2): 847-853.

AZUKA AI (2009) The influx of used electronics into Africa: a perilous trend. *LEAD Journal*, 5(1): 90-106.

BAL-PRICE A, BROWN GC (2001) Inflammatory neurodegeneration mediated by nitric oxide from activated glia-inhibiting neuronal respiration, causing glutamate release and excitotoxicity. *J Neurosci*, 21(17): 6480-6491.

BANCROFT JD, GAMBLE H (2008) *Theory and practice of histological technique.* 6th ed. Churchill Livingstone, New York.

BARBIER O, JACQUILLET G, TAUC M, COUGNON M, POUJEOL P (2005) Effect of heavy metals on, and handling by, the kidney. *Nephron Physiol,* 99: 105-110.

BARRETT KL, WILLINGHAM JM, GARVIN AJ, WILLINGHAM MC (2001) Advances in cytochemical methods for detection of apoptosis. *J Histochem Cytochem*, 49: 821-832.

BAS H, KALENDER S (2016) Antioxidant status, lipid peroxidation and testis histoarchitecture induced by lead nitrate and mercury chloride in male rats. *Braz Arch Biol Technol*, 59: e16160151.

BAS H, KALENDER S, PANDIR D (2014) In vitro effects of quercetin on oxidative stress mediated in human erythrocytes by benzoic acid and citric acid. *Folia Biol*, 62: 59-66.

BISHAK YK, PAYAHOO L, OSATDRAHIMI A (2015) Mechanisms of cadmium carcinogenicity in the gastrointestinal tract. *Asian Pac J Cancer Prev*, 16(1): 9-21.

BROWN GC (2010) Nitric oxide and neuronal death. *Nitric oxide*, 23: 153-165.

BUFFO A, RITE I, TRIPATHI P, LEPIER A, COLAK D, HORN A-P, MORI T, GÖTZ M (2008) Origin and progeny of reactive gliosis: A source of multipotent cells in the injured brain. *Proc Natl Acad Sci USA*, 105(9): 3581-3586.

CHEN A, DIETRICH KN, HUO X, HO S (2011) Developmental neurotoxicant in e-waste: an emerging health concern. *Environ Health Perspect*, 119: 431-438.

CHIURCHIÙ V, ORLACCHIO A, MACCARRONE M (2016) Is modulation of oxidative stress an answer? The state of the art of redox therapeutic actions in neurodegenerative diseases. *Oxid Med Cell Longevity*, 2016: 7909380.

CORDEIRO MF, GUO L, COXON KM, DUGGAN J, NIZARI S, NORMANDO EM, SENSI SL, SILLITO AM, FITZKE FW, SALT TE, MOSES SE (2010) Imaging multiple phases of neurodegeneration: a novel approach to assessing cell death in vivo. *Cell Death Dis*, 1: e3.

DEIANA L, CARRU C, PES G, TADOLINI B (1999) Spectrophotometric measurement of hydroperoxides at increased sensitivity by oxidation of Fe 2+ in the presence of xylenol orange. *Free Radical Res,* 31(3): 237-244.

DELCAMBRE GH, LUI J, HERRINGTON JM, VALLARIO K, LONG MT (2016) Immunohistochemistry for the detection of neural and inflammatory cells in equine brain tissue. *PeerJ,* 4: e1601.

DEMIR F, UZUN FG, DURAK D, KALENDER Y (2011) Subacute chlorpyrifos-induced oxidative stress in rat erythrocytes and the protective effects of catechin and quercetin. *Pestic Biochem Physiol*, 99: 77-81.

DRURY RA, WELLINGTON EA (1980) *Carleton Histological Technique.* 5th ed. Oxford University Press, New York.

ENG L, GHIRNIKAR R, LEE Y (2000). Glial fibrillary acidic protein: GFAP-thirty-one years (1969-2000). *Neurochem Res,* 25: 1439-1451.

FAIX A, FAIXOVA Z, BOLDIZAROVA K, JAVORSKY P (2005) The effect of long-term heavy metal intake on lipid peroxidation of gastrointestinal tissue in sheep. *Veterinární medicína*, 50(9): 401-405.

FERREIRO E, BALDEIRAS I, FERREIRA IL, COSTA RO, REGO AC, PEREIRA CF, OLIVEIRA CR (2012) Mitochondrial- and endoplasmic reticulum-associated oxidative stress in Alzheimer's disease: from pathogenesis to biomarkers. *Int J Cell Biol*, 2012: 735206.

FLORIANNE M, MARIE-GABRIELLE Z, CORINA B, ANNE C, PAUL H (2006) Involvement of environmental mercury and lead in the etiology of neurodegenerative diseases. *Rev Environ health,* 21(2): 105-117.

FLORA SJS (2009) Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. *Oxid Med Cell Longevity*, 2: 191-206.

FOYER CH, NOCTOR G (2005) Redox homeostasis and antioxidant signalling: a metabolic interface between stress perception and physiological responses. *Plant cell*, 17: 1866-1875.

GILGUN-SHERKI Y, MELAMED E, OFFEN D (2001) Oxidative stress induced-neurodegenerative diseases: the need for antioxidants that penetrate the blood brain barrier. *Neuropharmacol*, 40: 959-975.

HOEHN T, FELDERHOFF-MUESER U, MASCHEWSKI K, STADELMANN C, SIFRINGER M, BITTIGAU P, KOEHNE P, HOPPENZ M, OBLADEN M, BÜHRER C (2003) Hyperoxia causes inducible nitric oxide synthase-mediated cellular damage to the immature rat brain. *Pediatr Res*, 54: 179-184.

HOUSTON MC (2011) Role of mercury toxicity in hypertension, cardiovascular disease and stroke. *J Clin Hypertens*, 13: 621-627.

HUANG J, NKRUMAH PN, ANIM DO, MENSAH E (2014) E-waste disposal effects on the aquatic environment: Accra, Ghana. *Rev Environ Contam Toxicol*, 229: 19-34.

IBRAHIM NM, EWEIS EA, EL-BELTAGI HS, ABDUL-MOBDY YE (2012) Effect of lead acetate toxicity on experimental male albino rat. *Asian Pac J Trop Biomed*, 2(1): 41-46.

IBRAHIM U (2017) E-waste environmental pollution and health risk implications for early child care, growth and development in Nigeria. *Sustainable Human Dev Rev*, 9(2): 41-54.

IZAH SC, CHAKRABARTY N, SRIVASTAV AL (2016) A review on heavy metal concentration in potable water sources in Nigeria: human health effects and mitigating measures. *Expo Health*, 8(2): 285-304.

JADHAV S, SARKAR S, PATIL R, TRIPATHI H (2007) Effects of subchronic exposure via drinking water to a mixture of eight water-contaminating metals: a biochemical and histopathological study in male rats. *Arch Environ Contam Toxicol,* 53(4): 667-677.

JAMBUNATHAN N (2010) Determination and detection of reactive oxygen species (ROS), lipid peroxidation, and electrolyte leakage in plants. *Methods Mol Biol*, 639: 292-298.

JINHUI L, HUABO D, PIXING S (2011). Heavy metal contamination of surface soil in electronic waste dismantling area: site investigation and source-apportionment analysis. *Waste Manage Res*, 29: 727-738.

JOHANSSON C, CASTOLDI AF, ONISHCHENKO N, MANZO L, VAHTER M, CECCATELLI S (2007) Neu-

robehavioural and molecular changes induced by methylmercury exposure during development. *Neurotoxic Res*, 11: 241-260.

JOSEPH P (2009) Mechanisms of cadmium carcinogenesis. *Toxicol Appl Pharmacol,* 238: 272-279.

KALENDER S, APAYDIN FG, BAS H, KALENDER Y (2015) Protective effects of sodium selenite on lead nitrateinduced hepatotoxicity in diabetic and non-diabetic rat rats. *Environ Toxicol Pharmacol*, 40: 568574.

KARRI V, SCHUHMACHER M, KUMAR V (2016) Heavy metals (Pb, Cd, As and MeHg) as risk factors for cognitive dysfunction: A general review of metal mixture mechanism in brain. *Environ toxicol pharmacol*, 48: 203-213.

LIU W, TANG Y, FENG J (2011) Cross talk between activation of microglia and astrocytes in pathological conditions in the central nervous system. *Life Sci*, 89: 141-146.

MAHMOUD A, MOKHTAR MMT, HANAA GA (2017) Neurodegenerative disorders associated with mercuric chloride toxicity in mice and the role of some antioxidant. *Int J Sci Res*, 6(4): 1253-1260.

MAKANJUOLA VO, OMOTOSO OD, FADAIRO OB, DARE BJ, OLUWAYINKA OP, ADELAKUN SA (2016) The effect of parkia leaf extract on cadmium induced cerebral lesion in Wistar rats. *Br J Medic Med Res*, 12 (4): 1-7.

MARSLAND TA, GLEES P, ERIKSON LB (1954) Modification of Glee's silver impregnation for paraffin sections. *J Neuropathol Exp Neurol*, 13: 587-591.

MCCALL MA, GREGG RG, BEHRINGER RR, BRENNER M, DELANEY CL, GALBREATH EJ, ZHANG CL, PEARCE RA, CHIU SY, MESSING A (1996) Targeted deletion in astrocyte intermediate filament (Gfap) alters neuronal physiology. *Proc Natl Acad Sci USA,* 93: 6361-6366.

MESSARAH M, KLIBET F, BOUMENDJEL A, ABDENNOUR C, BOUZERNA N, OULAKOUD MS, EL FEKI A (2012) Hepatoproctective role and antioxidant capacity of selenium on arsenic-induced liver injury in rats. *Exp Toxicol Pathol*, 64: 167-174.

MISTRAL HP, FRIDOVICH J (1972) The role of superoxide anion in the antioxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem*, 247: 3170-3175.

NAGAJYOTI PC, LEE KD, SREEKANTH TVM (2010) Heavy metals, occurrence and toxicity for plants: a review. *Environ Chem Lett*, 8(3): 199-216.

NATIONAL ACADEMY SCIENCES (NAS) (2011) *"Guide for the Care and Use of Laboratory Animals".* Institute for Laboratory Animal Research. 8th ed. National Academies Press, New York.

NEAL AP, GUILARTE TR (2012) Mechanisms of heavy metal neurotoxicity: lead and manganese. *J Drug Metab Toxicol*, S5: 002.

NUDLER SI, QUINTEROS FA, MILER EA, CABILLA JP, RONCHETTI SA, DUVILANSKI BH (2009) Chromium VI administration induces oxidative stress in hypothalamus and anterior pituitary gland from male rats. *Toxicol Lett*, 185: 187-192.

OLAJIDE OJ, AKINOLA OB, AJAO MS, ENAIBE BU (2016) Sodium azide-induced degenerative changes in the dorsolateral prefrontal cortex of rats: attenuating mechanisms of kolaviron. *Eur J Anat*, 20(1): 47-64.

OYAGBEMI AA, OMOBOBOWALE TO, AKRINDE AS, SABA AB, OGUNPOLU BS, DARAMOLA O (2015) Lack of reversal of oxidative damage in renal tissues of lead acetate-treated rats. *Environ Toxicol*, 30(11): 1235-1243.

PACHER P, BECKMAN JS, LIAUDET L (2007) Nitric oxide and peroxynitrite in health and disease. *Physiol Rev*, 87: 315424.

PADURARIU M, CIOBICA A, LEFTER R, SERBAN IL, STEFANESCU C, CHIRITA R (2013) The oxidative stress hypothesis in Alzheimer's disease. *Psychiatria Danubina*, 25(4): 401-409.

PAGLIA DE, VALENTINE WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med*, 70: 158-169.

PARDEEP S, BIMLA N (2004) Lead intoxication: histological and oxidative damage in rat cerebrum and cerebellum. *J Trace Elem Exp Med*, 17: 45-53.

PATHAK N, KHANDELWAL S (2006) Influence of cadmium on murine thymocytes: potentiation of apoptosis and oxidative stress. *Toxicol Lett*, 165: 121-132.

PETRONI D, TSAI J, AGRAWAL K (2012) Low-dose methylmercury-induced oxidative stress, cytotoxicity, and tau-hyperphosphorylation in human neuroblastoma (SH-SY5Y) cells. *Environ Toxicol*, 27(9): 549-555.

PUSCHENREITER M, HORAK O, FRIESEL W, HARTL W (2005) Low-cost agricultural measures to reduce heavy metal transfer into the food chain- a review. *Plant Soil Environ*, 51: 1-11.

RAI A, MAURYA SK, KHARE P, SRIVASTAVA A, BANDYOPADHYAY S (2010) Characterization of developmental neurotoxicity of As, Cd, and Pb mixture: synergistic action of metal mixture in glial and neuronal functions. *Toxicol Sci,* 118(2): 586-601.

RAI NK, ASHOK A, RAI A, TRIPATHI S, NAGAR GK, MITRA K, BANDYOPADHAY S (2013) Exposure to As, Cd and Pb-mixture impairs myelin and axon development in rat brain, optic nerve and retina. *Toxicol Appl Pharm,* 273: 242-258.

RANJAN B, HUSAIN SMD, KUMAR K, MAHESHWARI TP (2015) Comparative study of histopathological effects of mercury on cerebrum, cerebellum and hippocampus of adult albino rats. *Ann Int Med Dent Res*, 1 (1): 21-24.

RECKZIEGEL P, DIAS VT, BENVEGNÚ DM, BOUFLEUR N, MOREIRA DOS SANTOS CM, FLORES EMM (2016) Antioxidant protection of gallic acid against toxicity induced by Pb in blood, liver, and kidney of rats. *Toxicol Rep*, 3: 351-356.

SAHA RN, PAHAN K (2006) Regulation of inducible nitric oxide synthase gene in glial cells. *Antioxid Redox Signaling*, 8: 929-947.

SANDERS T, LIU Y, BUCHNER V, TCHOUNWOU PB (2009) Neurotoxic effects and biomarkers of lead exposure: a review. *Rev Environ Health*, 24: 15-45.

SCHULZ JB, LINDENAU J, SEYFRIED J, DICHGANS J (2000) Glutathione, oxidative stress and neurodegeneration. *Eur J Biochem*, 267: 4904-4911.

SHAMIM A, MURSHEDA AK, RAFIQ I (2015) E-waste trading impact on public health and ecosystem ser-

vices in developing countries. *Int J Waste Resour*, 5 (4): 188.

SINGER SJ, NICOLSON GL (1972) The fluid mosaic model of structure of cell membranes. *Science*, 175 (4023): 720-731.

SOUDANI N, TROUDI A, AMARA IB, BOUAZIZ H, BOUDAWARA T, ZEGHAL N (2012) Ameliorating effect of selenium on chromium (VI)-induced oxidative damage in the brain of adult rats. *J Physiol Biochem*, 68(3): 397-409.

STOCKS J, DOMANDY TL (1971) The autoxidation of human red cell lipid induced by hydrogen peroxide. *Br J Haematol*, 20: 95-111.

STRUZYÑSKA L, BUBKO I, WALSKI M (2001) Astroglial reaction during the early phase of acute lead toxicity in the adult rat brain. *Toxicol*, 165(2-3): 121-131.

TRIOLO D, DINA G, LORENZETTI I, MALAGUTI M, MORANA P, DEL CARRO U, COMI G, MESSING A, QUATTRINI A, PREVITALI SC (2006) Loss of glial fibrillary acidic protein (GFAP) impairs Schwann cell proliferation and delays nerve regeneration after damage. *J Cell Sci*, 119(Pt19): 3981-3993.

UNITED NATIONS UNIVERSITY (UNU) (2013) Solve the E-waste Problem (StEP), Massachusetts Institute of Technology (MIT), National Center for Electronics Recycling (NCER). World e-waste map reveals national volumes, international flows. http://www.step-initiative.org/news/world-e-waste-map--reveals-national-volumes-internation-flows.htm, [Accessed 11 January 2017].

VALKO M, JOMOVA K, RHODES CJ, KUČA K, MUSÍLEK K (2016) Redox- and non-redox-metal-induced formation of free radicals and their role in human disease. *Arch Toxicol*, 90(1): 1-37.

VAN ZUTPHEN LFM, BAUMANS V, BEYNEN AC (1993) *Principles of Laboratory Animal Science*. Elsevier, Amsterdam.

WOLFF SP (1994) Ferrous ion oxidation in the presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. *Methods Enzymol*, 233: 182-189.

WORLD HEALTH ORGANIZATION (WHO) (2011) World report on disability 2011. http://www.who.int/disabilities/world_report/2011/en/index.html, [Accessed 11 August 2016].

WRIGHT RO, BACCARELLI A (2007) Metals and neurotoxicology. *J Nutr*, 137: 2809-2813.

ZENG X, XU X, BOEZEN HM, HUO X (2016) Children with health impairments by heavy metals in an e-waste recycling area. *Chemosphere*, 148: 408-415.

ZHANG D, HU X, QIAN L, O'CALLAGHAN JP, HONG JS (2010) Astrogliosis in CNS pathologies: Is there a role for microglia? *Mol Neurobiol*, 41(2-3): 232-241.

ZUCHERO JB, BARRES BA (2015) Glia in mammalian development and disease. *Dev*, 142: 3805-3809.