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Therapeutic Response of Sulforaphane on Oxidative Stress and Cognitive Impairment in Aluminum-Induced Alzheimer-type Hippocampal Neurodegeneration in Adult Wistar Rats

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ABSTRACT

The accumulation of Aluminum in the brain is believed to be involved in the pathophysiology of neurodegenerative diseases including Alzheimer's disease. This study evaluated the neuroprotective activities of sulforaphane (SFN) on aluminum chloride ($AlCl_3$)-induced hippocampal degeneration in Wistar rats. A total of 28 adult male Wistar rats were divided into four groups (n=7). Group A (control) received 200 mg/kg of normal saline solution *ad libitum*; Group B received 200 mg/kg body weight of $AlCl_3$; Group C received 200 mg/kg of SFN and 200 mg/kg of $AlCl_3$; Group D received 200 mg/kg of SFN alone. All administrations were done daily, through oral gavage for 45 days and the rats underwent behavioral tests such as Y-maze and Open field test. On day 46, blood samples were collected to obtain serum for analysis and the brains were harvested for assessment. The results showed significant memory decline, spatial memory impairment and learning deficit, decreased levels of oxidative markers with corresponding increase in stress markers among $AlCl_3$ only group compared to control but was attenuated with SFN administration. There were also significant differences in the levels of neurotransmitters in SFN and $AlCl_3$ before and after treatment with SFN. Histological examination revealed clear pathological alterations indicative of hippocampal degeneration, including distortion and pyknotic abnormalities in the pyramidal layer of the $AlCl_3$-only group's hippocampal region, which were mitigated by SFN treatment. SFN improved learning and memory, antioxidant enzymes, neurotransmitters, and morphology of hippocampus thereby attenuating the neurotoxic effects of $AlCl_3$.

Keywords: Alzheimer's disease, aluminum chloride, behavioural stress test, hippocampus, oxidative stress.

INTRODUCTION

Alzheimer's disease (AD) is a revolutionary neurodegenerative disease that imposes a tremendous socioeconomic burden on the society [1]. AD is typically categorized into two forms; sporadic and non-sporadic, in which 95% of cases account for the sporadic form and below 5% instances account for the non-sporadic form. The non-sporadic form of AD is early onset, usually manifests in individuals younger than sixty-five years [2]. The preeminence of AD doubles every 5 years, after the age of 65 in the late onset sporadic nature of AD [3]. AD is the most common form of dementia related to neuropathological and neurobehavioral changes, in which the symptoms aggravate progressively over the years [3]. AD can be grouped into three major stages according to the progression of the disease which includes mild, moderate, and severe. The first symptom of AD is memory impairment, specifically short-term memories, whereas the long-term recollections are properly stored.[4] The advancement of AD causes visible impairment in cognitive skills, executive decision-making, and ability to do daily chores drastically decrease.[5] The pathological hallmarks of AD consist of positive lesions such as amyloid plaques, neurofibrillary tangles, cerebral amyloid angiopathy and glial responses alongside negative lesions such as synaptic loss and neuronal loss [6,7].

The most important biomarkers for AD pathology include the 1-42 amino acid structure of beta-amyloid (Ab42) and phosphorylated tau protein (P-tau).[8] According to Hardy and Selkoe, [9] Ab42 fibril buildup is thought to be the catalyst for this neurodegenerative disease and a series of related events that include oxidative stress, neurotoxicity, and inflammation.

According to Jönsson *et al.*,[10] the primary cause of mortality in people with AD is typically not

connected to these brain changes but rather associated comorbidities such as pneumonia, immobility, and malnourishment as a result of difficulty swallowing. According to Albert *et al* [11] individuals with AD experience the disease in diverse ways and have varying symptoms and rates of development. The primary cause of this is the variation in variables including co-morbidities, age, education, and heredity [12].

Aluminum (Al) is the most numerous neurotoxicant worldwide with daily consumption estimated to be nearly 10–20 mg from cooking utensils, food additives and drinking water – probably due to water purification procedures and medicines (antacids) [13,14]. The accumulation of Al in the brain is believed to be involved in the pathophysiology of neurodegenerative diseases including Alzheimer's disease, amyotrophic lateral sclerosis, Guam-Parkinson's dementia, etc. [15] Since a range of biomolecules can bind Al, and it can displace other organic cations (such as calcium and magnesium) from their binding sites, nearly all metabolic pathways are possible targets for the adverse effects of Al [16]. Therefore, Al neurotoxicity is no longer brought about through a single alteration, but adverse effects at multiple cellular levels. Al salts in biological tissue enhance the potential of iron salts to cause the production of reactive oxygen species (ROS) rather than directly acting as prooxidants. Furthermore, Al improves peroxidative destruction to lipids, proteins and lessen antioxidant enzymatic condition [17]. The oxidative damage of Al is through its affinity to negatively charged phospholipids of the brain such as polyunsaturated fatty acids thereby permitting the reactive oxygen species (ROS) including H_2O_2, $O^{2}\bullet-$, $OH\bullet$, and $OH-$ to easily attack these fatty acids [18]. Al chloride has the potential of aggregating in specific brain regions, [19] which was correlated earlier with the degenerative changes observed.

Nutritional supplements derived from medicinal plants are known to exhibit a variety of biological effects including anti-inflammatory, antimutagenic, anticarcinogenic, antioxidant, and anti-aging activities [20]. Several clinical and laboratory studies have shown the activities of nutritional dietary intake in preventing and/or delaying AD due to their relatively minimal side effect or low drug to drug interaction [21,22]. Since there is no effective treatment for AD, recent studies have been focused on the use and development of nutritional supplements from plant sources with multifunctional properties [23, 24].

Sulforaphane (SFN) is the most naturally interesting and studied isiothiocyanates present in crucifers [25]. Recent study revealed that SFN has the capability to induce a wide variety of chemoprotective genes such as antioxidant proteins, anti-inflammatory molecules, and phase 2 enzymes by activating nuclear factor erythroid 2-related factor 2 (Nrf2) signaling [26]. SFN exhibits a wide range of biological effects such as antioxidant, antimicrobial, anticancer, anti-inflammatory, anti-aging and anti-diabetic [27, 28]. In addition, SFN supplementation prevents and/or improve skin erythema[29] and induces apoptosis and cell cycle arrest associated with regulation of many molecules such as Bcl-2 and Bax family proteins, caspases, p21, cyclins, and cyclin-dependent kinases [30]. Therefore, this study was aimed at evaluating the activities of SFN supplement on Al-induced Alzheimer's disease in Wistar rats.

MATERIALS AND METHODS

Chemicals

Aluminium chlorohydrate ($AlCl_3$) and Sulforaphane (SFN) were purchased from Sigma Corporation (100% purity and concentration) (USA, St. Louis, MO). The other chemicals used for this study were all of analytical reagent grade.

Animal Care and Use

From an animal house at Ladoke Akintola University of Technology (LAUTECH) Ogbomosho, a total of 28 adult male Wistar rats weighing between 150 and 240 g were procured. The rats were housed in the Animal House of Department of Human Anatomy, Federal University of Technology, Akure. The rats were kept in a wire-gauzed cage that was appropriately divided into four compartments and large enough to allow for good ventilation and unrestricted mobility. They were also exposed to a suitable temperature of 32 to 37°C and a 24-hour light supply. To act as a cushion, coarse sawdust was sprinkled on the carpet pieces that lined the cage's floor. To get rid of garbage and keep things clean, the coarse sawdust was replaced daily. The rats were given growers' marsh (pellets) (Agro Feeds and Flour Mills) and water throughout the experiment. The rats underwent a seven-day acclimation phase.

Ethical Approval

Ethical approval for the study was issued by Health Research Ethics Committee, Federal University of Technology, Akure for the use of the experimental rats in compliance with the Guide for the Care and Use of Laboratory Animals (HREC/2022/0155).

Experimental Design

Rats were divided into four groups (n= 7). $AlCl_3$ and SFN solutions were prepared freshly each day for administration. $AlCl_3$ was dissolved in distilled water

and administered orally at a dose of 0.5 mL/100 g bodyweight according to previous study [31]. The groups were as follows:

Group A: Control served as untreated control and received 200 mg/kg normal saline solution; Group B: $AlCl_3$ only received a dose of 200 mg/kg body weight (BW) of $AlCl_3$; Group C: SFN + $AlCl_3$ received 200 mg/kg BW of SFN simultaneously with 200 mg/kg BW of $AlCl_3$; Group D: SFN only received 200 mg/kg BW of SFN. Mode of administration was oral.

Over the course of 45 days, all the groups were kept in similar housing conditions. The animals were weighed, and behavioral observations were noted both at the beginning of the experimental investigation (baseline) and at its conclusion. Animals were sacrificed under intraperitoneal pentobarbital anesthesia at the conclusion of the experiment. A portion of the brains were preserved in Neutral Buffered Formalin for histology and immunohistochemistry after they were extracted, cleaned, and rinsed with saline (0.9% sodium chloride).

Neurobehavioural Study

Neurobehavioral tests were used to determine the impact of $AlCl_3$ intoxication on the various experimental groups' locomotor, learning, and memory abilities as well as the antioxidant molecules' capacity to return the body to a state of physiological homoeostasis. Animals underwent neurobehavioral studies before the experimental period to obtain the baseline results of the rats before administration of both $AlCl_3$ and SFN. Thereafter, at the end of the experiment, the rats underwent neurobehavioral tests to study learning and memory using Open field, Morris water maze and Y maze tests[32,33] and fear and anxiety using the open field apparatus.[34]

Open Field Test

The open field apparatus was a 100 cm x 100 cm wooden box with 38 cm high walls (opened from the tip) which was kept in an isolated room with normal lighting and temperature. The floor of the area was divided by straight lines into squares of 10 cm each. A video recording system was stationed at an angle to capture the movements of the rats within the boxing arena. Twelve hours after the last administration of SFN and $AlCl_3$, each group of rats was taken to the open field arena in separate cages while making sure there was no agitation to avoid stress in animals. Subsequently, each rat was placed in the open field arena, away from the others and their explorative movement was measured for 10 minutes using the video recorder. This was repeated for rats across the groups. Before each exercise, the apparatus was wiped with methylated spirit to abolish the odor of the previously tested rat. A neutral observer stayed away from the apparatus during each test.

Following the completion of the exercise, the video was analyzed by the neutral observer who counted the number of squares explored by each rat. The open field apparatus was constructed using plywood each measuring 100 cm and height of 50 cm. The floor was divided into square grids each measuring 25 cm in length with a blue marker and a Centre square of the same length was drawn using a red marker. Each rat was picked by the tail dropped in the Centre square and allowed to explore for 5 minutes while the video was captured by a camera from above the apparatus. Five behavioral patterns were scored: the number of lines crossed, Centre square entry, Centre square duration, rearing frequency, and stretch attend posture.

Morris Water Maze Test

Rats were tested on their spatial learning and memory using the Morris Water Maze (MWM). The process was carried out in compliance with Vorhees and Williams.[33] In short, a water tank with dimensions of 3ft in diameter and 2ft in depth was used for the test. An escape platform was positioned one-inch deep in the center of the North-East (NE), North-West (NW), South-East (SE), and South-West (SW) quadrants of the water-filled tank. The rats were trained before the main test and given a maximum of 60 seconds to locate the escape platform in each of the other three quadrants (NE, SE, and SW). The time it took them to locate the escape platform was measured as the escape latency duration on test day when they were put once in each of the three quadrants for a maximum of one minute. The escape latency period was 60 seconds, and those who were unable to locate the escape platform in that time were removed.

Y-Maze Test

The 100 cm x 100 cm x 50 cm tall open field apparatus was built out of plywood.[31,34] A blue marker was used to divide the floor into square grids, each measuring 25 cm in length. A red marker was used to create a center square with the same length. A camera mounted above the equipment recorded the video when each rat was released into the central square, where it was free to roam about for five minutes. The number of lines crossed, rearing frequency, center square duration, center square entry, and stretch attend posture were the five behavioral patterns that were graded. The frequency

with which the rat crossed a grid line with all four paws was measured as the number of lines crossed; the frequency with which it entered a central square using all four paws was measured as the center square duration; the frequency with which the rat stood on its hind limbs was measured as the rearing frequency; and the frequency with which it displayed a forward elongation of the head and shoulders followed by a retraction to the original position was measured as the stretch attend posture.

Tissue Processing

Fischer *et al*.[35] modified version of Pearse's method was used to stain brain sections. This approach was used to illustrate the prefrontal cortex and hippocampal general cytoarchitecture. Slide holders were used to hold slides holding paraffin sections, and the following reagents were used for deparaffinization and rehydration of the sections, respectively: excess water was wiped from the slide holder before placing them in hematoxylin. They were put in xylene three times for three minutes, 1:1 xylene with 100% ethanol three times for three minutes, 95% ethanol once for three minutes, 80% ethanol once for three minutes, and finally deionized H_2O once for five minutes. Then, slides were dipped 12 times in acid ethanol, rinsed twice with tap water for one minute each, rinsed once in deionized water for two minutes, and left overnight for hematoxylin staining. The process involved dipping slides once for three minutes with hematoxylin and once in tap water for five minutes to allow the stain to develop. Before adding eosin, extra water was wiped from the slide holder.

The protocols for eosin staining and dehydration were as follows: slides were immersed in eosin for 30 seconds (or 45 seconds for older batches of eosin), then in 95% ethanol three times for five minutes, then in 100% ethanol three times for five minutes (any excess ethanol was blotted before being placed in xylene), and finally three times for fifteen minutes in xylene. After that, a single drop of disperse plasticizer in xylene (DPX) was applied to each slide using a glass rod to coverslip the slides. The coverslips were inclined to provide for a gentle descent onto the slide. The slides were then left in the hood to dry for the whole night.

Biochemical Analysis

Superoxide Dismutase (SOD) Assay

After being homogenized, the brain tissues were submerged in a 0.25 M sucrose solution. Using a centrifuge (Model 90-1; Jiangsu Jinyi Instrument Tech, Jiangsu, China) at 3,000 rpm for 15 minutes, tissue homogenate was collected in a 5 ml sample container. Using Pasteur pipettes, the supernatant was collected into sample vials and frozen at –4°C. Spectrophotometric method was used for SOD.[36] The reaction was started using a 3 ml reaction mixture that included 2.95 ml of carbonate buffer, 0.02 ml of homogenate, and 0.03 ml of 2 mM SOD substrate in 0.005 N HCl. There were 2.95 ml of buffer, 0.03 ml of substrate, and 0.02 ml of water in the reference cuvette. For five minutes, the absorbance was measured at 480 nm at regular intervals of one minute. Values were expressed in nmol/mg of protein or U/mg protein.

Catalase (CAT) Assay

This was analyzed using the protocols of Clairborne,[37] in a solution containing 50mM phosphate buffer, 19 mM hydrogen peroxide and tissue homogenates. The reaction was ended by addition of dichromate/ acetic acid solution. Values are expressed as µmole of H_2O_2 consumed/mg protein/min.

Reduced Glutathione Level (GSH)

GSH was assayed using the protocols of Jollow *et al*.[38] in a solution containing tissue homogenates, 4% sulfosalicylic acid, and subsequently DTNB. Values are expressed in nmol/mg of protein.

Lipid Peroxidation (LPO) Level

LPO was quantified as Malondialdehyde (MDA), using the protocols of Farombi *et al*.[39] Tissue homogenates, 10% TCA, 0.75% TBA, and 5% (w/v) butylated hydroxytoluene (BHT) were added to 0.1 mol/L of HCl for the reaction. The formula below was used to determine MDA: R¼1.56_105 L/mol/cm is the extinction coefficient, in this case. Values are 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.*[40] Briefly, 0.5 ml of phosphate buffer saline (pH 7.4) is mixed with 2 ml of 10 mM sodium nitroprusside. Sample extract (0.5 ml) is then added, and the mixture is incubated at 25°C. 0.5 milliliters of the incubated solution are mixed with 0.5 milliliters of Griess reagent (1% sulfanilamide, 2% H_3PO_4, and 0.1% naphthylethylene diamine dihydrochloride) after it has been incubated for 150 minutes. The reaction mixture is re-incubated at room temperature for thirty minutes. At 546 nm, the absorbance rate is recorded, and the inhibition percentage is calculated. NO inhibition= (A_{546} Control−A_{546} Sample)/A546 Control ×100. Values are expressed in µM/g.

Determination of Hydrogen Peroxide (H_2O_2) Level

H_2O_2 level was assayed as described by the method of Aebi.[41] Briefly, after preparing a 40 mM hydrogen peroxide solution in 50 mM phosphate buffer (pH 7.4), the absorbance at 230 nm was determined. Next, 2 ml of hydrogen peroxide solution were added to 1ml of sample extract or standard. The absorbance was measured against a blank solution after 10 minutes. Phosphate buffer was used to create the blank solution; hydrogen peroxide was not added. The hydrogen peroxide percentage was then determined as follows:

H_2O_2 scavenge (%) = A_{230} Control−A_{230} Sample)/ A_{230} Control ×100. Values are expressed in mM/g.

Brain Monoamine Neurotransmitters Analysis

Monoamine neurotransmitter (DA, 5-HT and NE) level was estimated as follows; wet tissue was weighed and homogenized in 5 mL HCl butanol for about 1 min. Next, the material was centrifuged at 2000 rpm for 10 minutes. A 1 mL aliquot of the supernatant phase was taken out and put to a centrifuge tube along with 2.5 mL of heptane and 0.31 mL of 0.1 M HCl. The tube was centrifuged under the same circumstances as before to separate the two phases after 10 minutes of vigorous shaking. The organic phase that was covering the tube was disposed of. Next, 0.2 mL of the aqueous phase were extracted for the DA, 5-HT, or NE assays. All steps were carried out at 0ºC following the techniques of Pagel *et al*.[42]

Dopamine and Norepinephrine Analysis

To the 0.2 mL of aqueous phase, 0.05 mL of 0.4 M HCl, 0.1 mL of EDTA/sodium acetate buffer (pH 6. 9), and 0.1 mL of iodine solution (0.1 M in ethanol) for oxidation were added. After 2 minutes, the reaction was halted by adding 0.1 mL of Na_2SO_3. After 1.5 minutes, add the 0.1 mL of acetic acid. After the sample had returned to ambient temperature, the solution was heated to 100ºC for six minutes. The spectrofluorometer (model Jasco-FP-6500, Japan) was then used to read the dopamine and norepinephrine excitation and emission spectra.

Serotonin Analysis

0.2 mL of aqueous extract and 0.25 mL of OPT reagent were combined. After ten minutes of heating to 100oC, the fluorescence appeared. The samples were measured in the spectrofluorometer (model Jasco-FP-6500, Japan) once they had matched the ambient temperature.

Statistical Analysis

GraphPad Prism® software (version 3.05 for Windows, GraphPad Software, San Diego, California, USA) was used to do statistical analysis. Tukey's multiple comparisons test was used in conjunction with one-way analysis of variance (ANOVA). The results were presented as means ± SEM, with significance defined as differences between means at $p<0.05$.

RESULTS

Effect of Treatment ON Neurobehavioral Parameters

The Morris Water Maze (MWM) and Y-maze were used to quantify learning and memory (short- and long-term) activities. The escape latency duration was used in the MWM test to assess learning and memory state (Fig. 1A). The group treated with $AlCl_3$ had a significantly higher escape latency period ($p<0.05$) in comparison to the control group, as seen in Figure 1A. This finding demonstrated that the long-term memory index was lowered by $AlCl_3$. On the other hand, the long-term memory index was significantly higher in the SFN and $AlCl_3$ treatment group ($p<0.05$) than in the $AlCl_3$ treated group (Fig. 1A).

The Y-maze test was used to measure alternation in short-term memory, and the results showed that the group treated with $AlCl_3$ had a lower proportion of accurate alternations than the control group ($p<0.05$) (Fig. 1B). In addition, compared to the rats treated with $AlCl_3$, the group that received both SFN and $AlCl_3$ therapy exhibited a larger proportion of correct alternation (Fig. 1B). This outcome demonstrated that SFN can prevent Al-induced memory loss by restoring short-term memory.

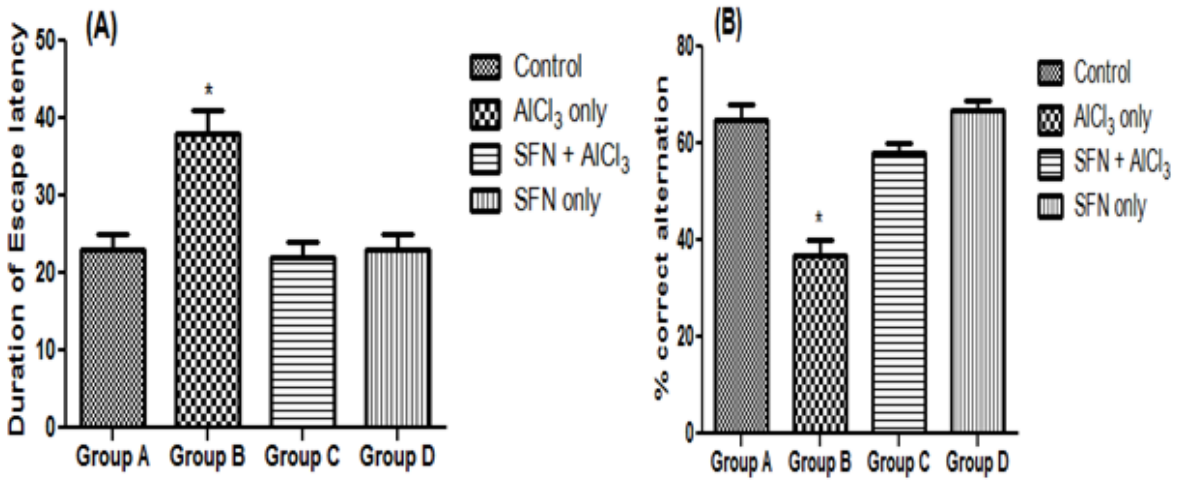

Figure 1: Role of Sulforaphane on Neurobehavioral stress tests (Y-maze and MWM) in aluminum chloride-induced neurotoxicity in normal and treated rats. * $p < 0.05$ as compared to group A.

The open field test was used to score exploratory drive and anxiety (Fig. 2). It was observed that $AlCl_3$ significantly reduced the number of lines crossed (measure of exploratory drive) relative to the control group ($p<0.05$) and the group co-administered with SFN and $AlCl_3$. The present result showed that the combined treatment group of SFN and $AlCl_3$ had a similar number of lines crossed with that of the control and SFN only groups (Fig. 2A).

In addition, $AlCl_3$ only group showed a reduction in center square duration, center square entry and rearing frequency while increasing the freezing duration and stretch attend posture frequency compared to the control (Fig. 2B, C, D & E). This observation can be attributed to anxiety-related behavior caused by $AlCl_3$ administration in the animal. However, the co-administration of SFN and $AlCl_3$ showed improvement in behavioral scores that was statistically significant compared with the control and SFN only groups except for the freezing duration which was not significant (Fig. 2B, C, D& E).

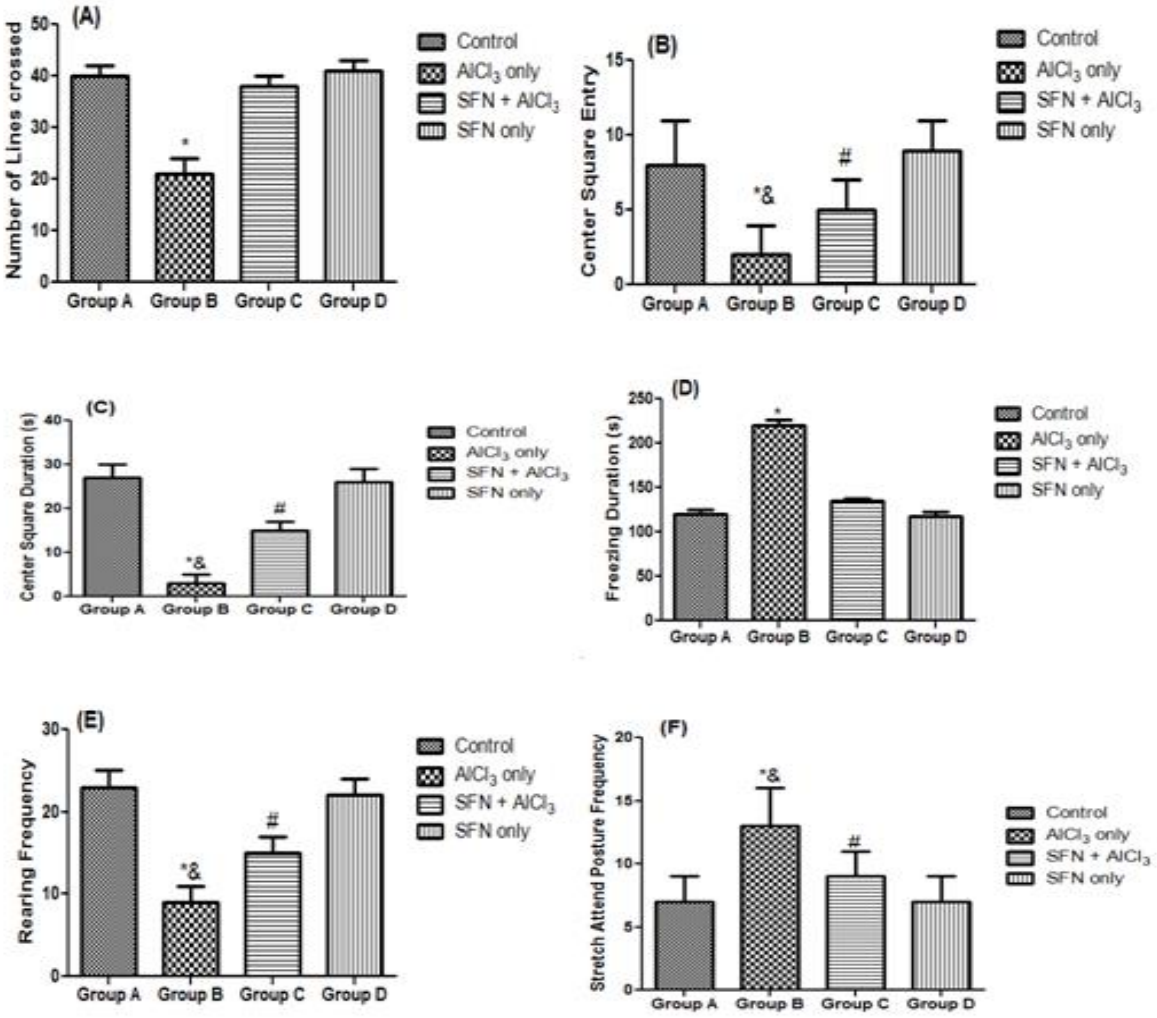

Figure 2: Role of Sulforaphane on Neurobehavioral stress tests (Open field tests) in aluminum chloride-induced neurotoxicity in normal and treated rats. * $p < 0.05$ as compared to group A; &: $p < 0.05$ as compared to group C; #: $p < 0.05$ as compared to group A and D.

Estimation of Antioxidant Enzyme (catalase, GSH, SOD) Levels by Sulforaphane

The $AlCl_3$ treated rats showed significant ($P< 0.05$) decrease in SOD, CAT and GSH activities compared to the control (Fig. 3). Sulforaphane significantly improved the antioxidant enzymes level compared to $AlCl_3$ treated group indicating the MDA properties and antioxidant properties of sulforaphane. However, there was a significant difference in the levels of CAT, SOD and GSH in SFN and $AlCl_3$ group compared with the control and SFN only groups.

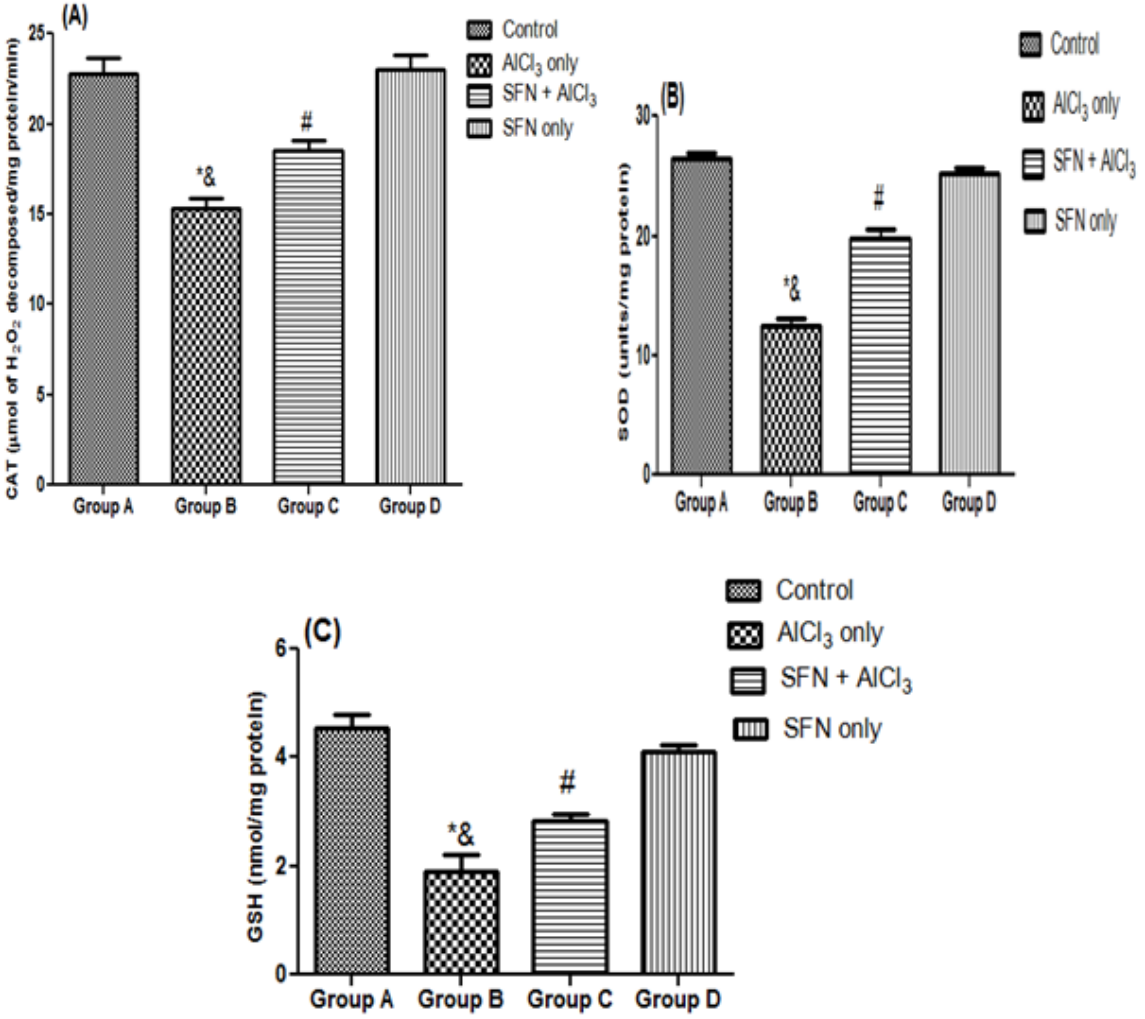

Figure 3: Role of sulforaphane on brain antioxidant parameters: (SOD) levels (units/mg protein), Catalase level (µmol of H_2O_2 decomposed/mg protein/min) and GSH level (nmol/mg protein) in aluminum chloride-induced neurotoxicity in normal and treated rats. * $p < 0.05$ as compared to group A; &: $p < 0.05$ as compared to group C; #: $p < 0.05$ as compared to group A and D.

Effects of Sulforaphane on Malondialdehyde, Hydrogen Peroxide and Nitrite Levels

In $AlCl_3$ treated rats, levels of malondialdehyde (MDA), Hydrogen Peroxide (H_2O_2) and Nitrite recorded were significantly increased compared to the control group (Fig. 4). Sulforaphane significantly reversed the elevated MDA, H_2O_2 and nitrite levels compared $AlCl_3$ treated group. However, there was no significant difference in the levels of MDA, H_2O_2 and Nitrite in the SFN and $AlCl_3$ group compared to the control and SFN only groups (Fig. 4)

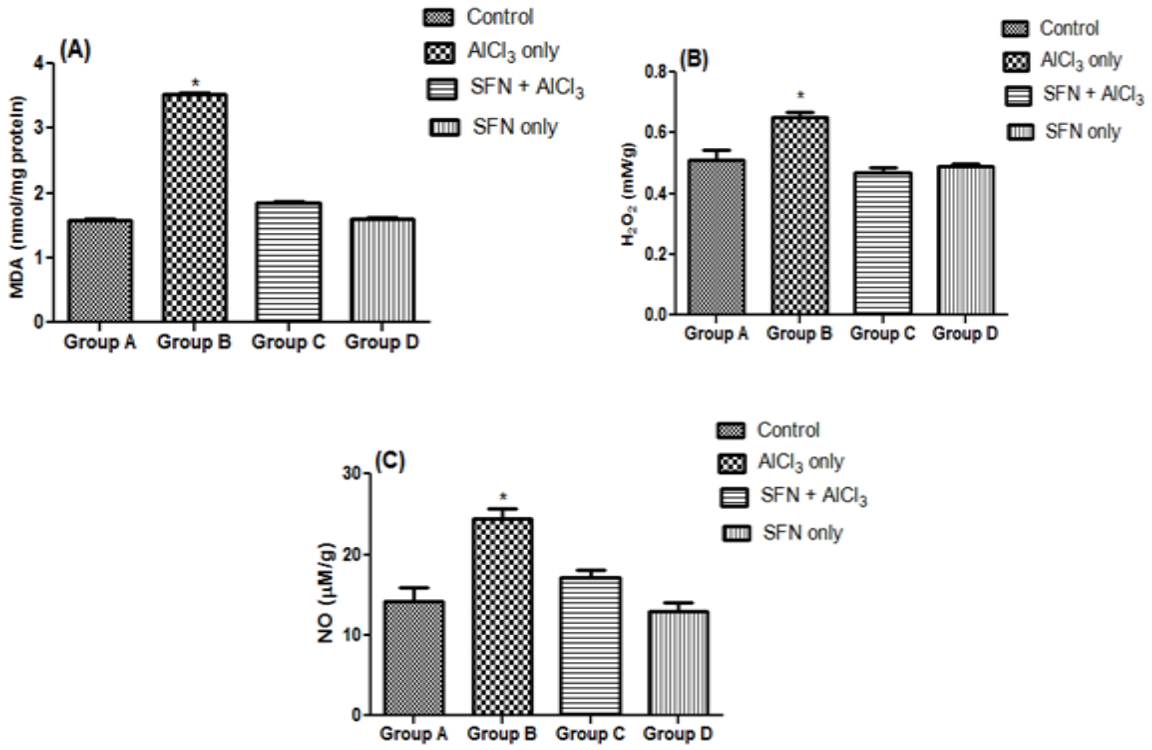

Figure 4: Role of sulforaphane on brain oxidative stress markers: MDA levels (nmol/mg protein), H_2O_2 (mM/g) and NO (µM/g) in aluminum chloride-induced neurotoxicity in normal and treated rats. * $p < 0.05$ as compared to group A.

Effect of Sulforaphane on Brain Monoamine Neurotransmitters

The administration of $AlCl_3$ showed significant decrease in the brain dopamine and serotonin level as compared with the control group (Fig. 5A & B) while the level of Norepinephrine was elevated compared with the control group (Fig. 5C). However, administration of SFN with $AlCl_3$ resulted in a significant improvement in the monoamine neurotransmitters compared with $AlCl_3$ only group. Although, there was statistically significant difference in the levels of dopamine, serotonin, and norepinephrine in SFN and $AlCl_3$ group compared with the control and SFN only group (Fig. 5).

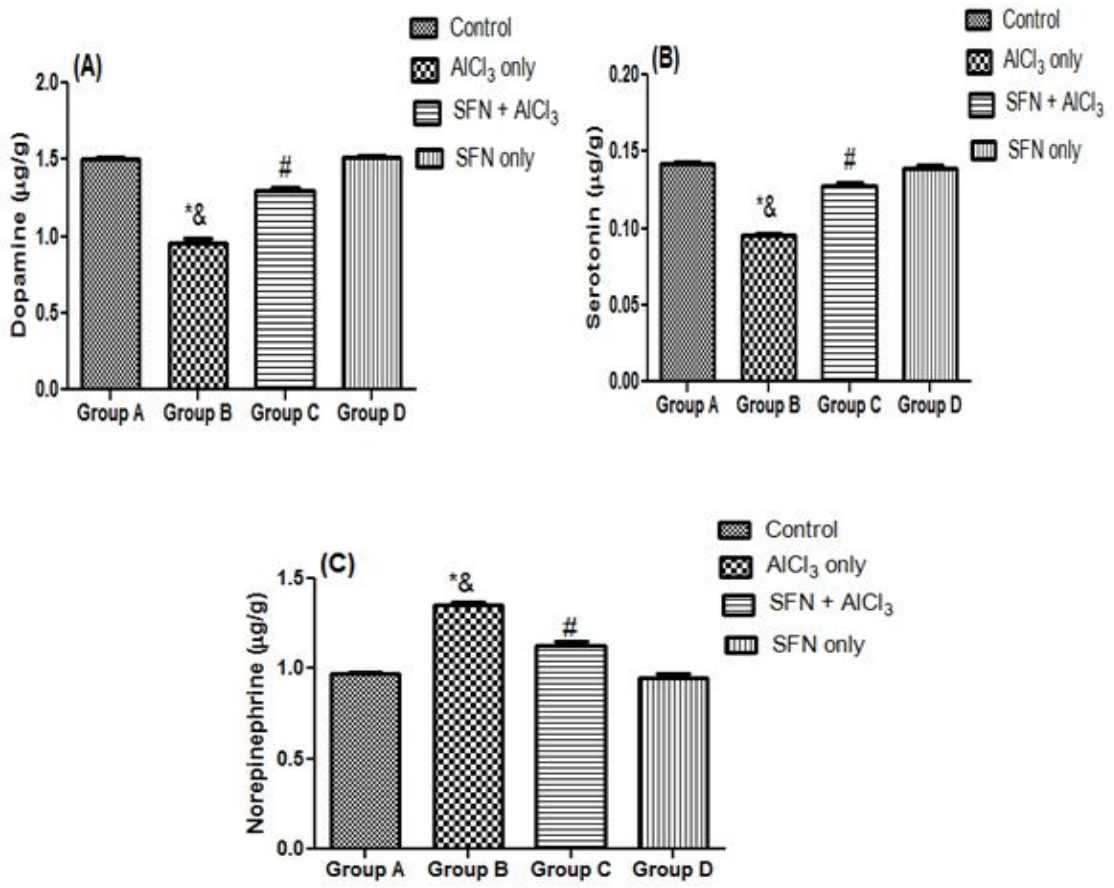

Figure 5: Role of Sulforaphane on brain monoamine neurotransmitters (DA, 5-HT and NE) in aluminum chloride-induced neurotoxicity in normal and treated rats. * $p < 0.05$ as compared to group A; &: $p < 0.05$ as compared to group C; #: $p < 0.05$ as compared to group A and D.

Histological Studies

The photomicrograph representation of the $AlCl_3$ only (group B) treatment group showed a mass proliferation of pyramidal cells especially in the CA3 region (Fig 7), and atrophy with alteration in Nissl bodies when compared with the control group (Group A) (Fig 7). In addition, photomicrographs of the group that received combined treatment of both SFN and $AlCl_3$ (group C) (Fig 7) showed a significant reduction in cell proliferation in all hippocampal regions as well as a presence of more erythrocytes similar to that of the control group (group A) (Fig 7).

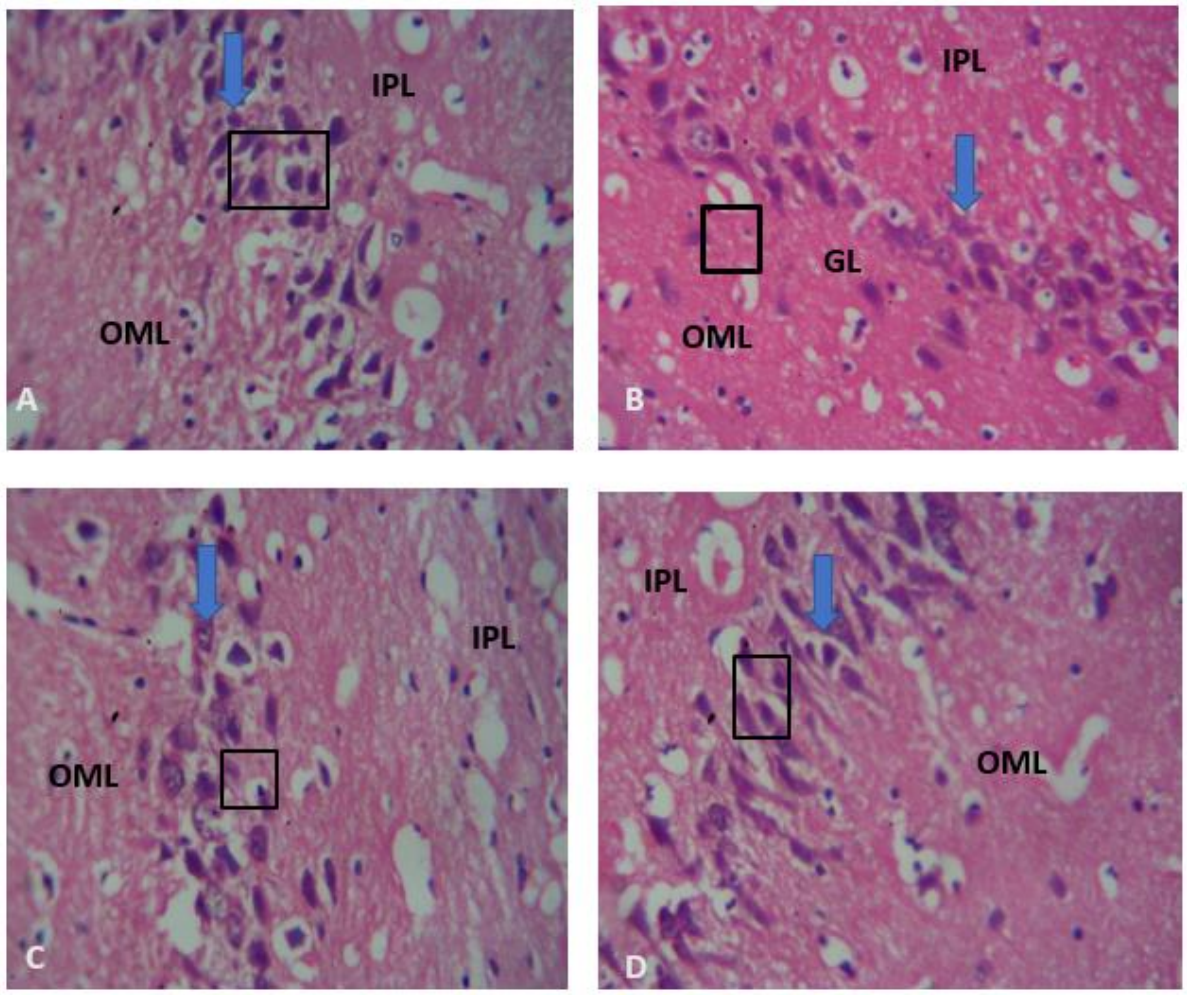

Figure 6: Histological examination revealed clear pathological alterations indicative of hippocampal degeneration, including distortion and pyknotic abnormalities in the pyramidal layer of the $AlCl_3$-only group's hippocampal region (Group B), which were mitigated by SFN treatment (Group C) showing normal hippocampus morphology with compact layers of small pyramidal cells and vesicular nuclei, many glial cells (Square) in the outer molecular layer (OML) and pyramidal cells (arrow) among neuronal process of the inner pyramidal layer (IPL) similar to control (Group A) and SFN only (Group D).

H and E: x400.

DISCUSSION

Aluminum neurotoxicity has been reported to cause and accelerate neuronal oxidative damage with its concentration elevated in the brains of patients with AD [43]. The high affinity for transferrin receptors potentiates its ability to cross the blood brain barrier thereby causing neuroinflammation, protein misfolding, damage to the synaptic morphology via the inhibitory mechanism of slow and fast axonal transports, self-aggregation of neurofilaments, microtubule-associated proteins and amyloid-b [44]. All the neurological deficits ultimately result in learning and memory deficits both in humans and animals.

In this study, it was observed that the behavioral and neuropathological effect of aluminum exposure showed neurological disorders such as learning deficits, memory impairment and neuronal loss when compared to the controls. In the Morris water maze test, decreased acquisition and retention latencies was observed in the $AlCl_3$ only group compared to the control. This observation revealed possible neurotoxicant activity of $AlCl_3$ in learning process failures and memory deficits. Previous studies have also observed that oral administrations of Al to rodents cause learning and memory deficits [45,46]. Similarly, impairment of rats learning and memory after Al treatment in drinking water in the passive avoidance task has been reported [47]. However,

groups treated with combination of sulforaphane and $AlCl_3$ showed improved acquisition and retention latencies compared to the $AlCl_3$ only group, this observation can be attributed to the protective role of sulforaphane against impairments in learning and memory skills deficits.

Generally, decrease in short-term memory and other forms of neurobehaviors have been implicated in experimentally induced diseases [33,44]. In the present study, the percentage correct alternation (a short-term memory index) measured in the Y-maze test showed observable reduction after administration of $AlCl_3$ thereby implicating the neurotoxicant impairment of learning and memory activities. The combined administration of sulforaphane and $AlCl_3$ ameliorated the memory declination compared to $AlCl_3$ only group thereby sustaining the learning capacity of the animals.

In addition, the crossing scores recorded using the open field test revealed alterations in the parameters measured such as rearing, sniffing and grooming after $AlCl_3$ induction. This observation is similar to the experiment carried out by two authors who observed significant reduction in the spontaneous locomotor activity (hypokinesia) after treatment with Al [48,49].

Apoptosis of the oligodendrocytes and death of the neuronal cells have been implicated to be caused by several factors such as oxidative stress, decrease in cytochrome oxidase and monoamine oxidase activities in the mitochondria [44,50]. Well-known significant consequences of oxidative stress are products of lipid oxidation, and the brain is regarded as most sensitive target to damage because of high level of lipid content and tissue oxygen consumption [51]. Previous studies have reported that the imbalance between generation of reactive oxygen species and antioxidants are consequences of Al-induced oxidative damage in the neurons of the brain[52,53]. The present study measured the endogenous antioxidants (CAT, SOD, GSH) and lipid peroxidation. Our study revealed increase in MDA (a marker for lipid peroxidation), H_2O_2 and NO levels with corresponding decrease in SOD, CAT and GSH levels in $AlCl_3$ only group compared to the control. These results corroborated previous observation that Al exposure activates the process of lipid production in rat brain [44]. In addition, the most significant scavengers of free radicals and cofactor of many detoxifying enzymes against oxidative stress is GSH.[54] In our study, decrease in GSH level in $AlCl_3$ only group suggested that antioxidant enzymes are the first cellular molecules needed for defense against generation of ROS. The decrease in antioxidant parameter is in agreement with the fact that Al reduced the total antioxidant parameter levels by promoting the imbalance between pro-oxidant and antioxidant potentials. Previous research reported similar observation in which Al chloride intraperitoneally administered resulted in increased $AlCl_3$ concentration in hippocampus and cerebellum in the Al-treated group compared to the control [44,55] with decrease in both the activity of GPx and GSH level.[56] However, the co-administration of $AlCl_3$ and sulforaphane significantly reverse activities of GSH, CAT, SOD and malondialdehyde concentration to the range of controls. This protective potential of sulforaphane could be because of its intrinsic antioxidant property [39,44].

The brain catecholamine neurotransmitters (DA, 5-HT and NE) can be altered due to increased production of free radicals that resulted in the oxidation of the neurotransmitters thereby causing neurodegenerative diseases such as Alzheimer's and Parkinson's diseases[57]. In the present study, there was a decrease in DA and 5-HT levels in $AlCl_3$ only group compared with the control. It was suggested that decreased levels of dopamine observed was due to altered activity of biosynthetic enzymes, or alteration to the availability of their precursor amino acid tyrosine[57] while decrease of serotonin might be due to conversion of serotonin to melatonin thereby interfering with oxidative stress caused by $AlCl_3$ exposure. In addition, the present study revealed elevation in norepinephrine level in $AlCl_3$ only group compared with the control. Previous observation revealed that increase in norepinephrine could be caused by $AlCl_3$ neurotoxicity thereby causing the activation of its synthetic pathway (conversion of dopamine to norepinephrine via hydroxylation)[58]. However, the co-administration of sulforaphane and $AlCl_3$ showed better improvement in the levels of brain catecholamine neurotransmitters compared with the intoxicated group by acting as an antioxidant in mopping up free radicals which could be detrimental to neuronal activities.

The region of the brain responsible for learning and memory formation is the hippocampus. Therefore, histoarchitectural alteration will affect the normal physiological attributes of the brain. In the present study, $AlCl_3$ exposure resulted in poorly stained granule cells of the dentate gyrus and cellular clusters with evidently pyknotic features with the hippocampus compared with control. The observed histomorphological malformations impair neuronal protein and cell membrane synthesis[57] thereby causing impairment in neurological functions of the brain. The neurological malfunctions produced due to $AlCl_3$ neurotoxicant could further potentiate impaired function arising due to axonal demyelination seen in multiple sclerosis. However, the combined administration of sulforaphane and $AlCl_3$ showed preserved Nissl granules against the insult of reactive oxygen species thereby enabling them to perform neuronal protein synthesis.

CONCLUSION

The results of this investigation showed that $AlCl_3$ can induce neuronal toxicity in the hippocampal regions that is associated with Alzheimer-type neurodegeneration. On the other hand, sulforaphane's antioxidant response protects neuronal cells and improves memory deficits, maintaining brain function linked to healthy neuronal integrity.

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