NEUROPROTECTIVE POTENTIAL OF FLOWERS OF *NYCTANTHES ARBOR-TRISTIS* ON ALUMINIUMCHLORIDE INDUCED NEGATIVE EFFECTS ONLEARNING ABILITIES IN RATS.

Authors

Arvind B. Naik [1,2] arvinbnaik@gmail.com

Deepti Bandawane [1]

Affiliations

1 Department of Pharmacology, Progressive Education Society's, Modern College of Pharmacy, Yamunanagar, Nigdi, Pune-411044

2 Department of Pharmacology, LSHGCT's Gahlot Institute of Pharmacy, Koparkhairane, Navi Mumbai-400709.

Abstract

This study investigated the neuroprotective effects of *Nyctanthes arbor-tristis* flower on memory and learning in aluminium chloride-induced brain dysfunction in rats. All Wistar rats in groups III and IV received the treatment dosages (100 and 200 mg/kg) 30 minutes prior to the oral administration of aluminium chloride (100 mg/kg), which was given every day for 42 days. The overall behavioural activity of rats was assessed using an open field arena (OFA) on days 20, 21, and 42 after the beginning of the administration of aluminium chloride. The parameters viz. line crossing, centre square entries, rearing, and grooming were used to score behavioural performance.

Morris Water Maze (MWM) and the Elevated Plus Maze(EPM) were used to assess cognitive performance. In MWM- rats were trained on day 19 and 20 to swim to a platform in a circular pool. The acquisition latency—the time it takes to get to the visual platform was measured. The first retention latency (1 RL) (21 day) and the second retention latency (2 RL) (42 day) were measured as the amount of time it took to identify the hidden platform,counted from the first day of aluminium chloride administration. On day 20, for EPM rats were trained, and each rat was placed at the end of an open arm. The initial transfer latency(ITL), which measures the time it took the rat to go from the end of the open arm to either of the closed arms, was then recorded. The rats were examined for retention latency on days 21 and 42, and the results were referred to as first RTL and second RTL, respectively. The respective treatment groups (100/200 mg/kg, p.o.) significantly defended against aluminum-induced brain damage, as evidenced bythe neuroprotective potential of *Nyctanthes arbor-tristis*.

Keywords: *Nyctanthes arbor-tristis*, Neuroprotective potential, cognitive performance

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Introduction

Alzheimer's disease (AD) is thought to have aluminium (Al) as a probable etiological component. Both clinical observations and animal tests have shown that an excess of aluminium can cause amyloid neurotoxicity. According to animal research, ingestion of too much aluminium may impair rat learning and memory, deposit amyloid beta (Aβ) protein in central nervous system cells, and increase expression of amyloid precursor protein (APP). (Gong *et al*, 2005).

The environmental factor(s) and susceptibility genes that trigger these formative events in plaque and tangle formation in the majority of Alzheimer patients have not received enough attention, despite significant advancements. In the brains of people with sporadic AD and in the brain tissue of transgenic mice used as a model for AD amyloidogenesis, for instance, oxidative stress is now understood to precede and contribute to the formation of β-amyloid deposits. However, the mechanism of this oxidative damage is unknown. Finding the primary source(s) of oxidative stress in AD will be crucial to comprehend its etiology.(Walton *et al*, 2007). According to reports, aluminium deposits in the cortex, cingulate bundles, corpus callosum, and hippocampus and has been shown to affect the blood-brain barrier.(Sethi *et al*, 2008). In the plasma and tissues of male rabbits, Yousef observed that aluminum-induced changes in hemato-biochemical parameters increased lipid peroxidation and lowered the activity of the antioxidant enzymes. (Yousef *et al*, 2004)

By 2030, there will be 66 million cases of dementia worldwide, and by 2050, there will be 115 million cases. The most common cause of dementia in elderly people is Alzheimer's disease. Alzheimer's disease can be categorized clinically into early-onset (affecting patients under 65) and late-onset (affecting patients over 65), whereas it is recognized pathologically by the presence of plaques of amyloid peptides and intraneuronal tangles of hyperphosphorylated forms of the microtubule-associated protein tau (MAPT). In our sample, those with AD accounted for 27.5% of fatalities over a 15-year period. (Ganguli *et al*,2005)

Aluminum induces harmful alterations to cholinergic neurotransmission by promoting the buildup of insoluble amyloid-protein, hyperphosphorylated tau-protein aggregates, and neurofibrillary tangles (NFTs). Additionally, Al enhances the oxidation brought on by a number of transition metals, such as copper and chromium. Al has also been linked to poor learning outcomes in Wistar rats, mice, and rabbits.(Sethi *et al*,2009).

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According to Banks et al., complexing with Al affects some aspects of blood-brain barrier (BBB) permeability, which is dependent on the facts that Al-Aβ complex clearance from blood is delayed compared to that of Aβ and that the central nervous system (CNS) absorbs the Al-Aβ complex from blood circulation more into brain parenchyma than Aβ. As a result, the BBB's capacity to transport Aβ into and out of the brain also influences the level of Aβ there, in addition to the CNS's rates of synthesis and breakdown. Considering this background, brain APP levels may be impacted by APP expression in other organs. (Li *et al*., 2012)

Recent research demonstrated that learning and the development of either short- or long-term memories might increase BDNF mRNA in the hippocampus, but the deprivation of endogenous BDNF resulted in special learning and memory damage in adult rats. It is yet unknown how exactly BDNF affects long-term memory formation, retention, and elimination. (Barde *et al*, 1982)

There are presently no effective or disease-modifying medications for AD. Clinical and molecular processes include amyloid accumulation, neuroinflammation, tau accumulation, neuronal degeneration, cognitive decline, and the appearance of behavioural and mental disorders all occurring in tandem with the advancement of AD. Since the amyloid theory has been challenged, there were much fewer anti-amyloid phase 3 trials in 2019. The goals of phase 1 and phase 2 studies can vary widely, but recent trends suggest that neuroprotection and anti-neuroinflammation are being targeted more frequently in both phases. (haung *et al*,2020)

At the end of the day, AD therapies have a lot of potentials. However, reversing AD remains outside the purview of straightforward antioxidant therapy until appreciable levels of oxidative damage have accumulated within the cell and the secondary diseases of AD become apparent. Because of this, while such a preventative therapy approach is suitable for the young-to-middle-aged population, it is not beneficial to those who exhibit the "oxidative steady state" within damaged cells. Secondary or symptomatic therapies must also be determined at that stage. (Bonda *et al*, 2010)

Carotenoids are essential in the prevention of several oxidative stress-mediated degenerative disorders, such as Alzheimer's disease and neurodegenerative diseases. Saffron is a common carotenoid-containing crude medicine that is a key component of many skin care products, particularly fairness creams. Crocin, an apocarotenoid is a major component in saffron stigmas. When examining the orange-colored tubular calyx of *Nyctanthes arbor-tristis* Lin., Gadgoli et al. noticed that the same component, crocin, is present in the saffron stigma. This observation was later validated by

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structural elucidation. (Gadgoli *et al*,2010)

Hence flowers of *Nyctanthes arbor-tristis*were investigated in detail for neuroprotective utilities tosubstitute crocin of *Crocus sativus* stigmas. Flowers being readily available in India, can be explored for phytochemical and pharmacological investigations.

Materials and Methods

Plant Material collection, authentication and extraction

The fresh flowers of *Nyctanthes arbor-tristis* were collected from the local area in the early morning, flowers were cleaned manually and dried under shadow and were used for the study. The crude drug was authenticated at Guru Nanak Khalsa College, Matunga, and Mumbai.

The dried flowers were extracted by macerating with ethanol in dark for 6-8 hrs at room temperature. The ethanolic extract was evaporated to syrupy consistency in the rotary flash evaporator and dried under a vacuum oven at a temperature not exceeding 45^{o}C. (Bhuskat *et al*, 2007). Ethanolic extract was fractionated using different solvents viz hexane, chloroform and ethyl acetate.

Animals

Male Albino Wistar rats weighing 180-300 g were purchased from the National Institute of Biosciences in Bhor, Pune, and kept under controlled conditions at 24 ^{o}C temperature, 60% humidity, and 12/12 h light/dark cycle with food and water *ad libitum*. The Gahlot Institute of Pharmacy's Animal Ethics Committee authorized the experimental methods, which adhere to the Compendium of CPCSEA, New Delhi, 2018, and were registered under registration number 1485/PO/Re/S/11/CPCSEA and proposal number GIP/IAEC/2021/13/2.

Chemicals

Aluminium chloride, Bovine Serum Albumin, Thiobarbituricacid, DTNB were purchased from Sigma - Aldrich, Mumbai, India. All other chemicals used were of analytical grade.

Dosage

In the $AlCl_3$- induced experimental model of AD, two different doses of fraction of*Nyctanthes arbor-tristis*flowers (NAF) (100 and 200 mg/kg) were used. It was observed that oral administration of $AlCl_3$ for 42 days caused memory dysfunction

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which was confirmed by behavioural studies using Morris water maze test,elevated plus maze testand open field test.

Experimental Design

For oral administration, the solutions of aluminium chloride and all drugs under evaluation were made freshly at the beginning of each experiment and ingested total volume is 0.5 mL/100 g body weight. Aluminium chloride dissolved in sterile water, administered orally at a dose of 100 mg/kg daily for 42 days to all groups excluding Group-I. Animals were divided into the following groups (each of 7 rats); as follows: Group I (Control): Included normal healthy rats that received vehicle P.O. (Saline) 0.5 mL/100 g body weight to be given daily for 42 days.

All the groups from II to IV will receive respective doses 30 minutes before oral administration of Aluminium chloride (100 mg/kg), daily for 42 days.

Group II (Untreated AD): Vehicle P.O. (Saline) 0.5 mL/100 g body weight.

Group III (NAF 100 mg/kg): Oral dose of NAF 100 mg/kg.

Group IV (NAF 200 mg/kg): Oral dose of NAF 200 mg/kg

On day 43 rats were sacrificed, brains were excised and used for the biochemical estimation.

Biochemical assessment

Measurement of brain Lipid peroxidation (Sridharamurthy et al,2012)(ohkawa *et al*, 1979).The level of Lipid peroxides was estimated by the Thiobarbituric acid (TBA) reaction method described by Ohkawaet al. The TBAtestis often saidtomeasuremalondialdehyde (MDA)formedin peroxidizinglipid systems. So, the results are expressed as nmoles/ mg protein.

Estimation of brain Nitrite (Green *et al*,1982) (Granger *et al*,1995)

Nitrite produced by spontaneous oxidation of NO under physiological conditions is detected using spectrophotometry and the Griess diazotization process. This method has a 1.0 M nitrite detection limit. The Griess reaction can also be used to investigate nitrate by catalytically reducing it to nitrite. In an acid solution, nitrite combines with Sulfanilic acidto quantitatively change it into a diazonium salt. The diazonium salt is then joined with N-(1-naphthyl) ethylenediamine to create an azo dye, whose absorbance at 548 nm may be quantitated spectrophotometrically.

Read nitrite concentrations corresponding to the absorbance of experimental samples from the standard plot**,** results are expressed as nmoles/ mg protein.

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Estimation of brain Reduced glutathione (Ellman,1959)

Glutathione reduction is determined by the technique of Elliman. When glutathione reacts with DTNB, a yellow chromophore is produced that may be detected spectrophotometrically at 412 nm. The GSH concentrations in the sample was calculated using the GSH standard curve in ug/mg protein.

Estimation of brain Superoxide dismutase (SOD) (Paoletti *et al*,1986)

As an initial line of defense against free radical damage, superoxide dismutase scavenges the superoxide (O*2). Superoxide anion (O*2) is dismutated into hydrogen peroxide and molecular oxygen by a family of metalloenzymes called SOD. SOD measurement was carried out on the ability of SOD to inhibit the spontaneous oxidation of epinephrine to adrenochrome.Units (U) of SOD activity per mg protein are used to express the results.

Estimation of catalase (CAT) (Beers *et al*, 1952)

H_2O_2 exhibits a continuous increase in absorption with decreasing wavelength in the U.V. range. The fast conversion of hydrogen peroxide to water is catalyzed by catalase. The drop in absorbance at 240 nm can be readily followed by the decomposition of H_2O_2. The amount of catalase activity is determined by the difference in absorbance per unit.

Estimation of brain Glutathione S-transferase (Habig *et al*,1974)

Glutathione S-transferases initiate the detoxication of alkylating agents by catalyzing their interaction with glutathione's -SH grouptherebyneutralizing their electrophilic sites and rendering the products more water-soluble. The final product, mercapturic acid, is assumed to be produced by further metabolizing glutathione conjugates by cleaving the glutamate and glycine residues, then acetylating the resulting free amino group of the cysteinyl residue. A unit of activity is defined as the amount of enzyme catalyzing the formation of 1μmole of product per min under the conditions of the specific assay.

Estimation of brain acetylcholinesterase (Ellman *et al*, 1961)

George Ellman created the approach for estimating AchE activity in 1961; it is commonly referred to as Ellman's method. Thiocholine is formed due to the breakdown

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ofacetylcholine iodide by AChE. Thiocholine is then permitted to react with the SH reagent 5, 5-dithio-bis-(2, nitrobenzoic acid) (DTNB), which is reduced to thionitrobenzoic acid, a yellow anion with an absorption peak at 412 nm. Thionitrobenzoic acid has an extinction coefficient of 1.36 $x10^4$ molar/cm. UV spectrophotometer was used to determine the amount of thionitrobenzoic acid formed and used as a direct indicator of the AChE activity, expressed in nmoles/min/gram.

Estimation of brain total proteins (Modified Biuret, End Point Assay) (Gornall *et al.*,1949)

The peptide bonds of proteins react with cupric ions in an alkaline solution to form a coloured chelate, whose absorbance is measured at 578 nm. Sodium-Potassium Tartrate, a component of the Biuret Reagent, aids in preserving the solubility of this complex at an alkaline pH. The absorbance of the final colour is proportional to the concentration of Total Protein in the Sample in g/dL.

Estimation of brain Aluminium (Zumkley*et al*,,1979)

The cortex and hippocampus of the brain were used to examine the aluminium using Zumkley's wet acid digestion procedure. The tissue was treated with 2.5 ml of a perchloric acid/nitric acid (1:4 by volume) solution before being submerged in a sand bath at 40°C to 50°C for 44 hours until a white ash or residue was produced. Then, 2.5 ml of 10 mM nitric acid was used to dissolve the residues. This sample was put in the sample holder of an atomic absorption spectrophotometer (in liquid form). The total aluminium content was calculated in μg/g of tissue.

Behavioral studies

Open Field Test(Thenmozhi *et al*,2015)

This has walls with 36 cm height, was made of white plywood, and measured 72 by 72 cm. Since one of the walls was made of transparent Plexiglas, rats could be seen inside the wooden box. The clear Plexiglas floor allowed for visibility of the green lines that had been marked with a marker on the ground. The floor was split into sixteen 18 × 18 cm squares by the lines. The open field had a central square (18 cm x 18 cm) drawn in the centre of it. Because some rat strains exhibit great locomotor activity and frequently cross the lines of the test chamber during a test session, the middle square is used. Additionally, the central square has enough room around it to indicate that it is different from the neighbouring areas. Rats were placed in the open field's middle or one of its

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corners, and they were free to explore the equipment for five minutes. The number of times each rat explored the wall's 12 nearby outer squares and the centre square was counted individually. Numerous grooming behaviours, such as licking the fur, washing the face, or scratching. For five minutes, the number of rearing, or standing on the hind limbs and occasionally leaning on the wall with the forelegs, smelling, and looking around, was noted. Rats were put back in their cages after the five-minute test, and the open field was wiped with 70% ethyl alcohol and left to dry in between experiments.

Rats were repetitively subjected to OFA for evaluation of gross behavioral activity on day 20, 21 and 42 starting from oral administration of Aluminium chloride.

Assessment of cognitive performance by the Morris water maze task-(kumar *et al*,2011)

The ability to memory acquisition and retention was tested using the Morris water maze. Two threads fixed at right angles to one another divided a large circular pool (150 cm in diameter, 45 cm in height, filled to a depth of 30 cm with water at 28±1°C), which served as the basis of the Morris water maze, into four equal quadrants. The pool was located in a room with plenty of coloured light cues and good lighting. These extraneous cues act as the reference memory and endure throughout the research. A circular platform with a diameter of 4.5 cm was placed in one pool quadrant during the acquisition phase, 1 cm above the water. The same platform was positioned 1 cm below the water's surface for the retention phase. During the evaluation of the two phases, the platform's position was not altered in any quadrant. Each animal underwent four consecutive trials, each separated by five minutes. Each trial's drop point was a different quadrant of the pool, and the animal was gently released into the water there. Then, the animal had 120 seconds to find the platform. The animal was then given 20 seconds to remain on the platform.If the animal failed to reach the platform within 120 seconds, it was guided to the platform and allowed to remain there for 20 seconds.

Maze acquisition phase (training)-

Animals received two consecutive daily training sessions. Each rat was placed into the water during the acquisition phase in one of four beginning positions, the order of which was chosen at random. The latency to reach the visual platform (acquisition latency) was measured.

Maze retention phase (testing for retention of the learned task)-

Animals were tested twice after the acquisition phase; to evaluate memory retention, each animal was released randomly from one of the pool's sides facing the wall. Time

latency to find the hidden platform first time was recorded and termed the first retention latency (1st RL) and the second time as second retention latency (2nd RL), respectively.

Rats were subjected to acquisition latency on day 19 & 20, and first & second retention latency on day 21 and 42 starting from oral administration of Aluminium chloride.

Elevated plus maze-(Kumar *et al*, 2008**)**

The elevated plus maze had two opposite open arms (50 × 10 cm) that were crossed by two closed arms of the same size but walls that were 40 cm high. The arms are connected with a central square (10 × 10 cm). After the administration of Al, memory acquisition was evaluated on day 20. Individual rats were positioned at the end of an open arm that was facing away from the center square. Initial transfer latency was measured as the amount of time it took to transition from an open arm to one of the closed arms (ITL). After recording ITL, animals were allowed to explore the maze for five minutes before being brought back to their original cage. By positioning a rat identically on an open arm, the retention of memory was evaluated. After recording the initial transfer latency, the retention latency was documented twice and is referred to as the first RTL and second RTL, respectively.Retention of memory was assessed by placing a rat similarly on an open arm and retention latency was noted twice after initial transfer latency and termed as first retention transfer latency (1st RTL) and second retention transfer latency (2nd RTL), respectively.

Rats were subjected to initial transfer latency on day 20, and first & second retention transfer latency on day 21 and 42 starting from oral administration of Aluminium chloride.

Brain histology

Rats were anesthetized using ketamine and xylazine before being decapitated and sacrificed. The brains were removed and put into a 10% formalin solution to preserve for 24 hours. We used a microtome to create coronal slices with a thickness of 5 µm following conventional paraffin processing. The sections had been stained with 1% cresyl violet.

Statistical Analysis

Values are expressed as the mean ± SD. The behavioral assessment data and biochemical estimations were analyzed using GraphPad Prism (version 9) by one-way

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analysis of variance (ANOVA) followed by Dunnett test. $p < 0.05$ was considered significant.

Results

Effect of NAF on biochemical parameters in whole brains of rats treated with aluminium chloride

In comparison to control rats, chronic treatment of aluminium chloride dramatically increased MDA and nitrite concentration, depleted reduced GSH, and decreased glutathione S-transferase, superoxide dismutase, catalase, and total protein levels in the whole brain ($p<0.05$). In contrast to untreated AD rats, chronic NAF (100 and 200 mg/kg) administration to the rats considerably reduced oxidative damage (as shown by decreases in MDA, nitrite concentrationand increases in reduced GSH, glutathione S-transferase, superoxide dismutase, and catalase activities). (**Table 1**).

Table 1: Effect NAF on lipid peroxidation, nitrite, reduced glutathione, glutathione S-transferase, superoxide dismutase, catalase activity and total protein in whole brains of rats treated with aluminium chloride

Groups	MDA (nmol MDA/ min/mg protein)	Nitrite (µmol/ mg)	GSH (µg/g)	SOD (unit/ mg)	Catalase (units/mg protein)	GST (µmol/ ml/min)	Total protein (g/dl)
Control	4.84**** ± 0.13	243.9**** ± 6.1	132.17**** ± 1.96	44.69**** ± 1.75	2.67**** ± 0.04	98.09**** ± 4.70	5.68**** ± 0.07
Untreated AD	8.00 ± 0.08	633.4 ± 25.0	43.66 ± 0.94	14.90 ± 0.66	1.38 ± 0.03	33.68 ± 1.80	1.69 ± 0.06
NAF 100	7.66*** ± 0.10	446.5**** ± 5.7	92.57**** ± 1.10	22.27**** ± 1.36	1.89**** ± 0.03	60.42**** ± 1.88	2.53**** ± 0.06
NAF 200	6.12**** ± 0.12	290.4**** ± 5.6	113.53**** ± 0.60	37.73**** ± 0.52	2.06**** ± 0.02	82.99**** ± 2.10	3.70**** ± 0.10

Effect of NAF on acetylcholinesterase (AChE) activity in aluminium chloride treated rats

When compared to control rats, chronic aluminium chloride therapy greatly raised the AChE activity throughout the entire brain. Chronic NAF administration (100 and 200 mg/kg) to the rats significantly decreased AChE activity, as compared to untreated rats ($p<0.05$). (**Fig.1**).

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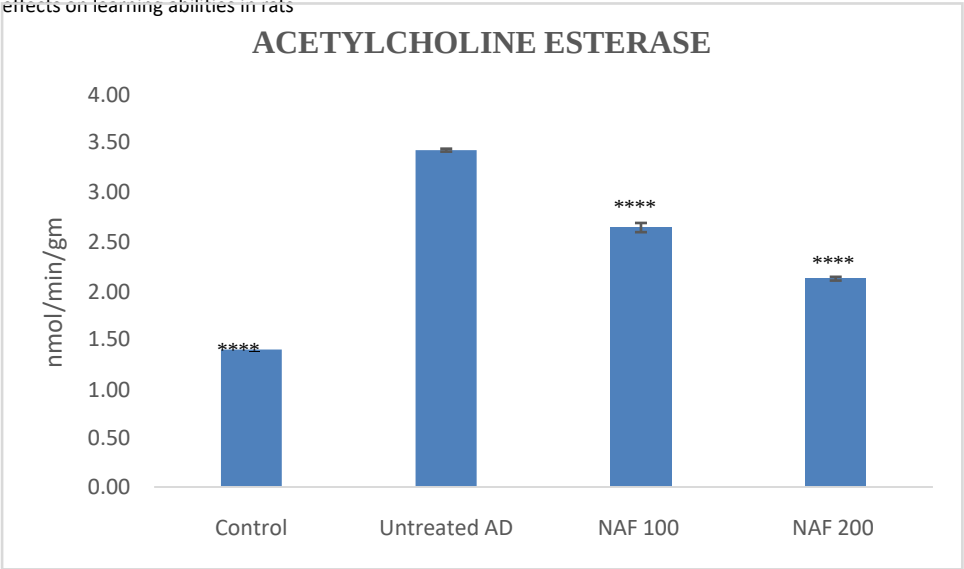

Fig. 1: Effect of NAF on acetylcholinesterase (AChE) activity in aluminium chloride-induced Alzheimer's Disease in rats.

Effect of NAF on aluminium concentration in aluminium chloride treated rats

Rats receiving an aluminium chloride therapy had considerably more aluminium in their cortex and hippocampus as compared to control. But compared to untreated rats, chronic NAF (100 and 200 mg/kg) therapy dramatically reduced the concentration of aluminium in the cortex and hippocampus. ($p < 0.05$) (**Fig. 2**).

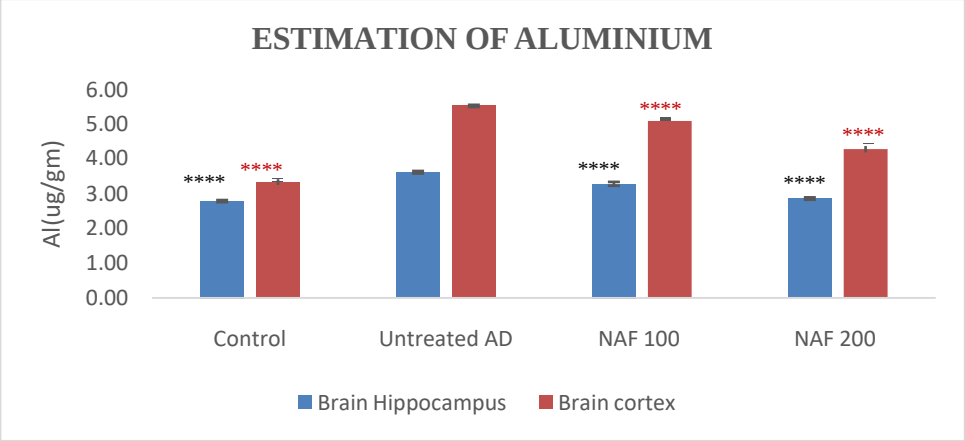

Fig.2: Effect of NAF on aluminium concentration in aluminium chloride- induced Alzheimer's Disease in rats.

Effect of NAF on Movement and activities of animals using open field-testing aluminium chloride-induced rats

Peripheral and central movements, as well as rearing and grooming behaviours, were significantly reduced ($P<0.05$) in the $AlCl_3$ group. However, the oral administration of

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NAF (each 100 and 200 mg/kg) to AlCl3-treated rats showed a significant increase in movement and activities (P<0.05) compared to the $AlCl_3$ group. (**Fig. 3 and 4**).

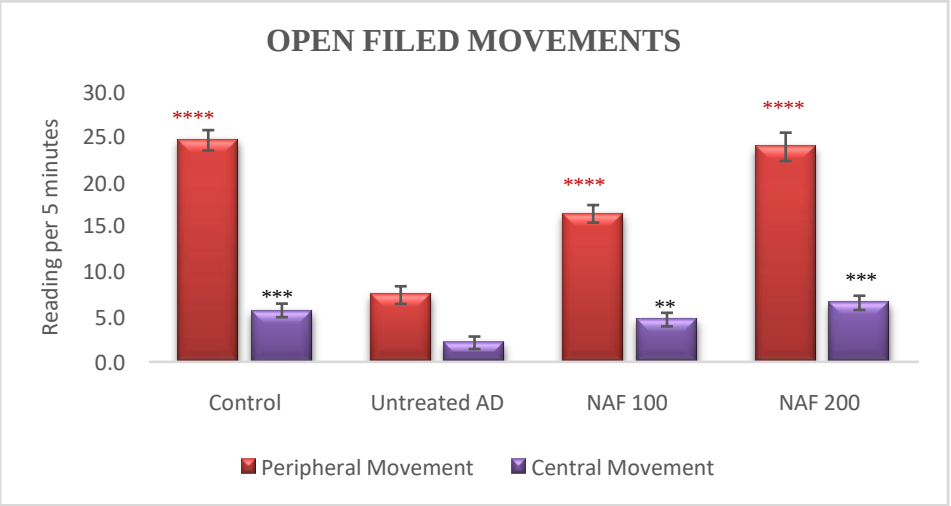

Fig.3 Effect of NAF on Movement of animals using open field test in aluminium chloride-inducedAlzheimer's Disease in rats..

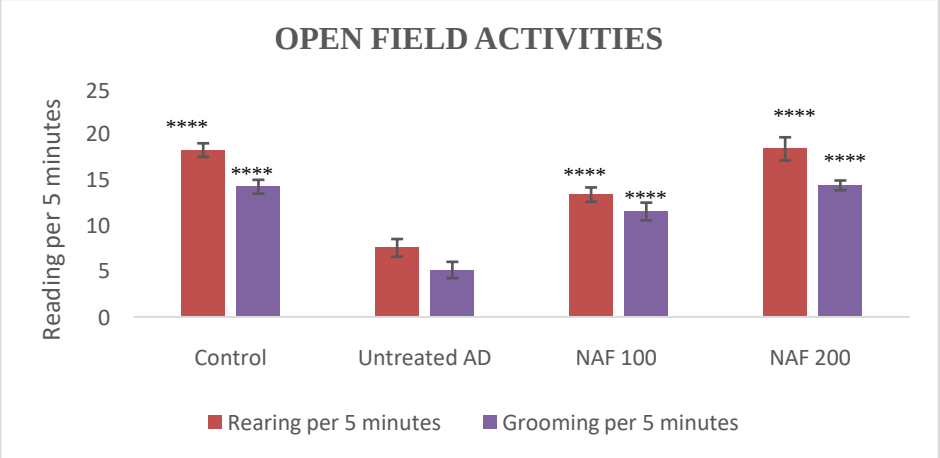

Fig.4 Effect of NAF on activities of animals using open field test in aluminium chloride- inducedAlzheimer's Disease rats.

Effect of NAF on memory performance in the Morris water maze task in aluminium chchloride-induced rats

In comparison to the control group, aluminium chloride-treated rats dramatically increased the acquisition latency to reach the visual platform, showing memory impairments. NAF treatment significantly improved this memory performance (i.e., shortened average acquisition latency) on day 19 and 20 ($p < 0.05$) in the aluminium chloride treated group. The visual platform was hidden after training. Aluminium

chloride induced group (Untreated) was then found to significantly delay average acquisition latency (on day 20) and retention latencies (1st and 2nd RL on day 21 and 42, respectively) to escape onto the hidden platform compared to the control group. These findings revealed that aluminium chloride significantly impaired cognitive function. Furthermore, compared to untreated rats, chronic NAF administration (each 100 and 200 mg/kg) significantly boosted memory retention for the first and second RL on days 21 and 42, respectively. (**Fig. 5**).

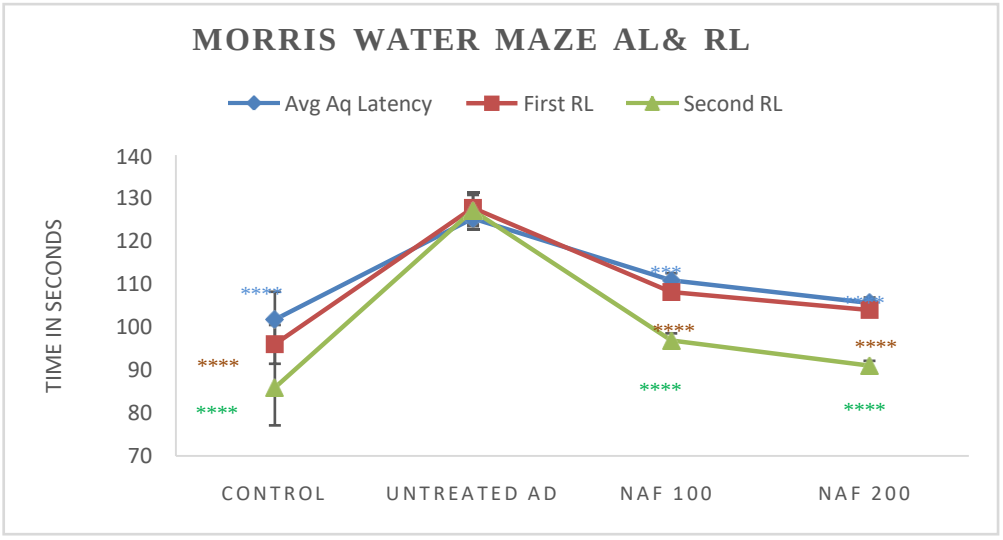

Fig.5 Effect of NAF on memory performance in the Morris water maze task in aluminium chloride-inducedAlzheimer's Disease in rats..

Effect of NAF on memory performance in the elevated plus maze in aluminium chloride-induced rats

On day 20, each rat's ITL was comparatively constant and didn't exhibit any appreciable fluctuations. Within 60 seconds, every rat entered the arm that was closed. Following training, rats in the Control, Untreated AD (Aluminum Chloride-induced), and chronic NAF treatment (each 100 and 200 mg/kg) groups quickly entered the closed arm, and the retention transfer latencies (first RTL and second RTL) to achieve this on days 21 and 42, respectively, were shorter than the ITL on day 20 for each group.

In contrast, Aluminium chloride induced rats performed poorly throughout the experiment and did not show any change in the retention transfer latencies on days 21 and 42 compared with the ITL on day 20, demonstrating that Aluminium chloride induces a marked memory impairment. Chronic administration of NAF treatment (each 100 and 200 mg/kg) 30 minutes before the Aluminium chloride administration

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significantly decreased the retention latencies on days 21 and 42 following Aluminium chloride administration ($p < 0.05$ as compared to Untreated AD group)(**Fig. 6**).

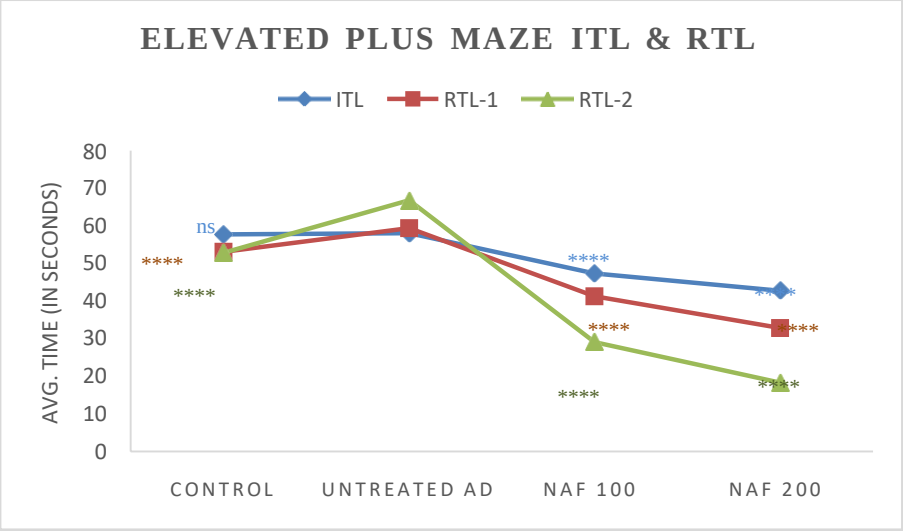

Fig.6: Effect of NAF on memory performance in the elevated plus maze in aluminium chloride-induced Alzheimer's Disease in rats.

Histopathology study of rat brain

As shown in Fig. 7, the histopathological evaluation of the brain of rats in the control group (fig.7.a) exhibited nneuronal integrity, and normal neuroglial cells arranged in several layers. While histology of the brain of untreated AD rats (fig.7.b) showed moderate to severe neuronal degeneration and proliferation of glial cells along with moderate chromatolysis. In the NAF treated groups exhibited moderate proliferation of glial cells in the brain section of rats of NAF (100 mg/kg,p.o.) (fig. 7.c) and high dose treated group i.e. NAF (200 mg/kg,p.o.) showed mild proliferation of glial cells and mild chromatolysis along with no lesions (fig. 7.d).

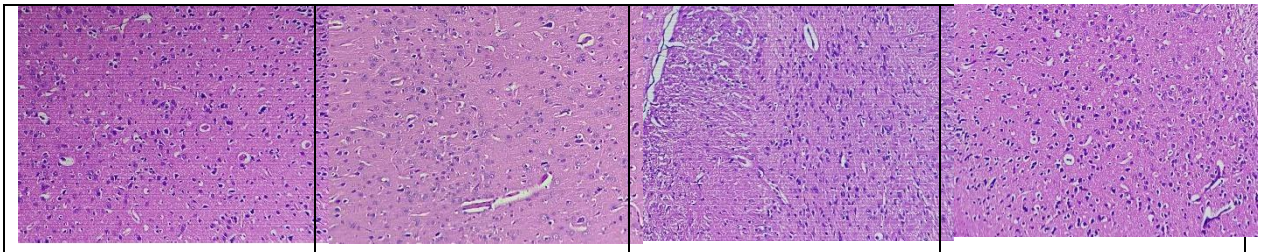

Fig.7.a. control	Fig.7.b. Untreated AD	Fig.7.c.NAF 100 mg/kg	Fig.7.d.NAF 200 mg/kg

Figure 7: Effect of NAF (100 and 200 mg/kg) on brain histopathology of Aluminium chloride-induced Alzheimer's Disease in rats.

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Discussion

A common metal, aluminium has been linked to the development of cognitive dysfunction and neurodegenerative diseases. It worsens brain oxidative damage, produces neuronal inflammation, and impairs working memory, visuoperception, attention, and semantic memory (Kumar *et al*, 2009; Platt *et al*, 2001). Additionally, aluminium modifies the function of the blood-brain barrier and cholinergic and noradrenergic neurotransmission (Yokel *et al*, 2000). It alters phosphoinositide metabolism, protein phosphorylation, lipid peroxidation, free-radical production, reduced glucose uptake, and phosphoinositide metabolism, resulting in severe neurotoxicity.

Our results showed that oral administration of $AlCl_3$ for 42 days caused a significant and greater aluminium accumulation in the hippocampus and cortex (Sakamoto *et al*, 2004;Abubakar *et al*,2004).

Specific high-affinity transferrin receptors (TfR) present in the blood-brain barrier allow aluminium to enter the brain (Roskms *et al*, 1990). It accumulated in all of the rat brain's areas, with the greatest amount in the hippocampus, the area responsible for memory and learning **(**kaur*et al*, 2006). Additionally, Crapper et al. noted that AD patients' brains had an increased Al concentration (crapper *et al*,1973).The present study examined the therapeutic potential of NAF in the prevention of the accumulation of $AlCl_3$.

The increased antioxidant enzyme levels associated with significant protection against oxidative stress may be the mechanism responsible for memory improvement and motor activity in this model.

In addition, brain AChE activity was also inhibited by NAF treatment. The observationsupports that modulation of cholinergic neurotransmission is involved in the improvement of memory function.

Administration of aluminium chloride resulted in progressive deterioration of spatial memory as determined by the Morris water maze, elevated plus maze which was regained due to treatment of NAF.

Therefore, the results of the current study show that NAF enhances behavioural activity, as measured by the OFA testand biochemical function in the brains of rats who have been exposed to aluminium; this effect may be partially attributed to NAF's antioxidant characteristics. However, additional cellular research is necessary to

eISSN 1303-5150

comprehend how NAF affects oxidative stress in many experimental systems.

Conclusions

In conclusion, the present study highlights that the Ethyl acetate fraction of *Nyctanthes arbor-tristis* treatment attenuated aluminium chloride-induced aluminium loading, cholinergic deficit and memory loss. However further research can be taken up to confirm the precise mechanism of action of NAF against Al-induced neurotoxicity.

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