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Sulphoraphane Supplementation Ameliorates Behavioural Impairments and Hippocampal Neurodegeneration Induced by Lead Exposure in Adult Wistar Rats

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ABSTRACT

Lead (Pb) exposure induces oxidative stress causing imbalance in antioxidant enzymes, cognitive impairments and neurodegeneration. This study investigated the neuroprotective and antioxidant properties of sulphoraphane (SFN) on Pb-induced neurotoxicity of adult Wistar rats. Forty animals (150 ± 20 g) were divided into four groups (n=10): Group A received normal saline as placebo; Group B received 50 mg/kg body weight (bw) of Lead only; Group C received a combination of 50 mg/kg bw of Lead and 50 mg/kg bw of SFN; Group D received 50 mg/kg bw of SFN only. All administration was through oral gavages for 28 days; animals underwent behavioural tests (Morris water and Y- mazes); and thereafter sacrificed and brains extracted. Biochemical estimations of antioxidants (superoxide dismutase, reduced glutathione, and catalase), oxidative stress markers (malondialdehyde, nitric oxide, and hydrogen peroxide), neurotransmitters (dopamine, serotonin, and norepinephrine) and hippocampal histology were done. The results showed significant increase in escape latency, norepinephrine and oxidative stress markers with concomitant decrease percentage correct alternation, serotonin, dopamine and antioxidant enzymes in Pb exposed rats compared with the control. However, the co-administration of SFN and Pb significantly attenuated Pb neurotoxicity. Sulphoraphane is capable of ameliorating oxidative stress induced neurobehavioural deficits and hippocampal neurochemistry caused by Pb exposure in Alzheimer's type animal model of neurodegenerative disorder.

Key words: *Lead; Hippocampal neurodegeneration; Sulphoraphane; Behavioural stress test; Oxidative stress*

INTRODUCTION

Neurotoxicity is one of the leading causes of neurodegenerative disorders with several complications such as cognitive impairment, disorders in memory (long and short term), mood swings and onset of psychiatric disturbances (Caban-Holt et al. 2005; Han et al. 2011; Mason et al. 2013). Alzheimer's disease (AD) is widely known for its prevalence, progression and fatality. It is usually associated with aging and it manifests clinically with symptoms including (but not restricted to) memory impairment and difficulty in the performance of normal daily activities (Bakulski et al. 2012). Within the last 50 years, AD has gone from a state of obscurity (although relative) to becoming a defining characteristic of a normal, industrialized society (Bertram et al. 2009). Research has provided evidence of an association between AD and over-exposure to environmental neurotoxicants (Hardy 1997). Neurotoxicants could exist in the form of elements such as aluminium, zinc, nickel, lead, cadmium, manganese and cadmium (Shcherbatykh

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and Carpenter 2007; Frisardi et al. 2010).

Lead is a soft, malleable, and extremely toxic heavy metal that occurs naturally in the earth's crust (Mason et al. 2013). It is found in all parts of the environment, derived majorly from human activities such as manufacturing, mining and burning fossil fuels (Sanders et al. 2009). It is utilized in a variety of products such as gasoline, paints, ceramics, ammunition, water pipes, solders, cosmetics, hair dye, farm equipment, airplanes, shielding for x-ray machines, manufacture of corrosion and acid resistant materials used in the building industry (ATSDR 2006).

Chronic Pb exposure has been associated with the incidence of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (Monnet-Tschudi et al. 2006). AD is characterized by the formation of waxy plaques consisting predominantly of β-amyloid protein between neurons (a known neuropathological hallmark of the disease), and Pb has been known to increase the expression of the amyloid precursor protein (APP) which precedes the formation of these plaques (Monnet-Tschudi et al. 2006).

Exposure to lead cause severe genetic, blood and mitochondria destructions (Anderson et al. 1996), resulting in several abnormalities such as; early onset of apoptosis, excitotoxicity affecting storage of neurotransmitters, release and modification of neurotransmitter receptors, mitochondria damage, cerebrovascular endothelial cells damage, astroglia and oligodendroglia destructions (Sharma et al. 2015). There is also indubitable evidence that increased exposure to lead acetate results in adverse effects on the central nervous system (CNS) and exposures in early developmental stages can result in neurodegeneration later in life (Bellinger et al. 2011).

Sulphoraphane (SFN) is a phytochemical compound; its precursor glucoraphanin is found in cruciferous vegetables, with concentrations in broccoli sprouts being the highest (Shiina et al. 2015). SFN belongs to a group of plant-derived compounds called isothiocyanates. It is a powerful inducer of the nuclear factor erythroid-related factor 2 (Nrf2) antioxidant response element (ARE) pathway which plays a major role in up-regulating cellular defences to prevent against oxidative stress (Trio et al. 2016). Several intense and in-depth researches have been carried out with the purpose of investigating the ability of SFN to decrease the risk of various cancers and reduce the damage associated with the various forms of oxidative stress (Kim et al. 2017). SFN has very strong antioxidant and anti-inflammatory properties which enable it to drastically reduce cytotoxicity in the CNS (Shapiro et al. 2006). Animal studies have suggested that SFN supplementation can modify many debilitating CNS diseases including Parkinson's disease, epilepsy and stroke among others (Shapiro et al. 2006). This study therefore attempts to provide an insight into SFN supplementation response on neurobehavioural stress test and Alzheimer's-type neurodegeneration subjected to chronic Pb exposure in adult Wistar rat.

MATERIALS AND METHODS

Chemicals

Lead acetate ($[C_2H_3O_2]_2Pb.3H_2O$) was obtained from Sigma Chemical Corporation (USA) and SFN was procured from Toronto Research Chemicals Inc. (Canada). All other chemicals used in the study were of analytical grade.

Ethics Approval

The experimental procedures were conducted in accordance with the National Institute of Health of the United States of America guidelines for the care and use of laboratory animals in line with guidelines of the Department of Human Anatomy, Federal University of Technology, Akure and the Health Research and Ethical Committee of the University of Lagos and Federal University of Technology, Akure (HREC/19/0120).

Animals and Experimental Design

Forty adult male Wistar rats weighing between 130 g and 170 g were obtained from the breeding colony of the Faculty of Agriculture and Agricultural Technology, Federal University of Technology, Akure, Nigeria. The rats were housed in the laboratory animal house, Department of Human Anatomy, Federal University of Technology, Akure, Ondo State, Nigeria. The rats were acclimatized for 7 days; maintained under standard laboratory conditions (12- hour light/dark cycles) and had free access to a commercial pellet diet and water *ad libitum*. The animals were maintained under a controlled room temperature of 25 ± 2 °C and relative humidity of 60 ± 5 gm^{-3}. The floor of the cage was lined with carpet pieces and sprayed with coarse saw dust which served as a cushion. The coarse saw dust was changed every day to dispose waste droppings and maintain proper hygiene. The use of the experimental animals was in accordance to the Guide for the Care and Use of Laboratory Animals and approved by the Health Research Ethics Committee of the Federal University of Technology, Akure, Ondo state, Nigeria. (HREC/19/0120).

Experimental Design

The animals were divided into four groups (n = 10). Lead acetate (Pb) dosage (Owoeye and Ojora 2015) and SFN dosage (Zhou et al. 2016) were dissolved in distilled water and prepared freshly each day for administration. The groups were as follows.

Group A (control) received normal saline solution (sodium chloride) orally as placebo; Group B

received 50 mg/kg body weight (bw) of lead acetate only orally; Group C received 50 mg/kg bw of SFN simultaneously with 50 mg/kg bw of lead acetate orally; Group D received 50 mg/kg bw of SFN only orally

All the groups were treated under the same housing conditions for 28 days. The animals were weighed and behavioural observations were recorded. The animals underwent behavioural studies after the last administration. At the end of the experiment, animals were sacrificed through cervical dislocation. The organs were excised, cleaned and washed with saline (0.9 % sodium chloride).

Behavioural Parameters

Prior to the commencement of the experiment, the rats underwent neurobehavioural training using Morris water maze (MWM) and Y maze tests for spatial memory and working memory (Vorhees and Williams 2006; Kim et al. 2013)

Morris Water Maze Test

This test was carried out to assess the spatial learning and memory of the rats. A pool of water measuring 100 cm in diameter and 30 cm in depth was used. An escape platform, 2.5 cm deep from the surface of the water, was placed in one of the quadrants outside of which was a visual cue. The animals were trained at day 0 before the commencement of the treatment. During the training, each rat was placed in each of the other three quadrants for a maximum period of 60 sec to find the escape platform at intervals of 25 min between quadrants until the escape latency reduced to less than 25 sec. During the test (after completion of the last administration), the pool was coloured and the animals were placed in each of the three quadrants different from the escape platform quadrant at an interval of 25 min between quadrants. The time taken to find the escape platform was recorded as the escape latency.

Y-maze Test

The rats underwent neurobehavioural training at day 0 after acclimatization period before the commencement of the experiment and behavioural testing at the end of the last administration. The Y-maze was carried out in a quiet dimly lit room between the hours of 9 am and 3 pm. All behavioural tests were videotaped and later scored by an independent observer who was unaware of the experimental protocol. All apparatus used for the tests were cleaned with 10% ethanol to remove possible bias due to odour left by the previous animal. This test was used to examine the working and cognitive memory of the rats. The animals were placed in a Y-maze whose arms measured 75 cm in length and 15 cm in breadth with an angle of 120° between the arms. The rats are placed on a predetermined start arm and were allowed to explore the maze for 5 min. Arm entry (hind limbs completely in the arm) was scored. A correct alternation was scored when the animal successfully explored each of the three arms of the maze per triad of exploration (i.e. entering all 3 arms in the overlapping triplet sets are defined as correct alternation) (e.g., XYZ, ZXY, or YZX). Once two arms were explored per triad of exploration (e.g., XYX, ZXZ, YXY), it was considered an incorrect alternation. The percentage of spontaneous alternation was calculated as: [correct alternation/ (total number of arm entries – 2)] × 100.

Tissue Collection and Processing

After the behavioural tests were concluded, the rats were subjected to cervical dislocation, and the brain tissues were immediately excised and dissected into two hemispheres. All the right hemispheres were fixed in 4% paraformaldehyde for histological processing, while the left hemispheres were rinsed three times in 0.25 M sucrose for 5 min and stored in 30% sucrose at 4°C. Paraffin wax sections were obtained for histological analysis. The hippocampus were excised from the fixed brain and dehydrated in ascending grades of alcohol (50%, 70%, 90%, and 100%). The tissues were then cleared in xylene twice for 15 min each. Infiltration was done with paraffin wax in Leica hot air oven at 56°C with tissues eventually embedded in paraffin wax at similar orientations. Tissue sections were obtained serially using a rotary microtome (Leica RM2245) and then mounted on glass slides. Sections were taken at 30 µm for haematoxylin and eosin (H&E) staining process using the method of Pearse (1975) as modified by Fischer et al. (2008). The slides were analysed using Leica®DM5000B microscope and photographed with Leica EC3 digital camera.

Quantitative Analysis

After the H&E staining process, quantitative assessment of the percentage number of surviving neurons was conducted. The assessment was based on selection of five sections from each rats viewed using light microscope under 400× (Leica®DM5000B microscope). The cells were counted stereologically for surviving neurons (healthy neurons with distinct nucleus) and the obtained data were statistically analysed (Wilcock et al. 2006; Kenawy et al. 2017).

Biochemical Analysis

The brain tissues were placed in 0.25 M sucrose solution and homogenized. Tissue homogenate was collected in a 5 mL sample bottle and centrifuged at 3,000 rpm for 15 min using a centrifuge (Model 90-1; Jiangsu Jinyi Instrument Tech, Jiangsu, China). The supernatant was collected with Pasteur pipettes into sample bottles and placed in a freezer at -4 °C.

Superoxide Dismutase (SOD) Assay: SOD assay was done using spectrophotometric technique (Sun and Zigman 1978). The reaction mixture (3 mL)

contained 2.95 mL carbonate buffer, 0.02 mL of homogenate and 0.03 mL of 2 mM SOD substrate in 0.005 normal hydrochloric acid (N HCl), used to initiate the reaction. The reference cuvette contained 2.95 mL buffer, 0.03 mL of substrate and 0.02 mL of water. The absorbance was read at regular interval of 1-5 min at 480 nm, with values expressed in U/mg of protein.

Catalase (CAT) Assay: CAT activity was analysed using the protocols of Clairborne (1995), in a solution containing 50 mM phosphate buffer, 19 mM hydrogen peroxide and tissue homogenates. The reaction was ended by addition of dichromate/ acetic acid solution, and values expressed as µmole of H_2O_2 consumed/mg protein/min.

Reduced Glutathione (GSH) Level: GSH was assayed using the protocols of Jollow et al. (1974) in a solution containing tissue homogenates, 4% sulphosalicylic acid, and subsequently 5,5'-dithiobis-2-nitrobenzoic acid, with values expressed in nmol/mg of protein.

Lipid Peroxidation (LPO) Level: LPO was quantified as malondialdehyde (MDA), using the protocols of Farombi et al. (2000). The reaction contained tissue homogenates, 5% (w/v) butylated hydroxytoluene (BHT), 10% tricyclic antidepressant and 0.75% in 0.1 mol/L of HCl. MDA was calculated using the following equation: R¼1.56_105 L/mol/cm, where R is the extinction coefficient, Values expressed in nmol/mg of protein or U/mg protein.

Determination of Nitric Oxide (NO) Level: Nitric oxide measured as nitrite was determined according to the method of Moshage et al. (1995). Briefly, 2 mL of 10 mM of sodium nitroprusside was prepared in 0.5 mL of phosphate buffer saline (pH 7.4). Next, 0.5 mL of sample extract was added and incubated at 25 °C. After 150 min of incubation, 0.5 mL of Griess reagent (1% sulphanilamide, 2% hydrogen tetraoxophosphate (VI), and 0.1% naphthyl ethylene diamine dihydrochloride) was added to 0.5 mL of incubated solution. The reaction mixture was re-incubated for 30 min at room temperature. The rate of absorbance was measured at 546 nm, and the inhibition percentage was calculated as below. Values expressed in µM/g.

$[(A546_{Control} - A546_{Sample})/A546_{Control}] \times 100$

Determination of Hydrogen Peroxide (H_2O_2) Level: Hydrogen peroxide level was assayed as described by Aebi (1984). Briefly, 40 mM of hydrogen peroxide solution was prepared in 50 mM phosphate buffer (pH 7.4) and the absorbance was measured at 230 nm. Then 1 mL of sample extract or standard was added with 2 mL of hydrogen peroxide solution. After 10 min, the absorbance was measured against blank a solution. The blank solution was prepared with phosphate buffer without adding hydrogen peroxide. Then, the percentage of hydrogen peroxide was then calculated as below. Values are expressed in mM/g.

$[(A230_{Control} - A230_{Sample})/ A230_{Control}] \times 100$

Brain Monoamine Neurotransmitters Analysis: Monoamine neurotransmitters (dopamine, serotonin and norepinephrine) level was estimated as follows; wet tissue was weighed and homogenized in 5 mL HCl for 1 min. The sample was then centrifuged for 10 min at 2000 rpm. An aliquot supernatant phase (1 mL) was removed and added to centrifuge tube containing 2.5 mL heptane and 0.31 mL HCl of 0.1 M. After 10 min of vigorous shaking, the tube was centrifuged under the same conditions as above in order to separate the two phases and the overlaying organic phase was discarded. The aqueous phase (0.2 mL) was then taken either for dopamine (DA), serotonin (5-HT) and norepinephrine (NE) assay. All steps were carried out at 0 ºC (Pagel et al. 2000).
For dopamine and norepinephrine analysis: 0.05 mL 0.4 M HCl and 0.1 mL of ethylene diamine tetra acetic acid /sodium acetate buffer (pH 6.9) were added to the 0.2 mL of aqueous phase, followed by 0.1 mL iodine solution (0.1 M in ethanol) for oxidation. The reaction was stopped after 2 min by addition of 0.1 mL di-sodium trioxosulphate (VI) solution. The 0.1 mL acetic acid was added after 1.5 min. The solution was then heated to 100 ºC for 6 min when the sample again reached room temperature; excitation and emission spectra were read for dopamine and norepinephrine from the spectrofluorometer (model Jasco-FP-6500, Japan).
For serotonin analysis: 0.25 mL of O-phthalaldehyde reagent was added to 0.2 mL aqueous extract. The fluorophore developed after heating to 100 ºC for 10 min. After the samples reached equilibrium with the ambient temperature, readings were then taken in the spectrofluorometer (model Jasco-FP-6500, Japan).

Statistical Analysis
Statistical analysis was performed using Graph Prism® software (version 3.05 for Windows, GraphPad software, USA) by one-way analysis of variance with Tukey's multiple comparisons test. The data were reported as mean ± standard error of Mean, while differences between means at $p < 0.05$ were considered significant.

RESULTS

Neurobehavioural Assessments
Neurobehavioural tests (MWM and Y-maze) were carried out to estimate the duration of escape latency and the percentage of correct alternation, respectively. The results showed that there was a

significant increase in the escape latency of Pb-treated rats (group B) in comparison with the control (group A) ($p<0.05$) (Fig. 1A) showing that Pb caused a decline in the long-term memory index. However, co-administration of SFN and Pb (group D) showed a significant decrease in escape latency period when compared with the Pb-treated rats (group B) ($p<0.05$) (Fig. 1a).

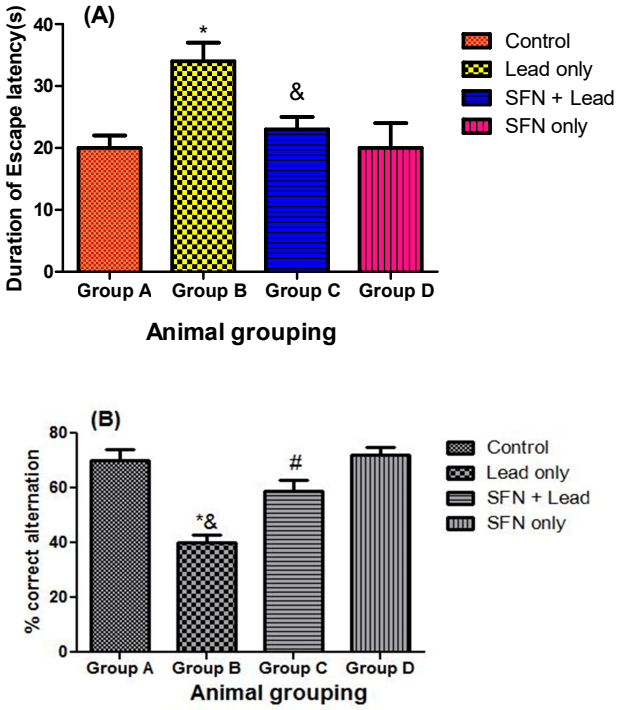

Fig. 1: Neurobehavioural stress tests in normal and treated rats. A: Morris water maze;; B: Y-maze test. * - $p < 0.05$ compared to group A; &: $p < 0.05$ compared to group B. # - $p < 0.05$ compared to group A

Furthermore, the percentage of correct alternation observed in the Y-maze test showed a significant decrease among Pb only rats (group B) in comparison with the control group (group A) ($p<0.05$) (Fig. 1b). However, the group treated with a combination of SFN and Pb (group C) showed significant increase in correct alternation compared with Pb only rats (group B) ($p<0.05$) (Fig. 1b), while the SFN only rats (group D) showed similar correct alternation with the control rats (group A) (Fig. 1b).

Sulphoraphane Response on Brain Monoamine Neurotransmitters (Dopamine, Serotonin and Norepinephrine)

In this present study, the result showed that Pb only rats (group B) had significant decrease levels of serotonin and dopamine with concomitant increase in norepinephrine level when compared with the control rats (group A) ($p<0.05$) (Fig. 2). However, combination of SFN and Pb (group C) significantly increased the levels of dopamine and serotonin with corresponding decrease in the level of norepinephrine when compared to the Pb only rats (group B) ($p<0.05$) (Fig. 2).

Sulphoraphane Response on Brain Antioxidant Parameters (CAT, SOD, GSH)

The levels of CAT, SOD and GSH were measured to estimate antioxidant activity in the brain of rats treated with SFN and Pb. The results showed a significant decrease in the levels of antioxidant enzymes among Pb only rats (group B) when compared with the control rats (group A) ($p<0.05$) (Fig. 3). However, the co-administration of SFN and Pb (group C) showed a significant increase in the levels of CAT, SOD and GSH when compared to Pb only rats (group B) ($p<0.05$). Although, the co-administration of SFN and Pb (Group C) showed a significant decrease in brain antioxidant enzyme levels when compared with control and SFN only rats (groups A and D) ($p<0.05$) (Fig. 3)

Sulphoraphane Effects on Brain Oxidative Stress markers (MDA, H_2O_2 and NO)

In the present study, there was a significant increase in the levels of MDA, H_2O_2 and NO in Pb only treated rats (group B) when compared with the control rats (group A)($p<0.05$) (fig. 4). However, there was a significant decrease in the levels of MDA, H_2O_2 and NO among the rats that was treated with SFN and Pb (group C) compared to Pb only rats (group B)

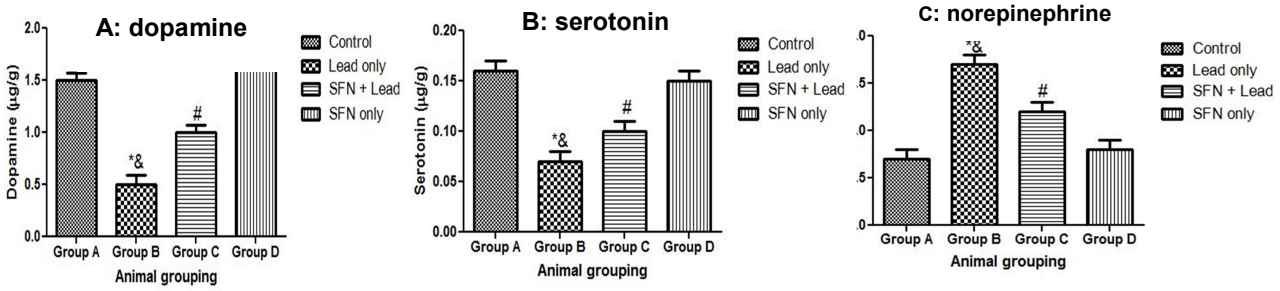

Fig. 2: Brain neurotransmitters (dopamine, serotonin and norepinephrine) in normal and treated rats. * - $p < 0.05$ compared to group A; & - $p < 0.05$ compared to group C; # - $p < 0.05$ compared to groups A

(p<0.05) (Fig. 4). Although, there was a significant increase in brain oxidative stress markers among the rats that were treated with SFN and Pb (group C) compared with control and SFN only rats (groups A and D) (p<0.05) (Fig. 4).

the hippocampal neurons showed a significant decrease in the percentage surviving neurons among Pb only treated rats (group B) in comparison with the control (group A) (p<0.05) (Fig.6). However, co-administration of SFN and Pb (group D) revealed a

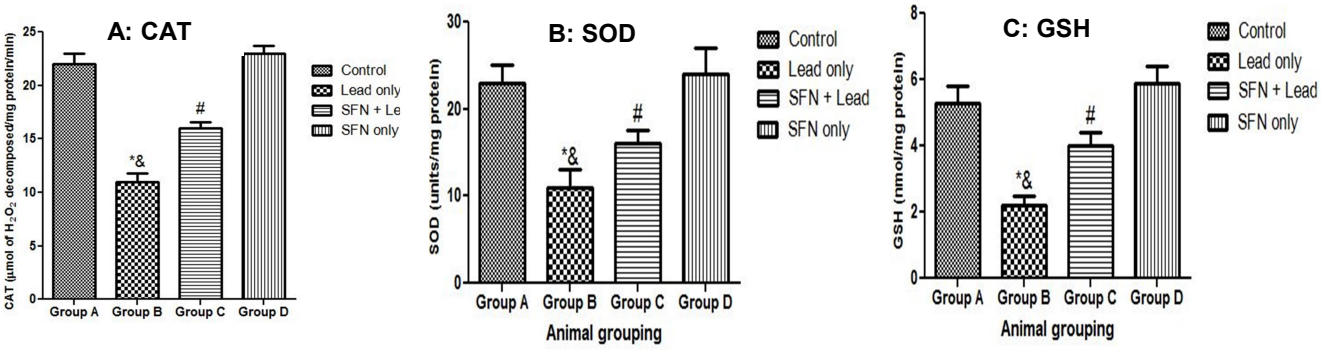

Fig. 3: Brain antioxidant parameters in normal and treated rats. *: p < 0.05 compared to group A; &: p < 0.05 compared to group C; #: p < 0.05 as compared to groups A.

Histological Observation of the CA3 Region

The representative photomicrograph of the Pb only rats (group B) showed protruding eosinophilic cytoplasm, pyknotic nuclei, decrease and shrinkage pyramidal cells (degenerated neurons) in the pyramidal layer, dilated bold vessels and dispersed vacuolization compared with the control rats (group A) (Fig. 5b). However, co-administration of SFN and Pb rats (group C) showed nearly normal morphological features of the hippocampus compared with Pb only treated rats (Fig. 5c). Additionally, the SFN only rats (group D) showed similar histological features with control rats (group A) displaying intact neurons with prominent nucleoli within the pyramidal layer of visible pyramidal cells (Fig. 5d).

Furthermore, quantitative assessments conducted on the hippocampal neurons showed a significant decrease in the percentage surviving neurons among Pb only treated rats (group B) in comparison with the control (group A) (p<0.05) (Fig.6). However, co-administration of SFN and Pb (group D) revealed a significant increase in the percentage surviving neurons compared with the Pb only treated rats (group B) (p<0.05) (Fig. 6). In addition, the co-administration of SFN and Pb (group C) showed a significant decrease in the percentage surviving neurons when compared with control and SFN only rats (groups A and D) (p<0.05) (Fig. 6).

DISCUSSION

The emergence of several debilitating disorders and diseases around the globe has drastically affected socio-economic growth. One of such disorders is AD, widely known for its negative influence on memory and cognitive skills, as well as on the ability to perform normal daily activities (Bakulski et al. 2012).

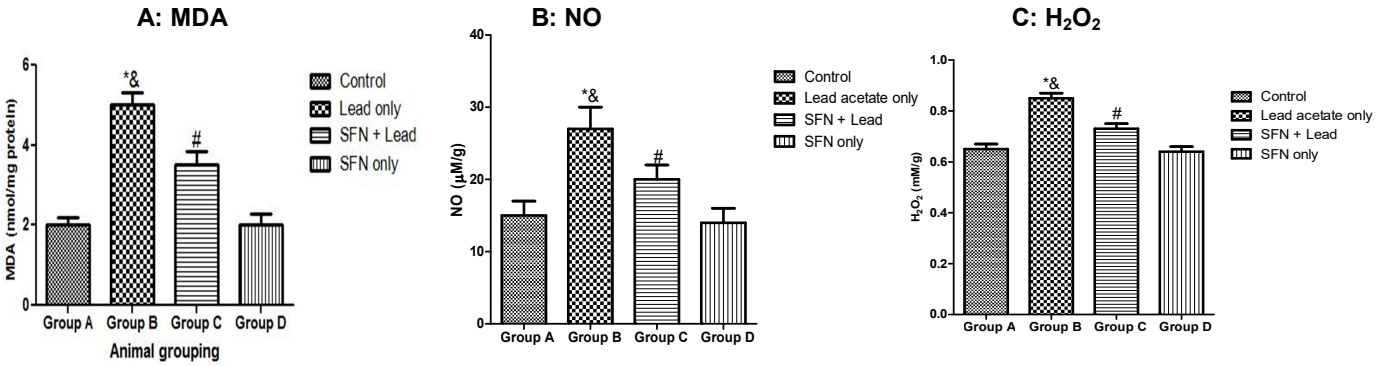

Fig. 4: Brain oxidative stress markers in normal and treated rats. * - p < 0.05 compared to group A ; & - p < 0.05 as compared to group C; # - p < 0.05 as compared to groups A

Several researchers in an attempt to source for potential ways to cure or manage the disease have made investigations into the root causes thereby linking heavy metal neurotoxicity to be one of the factors implicated in the pathogenesis of AD (Bakulski et al. 2012; Mason et al. 2013). Studies have indicated the occurrence of its adverse effects on the CNS by affecting cognition, learning and memory after chronic Pb-exposure (Bellinger 2011).

In this study, the Morris water and Y-maze tests are designed specifically to evaluate long and short term memory by measuring the duration of escape latency and percentage of correct alternation, respectively in rat model neural damage (Omotoso et al. 2018). The present result observed a drastic increase in the escape latency and concomitant decrease in percentage correct alternation in Pb-treated rats. This implies that Pb has a negative effect on cognitive capacity thereby inducing memory impairment as seen in AD patients. The memory alterations observed in this study is in accordance with previous studies that implicated memory and cognitive deficit to be induced by oxidative imbalance in which changes in antioxidant and pro-oxidants markers resulted in oxidative stress thereby damaging specific regions of the CNS (hippocampus) responsible for learning and memory (Riedel and Micheau 2001; Neves et al. 2015; Omotoso et al. 2020). However, the co-administration of SFN and Pb showed a reduced period of escape latency and higher correct alternation suggesting the ameliorative potential of SFN in reversing the memory impairment against Pb exposure. Previous studies have shown that SFN administration reduces memory deficits in mouse models due to its anti-oxidative and anti-inflammatory ability to scavenge free radicals and stop inflammatory responses in cells, thus improving learning and working memory (Hou et al. 2018; Lee et al. 2018). Similarly, SFN administration was reported to prevents or decelerates the process of normal brain aging and averts the occurrence of memory problems (Sunkaria et al. 2018), reduces the amount of amyloid beta (Aβ) and phosphorylated tau proteins as well as their aggregation (the typical

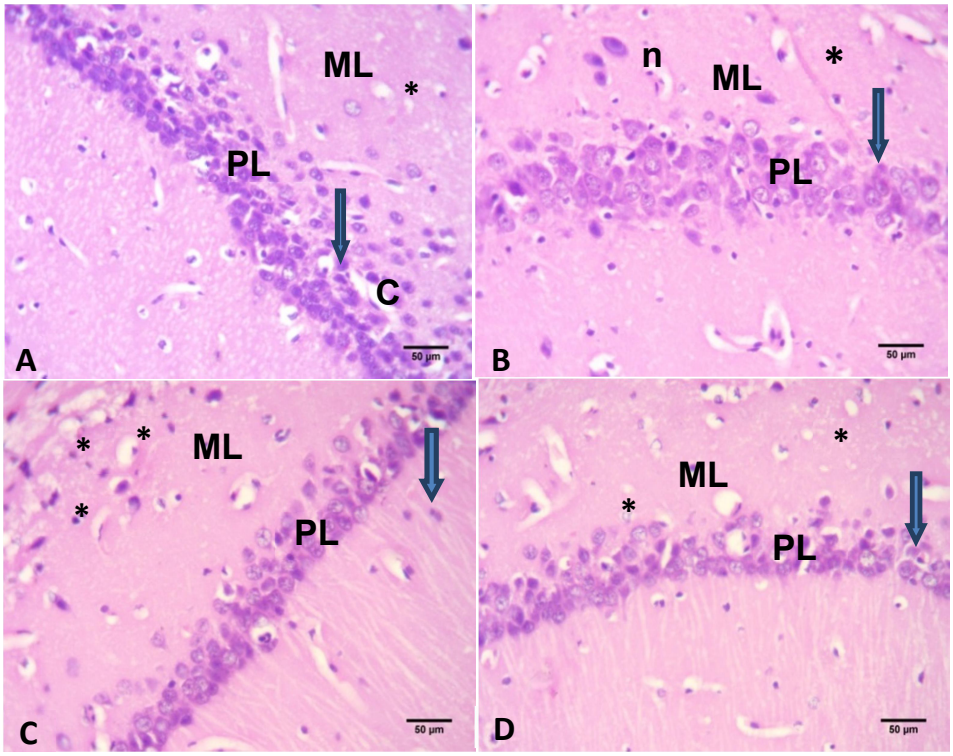

Fig. 5: A: Control showing normal hippocampus morphology of the Cornu Ammonis (CA3) region with compact layers of prominent pyramidal cells and vesicular nuclei, many glial cells (*) in the molecular layer (ML) and pyramidal cells (arrow) among neuronal process of the pyramidal layer (PL); B: Lead acetate only group showing loss and shrinkage in size of pyramidal cells (arrow), marked enlargement of neurons (n) and of glial cells (*) within the marginal layer (ML). C. SFN and lead acetate group showing preserved glial cells (*) in the molecular layer (ML) with few restored pyramidal cell (arrow). D. SFN only group showing normal orientation of glial cells (*) within the molecular layer (ML) and pyramidal cells (arrow) among neuronal process similar to the control. C - vacuolation; H&E, ×200

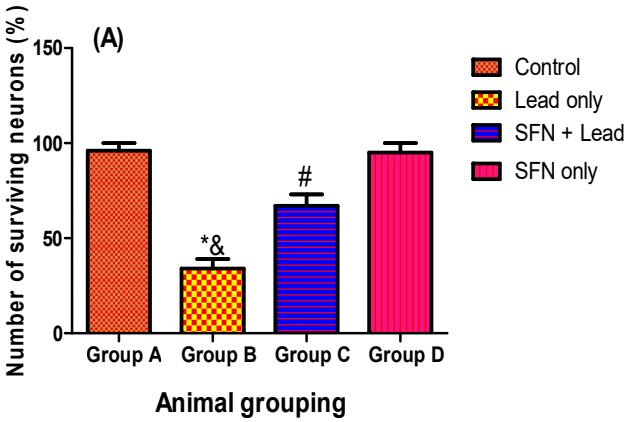

Fig. 6: Quantitative assessment of the number of surviving neurons in hippocampal sections * - $p < 0.05$ compared to group A; & - $p < 0.05$ compared to group C; # - $p < 0.05$ as compared to groups A

hallmarks of AD) (Hou et al. 2018; Lee et al. 2018).

The impairment of neurotransmission and deregulation of cell signalling are key aspects of Pb neurotoxicity (Sharma et al. 2015). The results showed a drastic depletion in dopamine and serotonin levels in the brain with corresponding increase in the level of norepinephrine after chronic Pb-exposure compared to the control, thereby affecting neurotransmitters storage within the brain. However, the combination of SFN and Pb significantly increased the levels of dopamine and serotonin with corresponding decrease in norepinephrine when compared to Pb only group. This observation revealed a boost in neurotransmitter levels initiated by SFN administration in the treatment of AD. Neurotransmitters are important for proper brain functioning because they are necessary for the transport and balance of signals between nerve cells in the brain (Sharma et al. 2015).

The defence against reactive oxygen species (ROS) and free radicals are important in proper brain function (Omotoso et al. 2018). Pb exposure is capable of causing neurotoxicity directly through oxidative stress or indirectly through lipid peroxidation resulting in the generation of ROS and direct depletion of antioxidant reserves (Khan et al. 2000; Melo et al. 2011; Tarozzi et al. 2013). In this present study, there was significant decrease in antioxidant reserves (CAT, SOD, GSH) and corresponding elevation in levels of oxidative markers (MDA, NO and H_2O_2) among the group treated with Pb only when compared to the control. This signifies that Pb has a deleterious effect on antioxidant enzyme levels and oxidative stress markers thereby increasing the levels of free radicals within the brain. However, the group treated with SFN and Pb combination showed significant decrease in the antioxidant enzymes levels (CAT, SOD, GSH) with concomitant decrease in oxidative stress parameters (MDA, NO and H_2O_2) compared to the Pb only group. This implies that SFN administration was capable of boosting antioxidant enzyme levels by mopping up the free radicals that induces oxidative stress. Previous studies revealed that SFN has very strong antioxidant and anti-inflammatory properties which allow for a significant reduction in cytotoxicity in the CNS (Shapiro et al. 2006) thereby scavenging the excess ROS within the brain tissue. SFN increases the release of GSH by up to 2.4 fold in cultured astrocytes (Steele et al. 2013) and has been shown to reduce oxidative stress in multiple disease states in cultured cells and animal models (Dauer and Przedborski 2003; Huo et al. 2018).

Although the molecular mechanisms involved in the pathogenesis of acute and chronic neurodegenerative diseases remain elusive, oxidative stress, misfolding, aggregation, accumulation of proteins, disturbed calcium ion homeostasis, excitotoxicity, inflammation, and apoptosis have been identified as possible causative agents of neurodegeneration (Dauer and Przedborski; 2003; Mandel et al., 2003). The brain is particularly susceptible to oxidative stress due to its high level of oxygen consumption, high oxidizable polyunsaturated fatty acid content, and low antioxidant defence capacities especially in old and aging brains (Halliwell 1992; Hamilton et al. 2001; Arouma 2002).

Chronic Pb exposure is capable of inducing damage in the prefrontal cortex of the cerebrum, hippocampus, and cerebellum which then result in various neurological disorders, including brain damage, mental retardation, behavioural problems, nerve damage, Alzheimer's and Parkinson's diseases, and schizophrenia (Sanders et al., 2009). The present study affirmed that Pb causes alteration in the hippocampal histomorphology characterized by disorganized neurons, eosinophilic stained cytoplasm, nuclei swelling and neuronal shrinking similar to the pathological hallmark of AD. These observable features were reported to be mediated by amyloid beta (1-42) accumulation within the hippocampus thereby inducing memory impairments associated with neuronal injury (Mason et al. 2014; El-Boshy et al. 2015; Zhang et al. 2016). Additionally, neurotoxicants such as Pb, aluminium, cuprizone, nickel, manganese) were reported to compromise structural composition of neurons through oxidative stress induced cellular damage by elevating neurotoxins levels capable of destroying the nuclear component of the cell (Omotoso et al. 2018). Pb is also known to restrict the release of neurotransmitters, thus affecting their availability in the brain and disrupting the function of GABAergic, dopaminergic and cholinergic systems (Hamilton et al. 2001; Sharma et al. 2015). Pb exposure is a major public health concern with evidence showing its adverse effects on the CNS as well as on motor and cognitive skills, and exposure during early life stages can cause neurodegeneration such as in AD in later life (Mason et al. 2014; Sharma et al. 2015). The photomicrograph of the SFN-treated group revealed mild neuronal degeneration with lesser number of eosinophilic stained neurons and increased number of healthy neurons with prominent nuclei thereby showing the attenuating potency of SFN on neurodegeneration and also preserving cognitive functions. The altered histological presentation observed in this study is in accordance with previous researchers (El-Boshy et al. 2015; Zhang et al. 2016) suggesting that neurotoxicant causes injury due to excess formation of free radicals in the brain. The administration of SFN further reinforced its capability to reverse the symptoms of Alzheimer's-type neurodegeneration brought about by chronic Pb-exposure. SFN is a potent activator of the Nrf2-ARE genetic pathway. Up-regulation of the Nrf2-ARE pathway increases the availability of multiple antioxidants in the brain, thus increasing its defence against oxidative stress and it effects (Klomparens

and Ding 2019). Studies have suggested that SFN supplementation could suppress many debilitating CNS diseases including AD, Parkinson's disease, epilepsy and stroke (Wang et al. 2016; Klomparens and Ding 2019).

Conclusion

It can thus be concluded that chronic Pb exposure is a major environmental neurotoxicant capable of causing memory impairments and hippocampal neurodegeneration in Alzheimer type animal model and SFN supplementation play a major role in preventing or mitigating the deleterious neurotoxicity of Pb exposure thereby allowing the proper cognitive function and normal homeostasis.

Conflict of Interest

None declared

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