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Research Article

Nigella sativa oil attenuates aluminum-induced behavioral changes, oxidative stress and cortico-hippocampal neuronal degeneration in rats

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Aluminum chloride, memory impairment, neuronal damage, Ki-67, nigella sativa oil

ABSTRACT

Background: Aluminum ($AlCl_3$) usage in both the industrial and domestic arena has dramatically risen over time owing to its ubiquity and utility for many activities despite frequent reporting of its neurotoxic effects over wide range of concentrations. The neuro-protective effects of Nigella sativa Oil (NSO) following intermediate exposure to aluminum salts has largely been unexplored. The present study explores the ameliorative and neuro-protective functions of NSO on aluminum chloride ($AlCl_3$)-induced damages in the frontal cortices and hippocampus of exposed rats. **Methods:** The study involved the use of thirty two adult male Wistar rats weighing 180 ± 20 g, randomly divided into four groups, in which group 1 received saline, group 2 received $AlCl_3$ (100 mg/kg), group 3 received $AlCl_3$ (100 mg/kg) followed by NSO (1 ml/kg) 30 min later, while group 4 received NSO (1 ml/kg) only. All administrations were done orally for 14 days. Standardized behavioural tests for anxiety and cognitive performance were carried on after the treatments prior to euthanizing (Ketamine 10 mg/kg, ip). On day 15, the rats were euthanized, and their brains excised, with the frontal cortex and hippocampus removed. Five of these samples were homogenized and centrifuged to analyze nitric oxide (NO) metabolites and total reactive oxygen species (ROS), and the other three were processed for histology (cresyl violet stain) and proliferative markers (Ki67 immunohistochemistry). **Results:** Increased Transfer latency, time in dark box, escape latency and reduced rearing frequency, percentage alternation and time in platform quadrant were observed in the $AlCl_3$ exposed rats. There was also an increased level of ROS and NO in the brain regions with marked inhibition of neuronal cell proliferation as evidenced by reduced Ki-67 protein expression in the brain of $AlCl_3$ only rats. However, rats co-administered $AlCl_3$ and NSO showed significantly reduced ROS and NO levels, improved anxiety-like and cognitive behaviors and increased Ki-67 expression when compared with $AlCl_3$ only treated rats. **Conclusion:** $AlCl_3$ exposure causes neuronal damage and impaired anxiety-like and memory indices which are associated with increased free radical generation and inhibited neuronal proliferation, whereas the antioxidant and neuro-protective properties of NSO were efficacious against the observed effects.

INTRODUCTION

Aluminium finds many uses in the daily routines of human existence, from purification of potable water, to vaccines, antacids, phosphate binders, parenteral fluids, inhalation fumes, preparation and storage of foods (cans and foils) subjecting humans to frequent exposure (Walton, 2012 a, b). Aluminium use has been identified as a long-familiar environmental neurotoxin to animals (Wu et al., 2012). Aluminium toxicity is directly linked to its bioavailability to biological systems since it has been reported to accumulate in many mammalian tissues such as the brain, bone, liver and kidney (Bhadauria, 2012), with an estimated long half-life in the brain. Aluminium accumulation and toxicity in the central nervous system induces increased formation of reactive oxygen species (ROS), leading to increased brain oxidative stress and lipid peroxidation thus increasing predisposition to neurodegenerative-like pathologies (Praticoet al., 2002). The human brain is particularly vulnerable to elevated ROS production because it

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metabolizes about 20% of the body oxygen and has determined amount of antioxidant capacity. Some genes coding for antioxidant enzymes [e.g, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GTH-Px)] are expressed during induction of aluminium treatment and oxidative stress suggesting a common mechanism of action (Rodriguez-Millaet al., 2002). Just like increased oxidative stress, aluminium exposure have been reported to cause neurobehavioral, neuropathological, neurophysiological and neurochemical changes (Yuan et al., 2012; Walton, 2013), and has been implicated to causes neurological symptoms and biological responses characterized by severe learning disabilities.

Nigella sativa (NS) belonging to the Ranunculaceae botanical family is also known as Black seed. It is most commonly found in Western Asia, Eastern Europe and the Middle East. The oil of NS (NSO) is a commonly used in traditional medical regimen, for the management of several diseases and has proven to be valuable (Imam et al., 2018a). Its efficacy has been studied broadly and reported to render protection against haematotoxicity (Ajao et al., 2017a), inflammation, psycho-cognitive disruptions (Imam et al., 2016 a, b), neurologic damage, memory and recall impairments (Mohammed and Shehab, 2016), and seizures (Farzaneh et al., 2015) among other conditions. Its therapeutic efficiency has been reportedly linked to its bioactive compound compositions which include thymoquinone, riboflavin, alkaloids, niacin, folic acid, piridoksin, proteins and minerals (Sarwar and Latif, 2015). The reported pharmacological properties of these bioactive compounds are efficient as antioxidant (Ashraf et al, 2011), anti-inflammatory (Alemi et al, 2013), neuroprotective (Imam et al., 2018 a, b, c), hepatoprotective (Ajao et al., 2017a), antidiabetic (Alli-oluwafuyi et al., 2017), hemato-protective, renal protective (Ajao et al., 2017b) substances with associated improvement in male infertility (Kolahdooz et al., 2014), efficacy in neurodegenerative diseases (Dariani et al., 2013) and memory enhancements (Imam et al, 2016a, b), although its efficacy is not limited to these properties.

Thus, this study was aimed to investigate the ameliorative effect of Nigella sativa oil in aluminium exposed rats as well as subsequent psycho-cognitive functions of the rats.

METHODS

Chemicals and Drugs

Crystalline salts of $AlCl_3$ was procured from Sigma-Aldrich (Germany) while normal saline solution was prepared in our laboratory. The *Nigella sativa* oil (concentration; 100%blackseed; HUSNA black seed oil, Fazhab Agency, Karachi, Pakistan) was purchased from a TIBB-medical store in Ilorin, Kwara state, Nigeria. Antibodies for Ki67 and the NO and ROS kits was obtained from abcam.

Animals and experimental design

Thirty-two (32) adult male Wistar rats weighing about 180 ± 20 g were obtained from the University of Ilorin Biological garden, Ilorin. They were housed in cages and fed with standard laboratory diet and water adlibitum, in the animal holding unit of the Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin. The rats were exposed to a 12 h light/dark cycle at room temperature for 7 days before the commencement of the experiments. All rats were handled in accordance with the standard guide for the care and use of laboratory animals.

Treatment plan

The rats were randomly divided into four groups (n=8) as described below:

Group 1 (control) - were given normal saline (1 ml/kg.bw orally) daily

Group 2 – were given $AlCl_3$ (100 mg/kg.bw orally) daily

Group 3 – were given $AlCl_3$ (100 mg/kg.bw orally) plus NSO (1ml/kg.bw orally) daily

Group 4 – were given NSO (1 ml/kg.bw orally) daily

The experiments were all conducted in the morning (between the 07:00 and 09:00 hours), and treatments with substances lasted for the period of fourteen (14) consecutive days.

Ethical Approval

This research work was approved by the University of Ilorin ethical review committee (UERC) (UIL/UERC/11/46KA072), following the recommendation of the College of Health Sciences ethical review committee, in compliance with the Institutional Animal Care and Use Committee (IACUC).

Evaluation of Brain and Body Weight

The body weights of all the rats were recorded after acclimatization on the first day of exposure, as 'the initial weight', and on the last day of exposure, as 'the final weight'. The differences between the two weights were thus calculated and recorded as the weight fluctuated or changed. The brain weight of all the rats were recorded after the sacrifice, and a ratio of the brain to final body weight was calculated and recorded.

Behavioural Evaluations

The rats were subjected to behavioural evaluations on the 14th day of the treatment to assess anxiety-like behaviors and cognitive indices using Y-maze, modified

Elevated Plus Maze, white and black box test and Morris water maze (MWM) paradigms respectively.

Modified Elevated Plus Maze Test

The apparatus consisted of two open arms, surrounded by a short edge to prevent falls, and two enclosed arms erected in such a way that the two open arms were opposite each other. The maze was raised about 35 cm above the ground with a stable stand and the arms of the maze were connected by a central platform. At each of the two trials, each rat was gently placed on an open arm, positioned to face away from the central platform and the closed arms. The time it took the rats to retreat and move to the closed arms was recorded as the transfer latency. While the first trial was for acquisition, the second was used as a measure of fear learning (Mutlu et al., 2015; Hlinak and Krejci, 1998, 2000, 2002). The principle of this experiment is primarily based on the aversion of rats to heights and open spaces.

White and Black box Test

The apparatus consisted of a white and a black compartment both separated by a wall of 70 x 70 mm opening in its base. The rats were accustomed to the behavioral apparatus during 2 consecutive days (5 minutes) before the training session. On the 3rd day, the rats were placed in the white compartment, and the time latency for entering the dark compartment (transfer latency) and the total time spent in the black box were recorded.

Y Maze Test

The Y-maze apparatus is made up of three equally spaced arms, labelled as A, B, and C which are 120 degree from each other, 41 cm long and 15 cm high. The floor of the apparatus is 5 cm wide and is levelled with saw shaves. Each rat was stationed in one of the arms and allowed to freely explore the apparatus. Data recorded include the total arm entries indicate the total number of a single arm entered (e.g. ABCBCABACBC, contain 11 entries), the sequence or consecutive entrance of the rats into the arms (an alternation), the total number of arms entered minus two (spontaneous alternations), and the percentage alternation (actual alternations/spontaneous alternations) X100. 5 min was assigned as the test time limit for each of the rats in the Y-maze apparatus.

Morris water maze (MWM)

The MWM apparatus in this study was used to assess the changes in memory indices following exposures to ALCL3 and/or NSO in Wistar rats. Each of the rats (n=32) was placed in a black, circular pool, which was filled with 23–24°C water (pool dimensions: 60cm deep × 136cm diameter). The pool was divided into four quadrants, labeled north (N), east (E), south (S) and west (W). It contained a circular platform (10cm diameter, 28cm high) that was submerged (about 2cm below water surface) in the central area of the SW quadrant of the pool. The rats swam until they found the platform (total time allowed on the platform = 15s). If the subjects were unable to find the platform after 60s of swimming, they were gently guided to the platform. The rats were then removed from the pool, dried and placed in their cage for 5min. Each of the trials was recorded with the aid of a video system. Animals received a training session (three trials per session), for three consecutive days (days 11, 12and 13 of the experiment). Each of the trials was a maximum duration of 60s. The time interval between trials was approximately 30s in duration. 24h after the acquisition phase, the time it took the subjects to find the hidden platform (referred to as 'escape latency') was recorded as long-term memory (LTM). An average of the escape latency of the two subsequent trials was recorded as short-term memory' (STM). A probe test was also conducted by removing the platform and allowing the rats to swim freely in the pool for 60s; the time spent in the target quadrant, which had previously contained the hidden platform, was recorded as the reference memory on day 14 of the experiment. The time spent in the target quadrant indicated the degree of relative memory consolidation, which had taken place after learning.

Biochemical Evaluation

Once the treatment period was completed, the rats were euthanized with an overdose of Ketamine (10 mg/kg i.p.) and their brains dissected and weighed. Tissue blocks (from Bregma 2–4 mm of frontal cortex, 2.5 mm to 4.5 mm of the hippocampal formation) were removed from the brains of five rats (from each of the four groups), dipped in 30% sucrose solution, homogenized and portions centrifuged at 2500 rpm for 10 min. The supernatant was then collected in tubes containing the compounds for NO metabolites and ROS analysis. ROS was measured by monitoring the increasing fluorescence of DCFH-DA using flow cytometry (Partec, Deutschland) equipped with a 488nm argon ion laser and supplied with the Flomax software and the signals were obtained using a 530nm band pass filter (FL-1 channel). Each determination was based on the mean fluorescence intensity of 10,000 counts. The remaining tissue homogenate was added to the Griess reagents, sulfanilamide and naphthyl ethylene diamine solutions to measure nitrate/nitrite production (NO metabolites). Absorbance was measured with the aid of a microplate

reader and the levels of NO metabolites were calculated from a standard curve.

Tissue processing and Histopathology

Following euthanasia and extraction of the remaining three rat brains from each of the groups, the brains were fixed in 10% buffered formalin for 24h. The frontal cortex blocks (from Bregma 2–4 mm) and hippocampus blocks(from bregma 2.5 mm to 4.5 mm) were removed, dehydrated through ascending grades of alcohol, cleared in xylene and embedded in paraffin blocks. Tissue sections (5µm in thickness) were stained with Cresyl fast violet (CFV) for Nissl substances or immunestained to reveal Ki67 protein containing nuclei in the tissues. The sections were mounted, cover slipped and finally examined under an AmScope 40X-2500X LED Lab Compound Microscope, and photographed using the AmScope 5.0 MP USB Still Photo & Live Video Microscope Imager Digital Camera 5 MP (iSCOPE corp., USA).

Immunohistochemistry for Ki-67

Ki-67 is a chromosome-associated protein present during division (G1, S, G2, and M phases but absent from cells at rest, G0). Paraffin embedded sections were incubated for epitope retrieval in citrate buffer, pH6.0, at 90°C for 40min, followed by incubation in endogenous peroxidase blocking reagent, 0.6% H_2O_2 in Tris-buffered saline (TBS) – Triton (0.05% TritonX – 100 in TBS, pH7.4) for 30 min at room temperature. Thereafter, sections were pre-incubated in 2% serum (normal goat serum) + 0.1% bovine serum albumin (BSA) + 0.25% Triton in TBS for 60min at room temperature. The sections were then incubated with polyclonal rabbit anti-lyophilized Ki-67 antibody (Novocastra, Newcastle, UK; 1:5000 in pre-incubation solution) overnight at 4°C. Incubation with biotinylated goat anti-rabbit IgG (1:1000 + 2% normal goat serum + 0.1% BSA in TBS; Vector lab, CA, USA; 1:250) was performed for 2h at room temperature followed by incubation with streptavidin – biotin complex (Vecta stain Elite ABC kit) and stained with 3,3′-diaminobenzidine (DAB) as chromogen. All rinses prior to incubation with primary antibody, were made with TBS - Triton, afterwards with TBS alone.

Statistical Analysis

Data from the morphometry, behaviour and biochemical outcomes were analyzed using one-way analysis of variance (ANOVA) and subjected to post hoc Bonferroni's multiple comparison tests. The results are expressed as mean ±SEM. Statistical analyses were performed using Graphpad Prism software (version 5.0, La Jolla, CA). Values of $p< 0.05$ were considered statistically significant.

RESULTS

Effects of NSO and $AlCl_3$ on Body Weight Changes and Relative Brain Weight

Aluminium chloride caused a marked loss in body weight of exposed rats, while NSO treatment caused gain in body weight when compared with the $AlCl_3$ only or the saline treated rats. There is however no significant differences between the observed body weight changes (fig 1A). However, the brain- body weight ratio was significantly ($p\leq0.05$) reduced in the group administered $AlCl_3$ alone compared to the control), while NSO treatment caused significant ($p\leq 0.05$) increase in the brain body weight ratio of the NSO alone and $AlCl_3$ + NSO group compared to the $AlCl_3$ alone group (fig 1B).

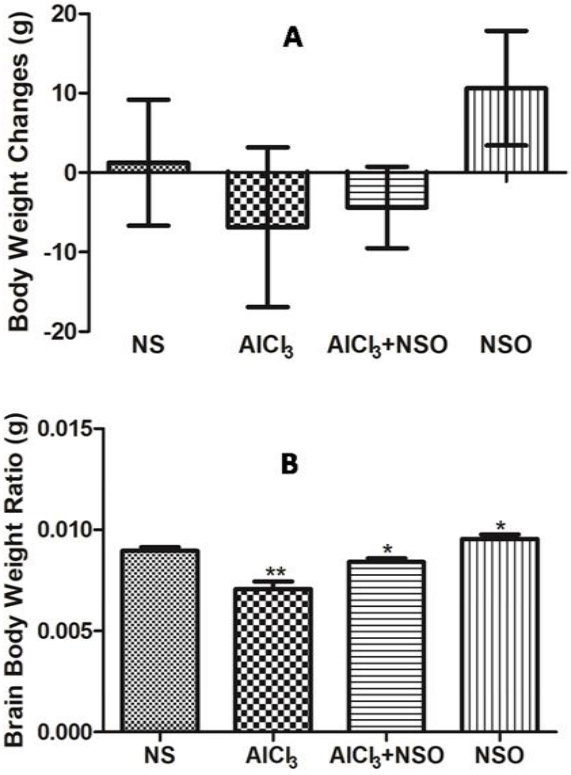

Fig. 1: The (**A**) body weight changes and (**B**) relative brain weight of rats exposed to NS, $AlCl_3$, $AlCl_3$+ NSO and NSO respectively. Data are presented as mean ± SEM (n=8 in each group) *p <0.05 indicate significant increase from $AlCl_3$ exposed, while **p<0.05 indicates significant reduction from the Saline treated rats. NS= Normal saline, $AlCl_3$ = Aluminum chloride, NSO = *Nigella sativa*; SEM= standard error of mean.

Anxiety-like behaviors following exposures to $AlCl_3$ and NSO

Repeated $AlCl_3$ ingestions caused observable high exploration and time spent in the dark box, and a reduced

rearing frequency in the white box of the dark and white box paradigm, while co-administration with NSO and NSO only reduced time spent in the dark box and increased rearing frequency in the white box when compared with the $AlCl_3$ and saline treated rats (Fig. 2).

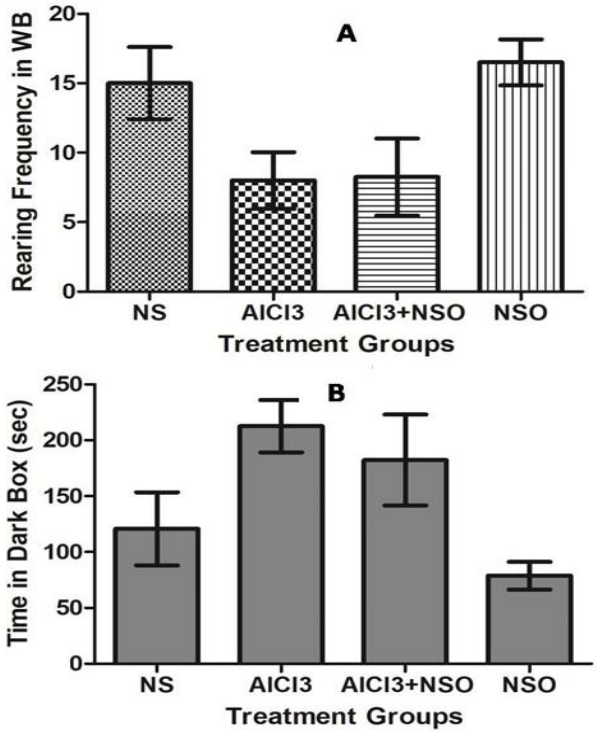

Fig. 2: The exploratory and anxiety related behaviors (**A**) rearing frequency in the white box and (**B**) time spent in the dark box following exposures to NS, $AlCl_3$, $AlCl_3$+ NSO and NSO respectively. Data are presented as mean ± SEM (n=8 in each group). NS= Normal saline, $AlCl_3$ = Aluminum chloride, NSO = *Nigella sativa*; SEM= standard error of mean.

Fear related spatial memory index of rats exposures to $AlCl_3$ and NSO

Transfer latency which a measure of fear learning in the mEPM was found to be delayed significantly ($p>0.05$) in the $AlCl_3$ exposed rats when compared with all other groups, while NSO co-treatment and alone significantly reduced transfer latency in the treated rats when compared with the $AlCl_3$ exposed rats (Fig. 3).

Effects of $AlCl_3$ and NSO on Spatial working memory in the Y maze test

$AlCl_3$ exposures led to a significant ($p<0.05$) reduction in the percentage alternation of the exposed rats when compared with the saline treated rats. Although in the $AlCl_3$ and NSO co-treatment, there was no significant increase in the percentage alternation, there is observable trend in increased memory index of the treated rats, which was complemented by the significant increase in the indices of rats treated with NSO only when compared with the $AlCl_3$ exposed rats (Fig. 4).

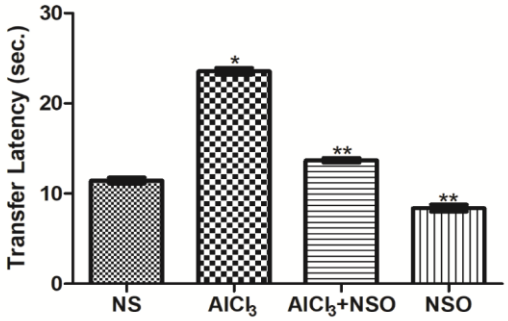

Fig. 3: The transfer latency of rats in the mEPM following exposures to NS, $AlCl_3$, $AlCl_3$+ NSO and NSO respectively. *p <0.05 indicate significant increase from all groups, while **p<0.05 indicates significant reduction compared to $AlCl_3$ exposed rats. Data are presented as mean ± SEM (n=8 in each group). NS= Normal saline, $AlCl_3$ = Aluminum chloride, NSO = *Nigella sativa*; SEM= standard error of mean.

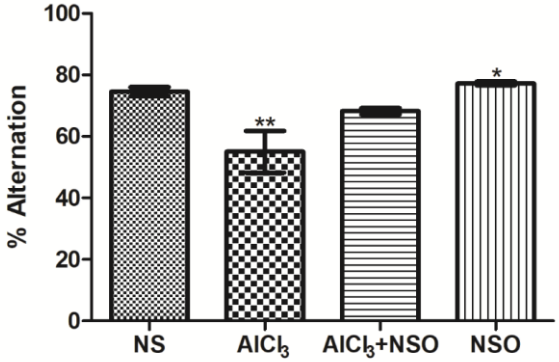

Figure 4: The percentage alternation of rats in the Y-maze paradigm following exposure to NS, $AlCl_3$, $AlCl_3$+ NSO and NSO respectively. Data are presented as mean ± SEM (n=8 in each group) **p<0.05 indicates significant reduction compared other groups, while *p <0.05 indicate significant increase compared to the $AlCl_3$ exposed rats. NS= Normal saline, $AlCl_3$ = Aluminum chloride, NSO = Nigella sativa; SEM= standard error of mean, LTM= Long time memory, STM= Short Time Memory, RM=Retentive memory.

Effects of $AlCl_3$ and NSO on Learning and Memory indices in the MWM test

The average escape latency during the 3 training trials appear to be slightly higher in the $AlCl_3$ exposed rats when compared with the control, but only the low escape latency in the NSO only treated rats is statistically ($p<0.05$) significant (Fig. 5A). Relative to the learning latency, $AlCl_3$ exposure caused marked ($p<0.05$) delay in the latency to find the hidden platform during the short- and long-term memory tests when compared with the saline treated rats (Fig. 5B&C). Just like in the learning latency, NSO co-treated or NSO alone rats display early latency to find the hidden platform, and this is significant when compared with the $AlCl_3$ exposed rats (Fig. 5B&C). In the reference memory test, the $AlCl_3$ exposed rats spent significantly less time exploring the quadrant that previously contained the hidden platform (Fig. 5D).

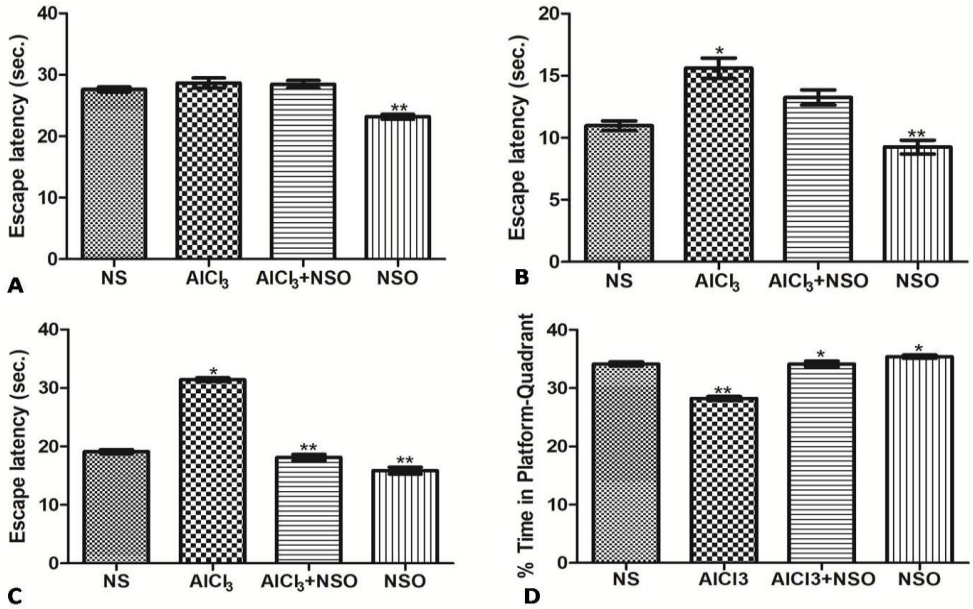

Figure 5: The escape latency (A-Learning; B-LTM; C-STM) and percentage time in platform quadrant (D-RM) in rats in the MWM after exposure NS, $AlCl_3$, $AlCl_3$+ NSO and NSO respectively. Data are presented as mean ± SEM (n=8 in each group), **p<0.05 indicates significant reduction compared with $AlCl_3$ (Fig. 5A,B,C) or control (Fig 5D), while *p <0.05 indicate significant increase compared to the control rats (Fig. 5A,B,C) or the $AlCl_3$ exposed rats. NS= Normal saline, $AlCl_3$ = Aluminum chloride, NSO = Nigella sativa; SEM= standard error of mean, LTM= Long time memory, STM= Short Time Memory, RM=Retentive memory.

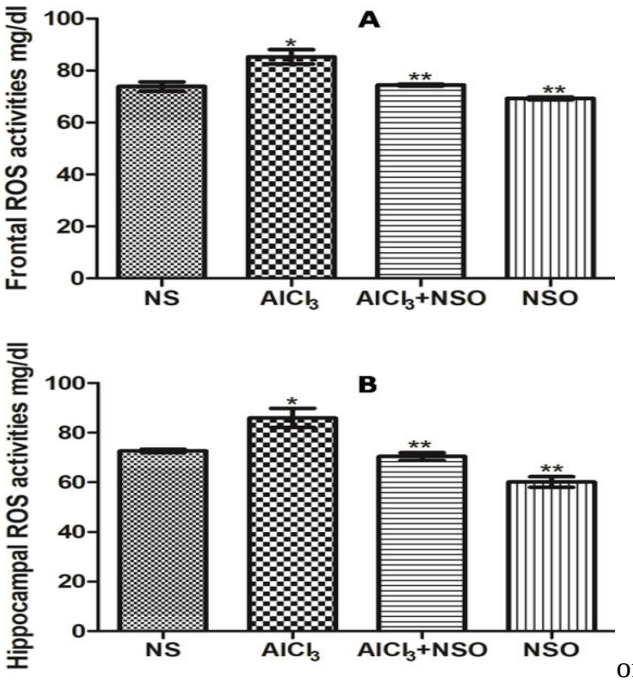

Fig. 6: ROS concentration in the frontal cortex (A) and Hippocampus (B) region of rat brain, after exposure to NS, $AlCl_3$, $AlCl_3$+ NSO, NSO respectively. Data are presented as mean ± SEM (n=8 in each group) **p<0.05 indicates significant reduction compared with $AlCl_3$, while *p <0.05 indicate significant increase compared to the control rats. NS= Normal saline, $AlCl_3$ = Aluminum chloride, NSO = Nigella sativa; SEM= standard error of mean.

Effects of $AlCl_3$ and NSO on the Total Reactive Oxygen Species (ROS)

The total reactive oxygen species (ROS) levels evaluated in the frontal cortices and hippocampus was significantly ($p<0.05$) elevated in the $AlCl_3$ exposed rats when compared to the saline treated rats. Expectedly, the co-administration with NSO and NSO only treatment caused significant ($p<0.05$) reduction in the total reactive oxygen species (ROS) in both the frontal cortices and hippocampus of the treated rats when compared with the $AlCl_3$ exposed rats (Fig. 6A&B).

Effects of $AlCl_3$ and NSO on the Nitric Oxides metabolites

$AlCl_3$ exposure in rats caused significant increase in Nitric oxides concentrations in the frontal cortices and hippocampus when compared with the saline treated rats. In a relative pattern to the ROS levels, the co-administration with NSO and NSO only treatment significantly ($p<0.05$) lowered NO metabolites levels in both the frontal cortices and hippocampus of the treated rats when compared with the $AlCl_3$ exposed rats (Fig. 7A&B).

Effects of NSO on $AlCl_3$ induced changes in the Histoarchitectural integrities

The result of the histological stained tissues of the saline and NSO treated rats showed the three layers of the hippocampus; the molecular, pyramidal and polymorphic layer, the pyramidal cells with large circular vesicular nuclei and lengthened cytoplasmic processes protruding towards the neighboring layer (molecular layer). The pyramidal cells of the CA regions appear to have normal shape in all the groups, and there is no marked alteration observable in the pyramidal cells of the frontal cortex and the hippocampus of the $AlCl_3$ exposed rats (Fig. 8).

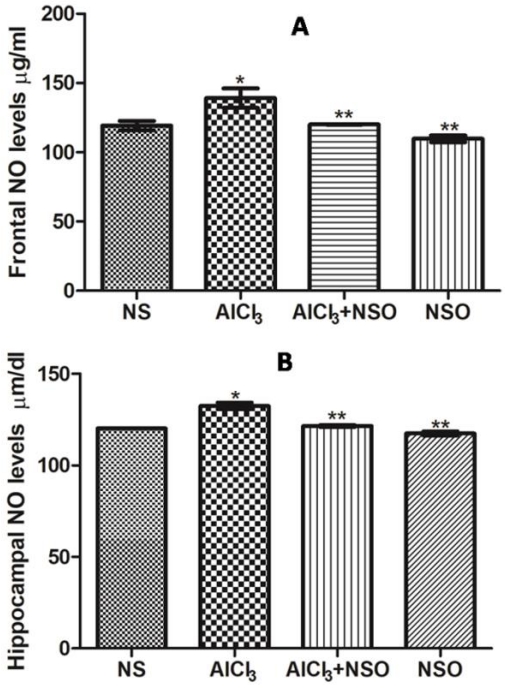

Figure 7: NO concentration in the frontal cortex (A) and Hippocampus (B) region of rat brain, after exposure to NS, $AlCl_3$, $AlCl_3$+ NSO, NSO respectively. Data are presented as mean ± SEM (n=8 in each group) **p<0.05 indicates significant reduction compared with $AlCl_3$, while *p <0.05 indicate significant increase compared to the control rats. NS= Normal saline, $AlCl_3$ = Aluminum chloride, NSO = Nigella sativa; SEM= standard error of mean

Effects of NSO on $AlCl_3$ induced changes in the distribution of immunoreactive Proliferative neurons

We immunostained for ki-67 protein using anti-ki67 antibody, to detect the effects of $AlCl_3$ and NSO on neuronal proliferation in the dentate gyrus and frontal cortex of the exposed rats. The results showed a reduced distribution of reactive cells in both the frontal and hippocampal dentate gyrus of $AlCl_3$ exposed rats when compared with the other groups. Meanwhile, a comparably more population of immunoreactive cells was observed in the subgranular zone of the dentate gyrus and the frontal cortex of the group administered NSO alone, and in the co-administered rats (Fig. 9).

DISCUSSION

Administration of $AlCl_3$ to rats has been shown by earlier workers (Buraimoh and Ojo, 2014; Nam et al., 2014) to induce marked reduction in body weight and relative brain weight, the reduction in the relative brain of $AlCl_3$exposed rats in the presentstudy is therefore not surprising. Similarly, the improvement in the relative brain weight of rats co-administered $AlCl_3$and NSO is in line with the previously reported activities of NSO following exposure to other toxicants (Imam et al., 2018a, b).

Aluminium exposure also caused impaired cognitive, exploratory, anxiety and working memory functions in the ingested rats. This suggests that $AlCl_3$ generates an ion-induced degenerative cascade within the hippocampus and prefrontal cortex which causes the cognitive and working memory decline. NSO prevented orreversed the cognitiveand working memory decline to normal and as well improved the willingness of the experimental animals to explore new environments. These maybe due to the reported potential of NSO in enhancing indices ofpsycho-cognitive functions in models of neurodegenerative disorders and neurotoxicity (Hosseini et al., 2015; Imam et al., 2016 a,b).

The effects of $AlCl_3$exposure causing an increase in the anxiety-like behavior in the exposed rats, supports the result of some workers, who observed that$AlCl_3$affected behavioural phenotypes of rats (Buraimoh et al., 2011, 2012; Nam et al., 2014; Nampoothiri et al., 2015; Kuznetsova et al., 2017; Said and Rabo, 2017).While the efficacy of NSO against these effects can be strengthened with our previous reports on the therapeutic efficacies of NSO are related intervention measures (Ajao, et al., 2017 a,b; Imam et al., 2016a, b, 2018 a, b, c).

Although there is minimal effect of $AlCl_3$ingestion on spatial working memory of the exposed rats in the Y maze test, substantive loss other cognitive indices using the MWM were observed; in this light, we found NSO to be efficient in restoring the long term and retentive memory of the rats impaired markedly following $AlCl_3$exposure. This can be supported with previous reports on the efficacy of NSO in enhancing spatial memory performance of rat (Khairul et al., 2013; Norouzi et al., 2018).

Earlier reports on the pathophysiology of $AlCl_3$induced neurotoxicity have largely implicated disrupted oxidative activities in the cellular environment as a contributor to the underlying molecular mechanism. Of note are the imbalances in neuronal oxidative – antioxidant systems in discrete brain regions following $AlCl_3$, leading to synaptic instabilities, reduced neurogenic cells

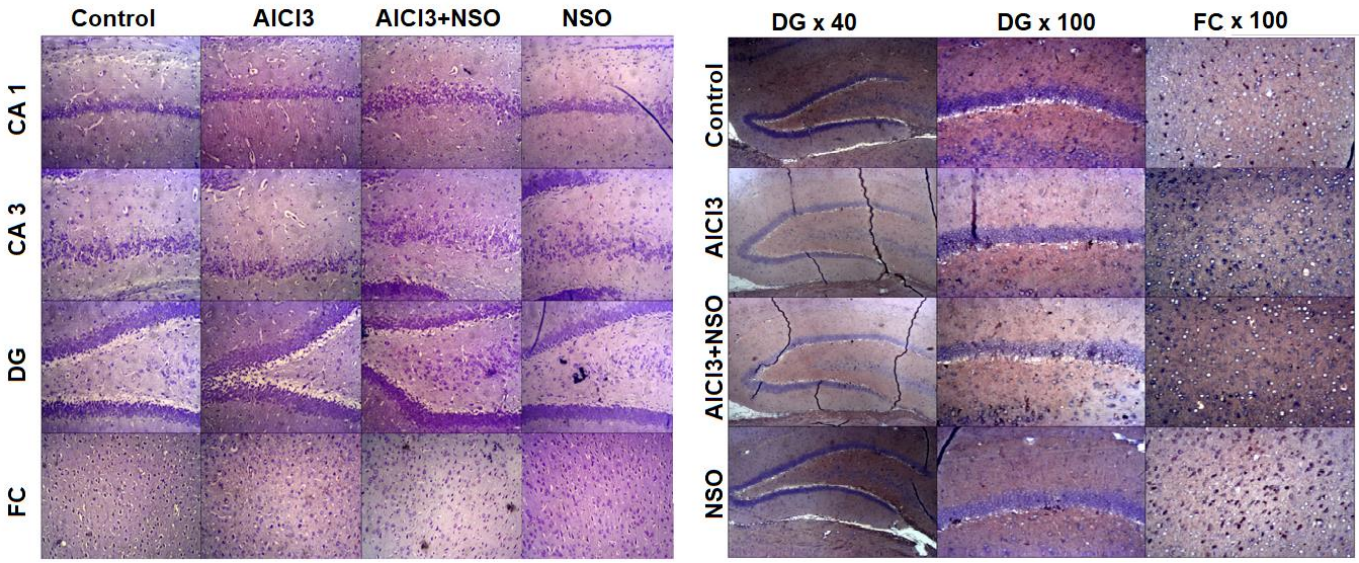

Fig. 8 (left): Representative photomicrographs of the hippocampus and frontal cortex histoarchitecture following exposure to NS, $AlCl_3$, $AlCl_3$+ NSO, NSO alone. NS= Normal saline, $AlCl_3$ = Aluminum chloride, NSO = Nigella sativa, CA1= Connus Amonis 1, CA3= Connus Amonis 3, DG= Dentate gyrus, FC = Frontal cortex ; Stain: Cresyl fast violet; magnification; CFV X100; **Fig. 9 (right)**: Representative micrographs of the distributions of Ki67 immunoreactive cells in the hippocampal dentate gyrus (DG) and frontal cortex (FC) of rats following exposure to NS, $AlCl_3$, $AlCl_3$+ NSO and NSO alone. NS= Normal saline, $AlCl_3$ = Aluminum chloride, NSO = Nigella sativa; Stain: Anti-Ki67 immunostaining; magnification; X40 and 100

survivals, apoptosis, increasing free radicals, and ultimately causing brain damage (Singh and Goel, 2015; Cao et al., 2016; Said and Rabo, 2017)**.**

Reactive oxygen species (ROS) and Reactive Nitrogen species (RNS) have been widely investigated using, total ROS and Nitric oxide (NO) (nitrite/nitrate concentration) in the brain. NO and ROS are signaling molecules that are very critical in the pathogenesis of inflammation, while, NO plays a regulatory role in homeostatic processes, such as in the regulation of the oxidation or reduction reaction; thus, its concentration determines if its activities would be protective or injurious (Mahindrakar et al., 2014). Here, as implications for the reported psycho-cognitive phenotypes above, $AlCl_3$ causes pro-inflammatory and oxidative responses through the induction of nitro-sative and oxidative stress by increasing the NO and ROS levels in both the hippocampus and prefrontal cortices of the exposed rats, which has been implicated in several neurological disorders and in $AlCl_3$exposure in rats (Kamel and Mostafa, 2013). Just like in other studies, this study showed that most of the cytotoxic activities of$AlCl_3$ in neurons can strongly be linked to the disruption of oxidative redox and increased ROS production. ROS attack cellular components, inflicting degenerative modifications to DNA, protein and lipid molecules in the process, thereby promoting apoptosis through activation of cell cycle regulatory proteins like p53 and bax. Co-administration of AlCl3 with NSO was sufficient to causing a reasonable depletion in the over-expressed NO and ROS. This finding is strongly supported by previous reports that corroborates this (Abdel-Zaher et al., 2010; Imam et al., 2018a, b, c; Kamel and Mostafa, 2013). Thus, further confirming the efficacy of an antioxidant NSO against both the neurochemical and behaviours phenotypes of $AlCl_3$ neurotoxicity

It has been suggested that Ki-67 protein plays a major role in cell proliferation by binding to the cell's DNA to regulate and maintain cell division during cell cycle. While increase or accumulation of Ki-67 protein immunoreactivity may suggest increased cellular proliferation, its decrease has been associated with degeneration of neuronal cells (Mohamadin et al., 2010; Kamel and Mostafa, 2013; Imam et al., 2018 a,b). Ki67 protein immune-reactivity was markedly low in the hippocampus dentate gyrus and the frontal cortex of the

$AlCl_3$ exposed rats, this finding agrees with the work of Nam and its colleague, that reported reduced neural stem cells, cell proliferation and neuroblast differentiation in the dentate gyrus of $AlCl_3$exposed rats (Nam et al., 2014, 2016). Interestingly, intervention with NSO prevented the loss ki-67 protein immunoreative cells, a report strengthened by other workers who reported the inhibition of cell death events and improvement of survival situations by NSO (Kamel and Mostafa, 2013; Sjoukjeet al., 2015).

In this regard, it is suggestive that the therapeutic mechanisms of NSO against $AlCl_3$ neurotoxicity may involve inhibition of cell death stimulus in neurons, through its roles in augmenting neuronal antioxidant defense system; subsequently promoting integration of new neurons into social, learning and memory circuitries resulting in psycho-cognitive function improvement.

CONCLUSION

In conclusion, oral administration of aluminum chloride for 2 weeks reduce the neuronal cell proliferation, with impaired memory indices in the exposed rats, observations which are linked to altered oxidative functions and depletion of neurogenic protein, while the antioxidant efficacy of NSO was sufficient in restoring the biochemical and behavioural dysfunctions.

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