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ARTICLE

Inflammation Versus Oxidative Stress in Pathophysiology of Alzheimer's Disease in Rat Model

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Abstract

Background: Alzheimer's disease (AD) is a highly debilitating neurodegenerative disorder characterized by cognitive dysfunction. Inflammation and oxidative stress are thought to play major roles in the pathophysiology. Which one has the principle role is unclear. **Objectives:** The role of brain growth factors, cytokines and oxidative biomarkers in cognitive dysfunction induced by Aluminium chloride ($AlCl_3$) in rats with application of an anti-inflammatory (Cilostazol) and an antioxidant (N-acetyl cysteine, NAC) were investigated to clarify the predominant pathophysiological mechanism involved. **Methods:** Alzheimer's model group was given $AlCl_3$ (100 mg/kg) orally for six weeks. Alzheimer's model + NAC, and Alzheimer's model + Cilostazol groups were given (NAC) and Cilostazol respectively one hour before $AlCl_3$ for the same duration. **Results:** Anti-inflammatory or antioxidant interventions significantly improved memory retention, which was evaluated by Morris Water Maze, passive avoidance task, and eight-arm radial maze. This improvement was consistent with histological recovery and was mediated by reduction $AlCl_3$ concentration in the brain hippocampus and frontal cortex, interference with the cholinergic dysfunction, as well as prevention of oxidative damage. In addition, anti-inflammatory agents can modulate superiorly the inflammatory response via reduction of the levels of inflammatory cytokines and adjustment of the levels of brain–derived neurotrophic factors and transforming growth factor B. **Conclusions:** These finding support the principal role of inflammation in pathophysiology of AD and suggests the potential therapeutic application of anti-inflammatory agents for this condition.

Key words: Alzheimer's disease, Cilostazol, N-acetyl cysteine, NAC.

Introduction

Alzheimer's disease is the most common form of dementia in the elderly. It affects more than 30 million people worldwide. The number of affected people is expected to double within the next 20 years (1). Aluminum (Al) is one of the most abundant metals in the earth's crust. Daily exposure to Al occurs mainly by exposure to food products and drinking water, with multi-detrimental effects throughout the body, such as dialysis encephalopathy, osteomalacia and microcytic anemia (2). Moreover, numerous studies have observed that concentrations of aluminum are elevated in the brains of patients suffering from senile dementia of Alzheimer's type (3). $AlCl_3$ generates excessive free radicals and thereby is implicated in the etiology of AD (4). Nevertheless, the core mechanism of $AlCl_3$'s damaging effect is still debated. The frontal cortex and hippocampus, areas of the brain (important for learning, memory and mood) are highly vulnerable to normal aging and AD (5).

The anti-inflammatory agent Cilostazol inhibits phosphodiesterase III with a therapeutic focus on increasing cAMP that increases the active form of Protein Kinase A, which is directly related to inhibition of platelet aggregation (6). Moreover, Cilostazol induces inhibition of inflammatory cytokine release from macrophages (7), and reduced the cell damage caused by oxidative stress (8). However, the ameliorative effect of Cilostazol on the learning and memory impairment has not been studied, and its mechanism remains unclear.

Several antioxidants are used for therapy of neurodegenerative disorders. Antioxidant agent, N-acetylcysteine (NAC) is a precursor of glutathione which plays an essential role in oxidative damage (9). Also, NAC could attenuate neuroinflammation (10). Based on this finding, the current investigation was designed to evaluate the neuroprotective effect of NAC against aluminum-induced cognitive impairment. Transforming growth factor ß (TGF-ß) plays an important role in brain response to injurious agents (11). Many studies reported increased TGF-ß associated with a reduction of brain derived neurotrophic factors (BDNF) in cases of cortical dysfunction of AD patients (11). Elucidating the pathophysiological mechanisms underlying in AD will be an attractive and necessary path to identify efficacious strategies that can ultimately lead to reduced incidence of this disease. In this study, we used $AlCl_3$-exposed rat as a model of AD to examine the pathophysiological mechanisms by which the anti-inflammatory agent (Cilostazol) and antioxidant agent (NAC) exert their effects on learning and memory impairment in order to prove the predominant mechanism involved. The study also highlights the relationship between brain growth factors and cytokines to cognitive impairment. By using behavioral tests, biochemical assays, and histological study, we compared the effects of anti-inflammatory agents on learning and memory impairment induced by $AlCl_3$ with those of antioxidant agen ts.

Material and methods

Chemicals

$AlCl_3$, NAC (Aldrich Chemicals Co., Sigma Chemicals Co. St Louis, MO, USA) and Cilostazol (Pharmatic Biotech, Sweden). Their solutions were freshly made at the beginning of each experiment. For oral administration, $AlCl_3$ was dissolved in distilled water (DW) while NAC and Cilostazol were suspended in 0.5% carboxymethyl cellulose sodium salt (Aldrich Chemicals Co.).

Animals

Adult male albino rats weighing 100-150 g at the start of the study were used. They were disease-free, obtained and maintained in the Assiut University Animal Nutrition and Care House, and were acclimatized to the laboratory conditions at room temperature before the experiments. The rats were housed in metal cages (5 per cage) and they were allowed free access to rodent laboratory food and water throughout the experiment. The housing facility was maintained at 25 °C with a 12 hour light, 12 hour dark cycle under a controlled humidity environment. The animals were carried out and all manipulations were done in the light phase between 0900 and 1600. The experimental protocol was approved by the Institutional Animal Research Committee of the Faculty of Medicine, Assiut University, Egypt. It was carried out in accordance with the published guidelines and regulations for the use and care of animals.

Treatment protocols

The animals were equally alienated into six groups of ten animals each. In group I (control DW), II (control antioxidant) and III (control anti-inflammatory), rats were given distilled water orally, 100 mg NAC/kg^{-1} b.w./ day IP and 30 mg Cilostazol /kg^{-1} b.w./day orally respectively for six weeks. Animals of group IV (Alzheimer's model) were given C (100 mg/kg^{-1} b.w.) through oral gavages for the same duration, while those of group V (Alzheimer's model + antioxidant) and group VI (Alzheimer's model + anti-inflammatory) were given the same dose of NAC and

Cilostazol one hour before administration of $AlCl_3$ for the same duration. The doses of $AlCl_3$, NAC and Cilostazol were selected based on those reported in the literatures (12-14 respectively). $AlCl_3$, NAC and Cilostazol solutions were freshly prepared before administration. Drugs were given daily at 0800 while behavioral tests were performed at 1100.

Laboratory methods

Body weight: All animals were weighed at the beginning (baseline) and at the end of the experiment (0900).

Learning and memory behavioral tests: The ability to navigate and complete a maze task requires the ability to create and store a cognitive map. AD affects the mechanisms by which visuospatial memory is formed and recalled in a spatial navigation task. The learning and memory tests were performed on day 0 (before start of the experiment) and on days 1, 15, 30 and 45 following $AlCl_3$ administration. The tests included:

a. Morris water maze test (MWM): evaluates spatial learning and memory in rodents (15).The rats can escape from the water onto the hidden platform after being placed randomly at one of four sites in the pool to test for learning and memory response. Latencies of the four trials /d were recorded and averaged to obtain a measurement for the performance of each animal on a given day.

b. Step-down type passive avoidance task: The method used as described previously (16). Each rat was placed in the illuminated compartment and ten seconds later the door was raised and the latency (initial latency) to enter the dark compartment was noted and upon entry, the door was closed and a foot shock administered (0.3 m A, 3 s), using a shock generator-scrambler (Muromachi-Kikai Co Ltd, , Chuo-ku, Tokyo, Japan). Twenty-four hours after the training trial (day 1 of $AlCl_3$ administration), the rat was again placed in the illuminated chamber and the latency was again noted up to a maximum of 300 seconds (retention trial). The tests were carried out again on day 15, 30 and 45 of $AlCl_3$ administration to record the onset of memory impairment.

c) Eight-arm radial maze: (index of hippocampus-dependent spatial memory). Four fixed arms were baited with food (cheese) in the food plastic cups. Four food pellets were placed just outside the unbaited arms to provide symmetrical food odor all around the maze. After the overnight fast, individual animals were placed in the central area of the maze, timing was begun and the rat was free to discover. Arm choices were recorded after the rat completely entered into the arm, and the trial was judged completed when the rat had chosen all baited arms, or had spent 10 minutes (17). The following parameters were calculated: working memory (the number of repeated entries to baited arms), reference memory (the number of entries to unbaited arms) and completion time (the mean latency required to complete the task).

Biochemical assays

At the end of the experiment the rats were sacrificed by cervical dislocation, the hippocampus and frontal cortex were micro-dissected, washed in isotonic saline, and dried. Each brain sample was mid-sagittally divided into two portions. The first portion was fixed in formalin buffer for histopathological study. The second portion of brain was weighed and homogenized immediately to give 10% (w/v) homogenate in ice-cold medium containing 50 mM Tris-HCl and 300 mM sucrose. The homogenate was centrifuged at 3000 rpm for 10 min. The supernatant (10%) was used for determination of $AlCl_3$ concentration, acetylcholinesterase activity (AChE), lipid peroxidation and nitric oxide, antioxidant enzyme activities (superoxide dismutase activity, catalase, reduced glutathione-GSH) and protein estimation. Also, inflammatory cytokines as IL-2, and TNF-α as well as brain growth factors (TGF-ß & BDNF) were estimated in supernatant. Lipid peroxidation products were measured in tissue homogenates as described previously (18). Nitric oxide in tissue homogenates was determined by evaluating its oxidation products (nitrates and nitrites) using Griess reagent (19). Antioxidant enzymes as superoxide dismutase activity (SOD), reduced glutathione and catalase activity in tissue homogenates were assayed by the methods of Kono, Ellman, and Luck respectively (20-22). Quantitative estimation of total protein concentration in the brain homogenate was carried out to express the concentration of each studied parameter per mg protein (23). Enzyme-linked immunosorbent assays (ELISA) were performed to measure concentrations of TNF-α (Cat. No. KAC 1571), IL-2 (Cat. No. M1916), TGF-ß (Cat No. KEC 2567), and BDNF (Cat. No. M 1712) by using commercial kits [Biosurce Europe, S.A. (Zoning Industriel, Nivelles, Belgium)] with monoclonal antibodies against each substance according to manufacturer's protocol. The apparatus used was Anthos 2000 (Anthos Labtec Instruments, Salzburg, Austria). AChE activity in the brain tissue was measured colorimetrically (24). Aluminum concentration was estimated as described

using atomic absorption spectrophotometer (25). The total concentration of aluminum was calculated in µg/g of tissue.

Histopathological examination: frontal cortex and hippocampus were examined, and the sections were stained with hematoxylin and eosin stain (26), then examined by light-microscope**.**

Data analysis: Statistical analysis was carried out using Graph Pad Prism software (version 4.03). Data were analyzed non-parametrically (due to the small number of animals in the groups and the cut-off time of latency performance). First, to compare the difference within groups the Friedman analysis of variance (ANOVA) followed by Dunn's method was used. Second, to compare the differences between the control and other group, the Mann/Whitney U-test was used. The level of statistical significance was $P < 0.05$ for all statistical evaluations.

Results

Results of control groups

Body weight and survival rate changed non-significantly in control DW animals (table 1). Meanwhile time course monitoring of learning and memory tests revealed that the latencies recorded by MWM (Figure 1a) and the errors of both working and reference memory decreased significantly as well as the completion time shortened progressively from day 0 to day 45 (figure 2). However, retention latencies recorded by step-through passive avoidance at day 1 increased significantly relative to the results of day 0 (figure 1b). Also, biochemical assays of control DW group showed a significant ($P < 0.001$) increase in the levels of LP, catalase, reduced-glutathion, TGF-ß, BDNF and TNF-α while decrease ACh E ($P < 0.05$) in hippocampus relative to that of frontal area as shown in table 2 and figures 3 & 4. In addition, histopathological studies showed normal cellular structure of frontal cortex and hippocampus areas (H&E x400) which confirms the establishing of normal working and reference memories (figure 5, 6). It was observed that administration of an antioxidant or anti-inflammatory alone did not significantly influence any of the previous parameters compared to the control DW rats.

Results of Alzheimer model

A) Body weight, survival rate, and learning and memory tests. Administration of $AlCl_3$ for 45 day significantly reduced body weights and survival rates at the end of experiment (table 1). Similarly, the latencies recorded by step-through passive avoidance decreased significantly after 15 days with progression until a maximum was reached at day 45 (figure 1). Meanwhile, it induced a significant increase in the latencies recorded by MWM on days 30 and 45 (figure 1), the number of errors among days 15, 30 and 45

Table 1. The body weight (grams) and survival rate in the different groups of rats.

Body weight (g)	Control distilled water	Control antioxidant	Control anti-inflammatory	Alzheimer model	Alzheimer + antioxidant	Alzheimer + anti-inflammatory
Initial body weight (g)	138.0 ±3.3 (100%)	139.2±3.2 (100%)	140.4±2.2 (101%)	143 ±1.8 (103%)	143.4±2.1 (104%)	144.2±1.8 (105%)
Final Body Weight (g)	198.5±3.0 (100%)	195.5±2.4 (98.5%)	196.5±2.8 (99%)	184.5±2.6** (93%)	189.1±1.5 (95%)	189.3±1.1 (95%)
Survival rate /total	10 of 10 (100%)	10 of 10 (100%)	10 of 10 (100%)	7 of 10*** (70%)	8 of 10# (80%)	9 of 10### (90%)

Values represent mean±SE and were analyzed by a t -test (n= 10 per group).
*** P < 0.01, *** P < 0.001 relative to control DW, # P < 0.05, # # # P <0.010 versus Alzheimer model. %= percentage difference from control*

Table 2. Effects of antioxidant and anti-inflammatory agents on and brain growth factors and inflammatory cytokines in frontal cortex and hippocampus of Alzheimer model rats.

Animal Groups	Brain growth factors		Brain cytokines	
	BDNF (ng/mg protein)	TGF-ß (pg/mg protein)	IL-2 (pg/mg protein)	TNF-α (pg/mg protein)
Frontal Cortex				
Control DW	0.35±0.02	640.2± 2.71	16.07 ±0.4	7.53±0.16
Control antioxidant	0.38±0.02	641.4±2.69	15.80±0.31	7.13±0.23
Control anti-inflammatory	0.36±0.02	644.6±1.66	15.65±0.38	7.4±0.27
Alzheimer model	0.23±0.01***	657.0±5.25 *	32.70±0.92***	10.11±0.55***
Alzheimer's +Antioxidant	0.25±0.01 (71%)	654.5±4.36 (102%)	31.10±0.77 (193%)	9.83±0.54 (130%)
Alzheimer's + anti-inflammatory	0.30±0.02 (86%) # #	656.5±3.67 (103%)	27.80±1.32 (173%) # #	9.16±0.39 (122%)
Hippocampus				
Control DW	0.44±0.02	742.2±4.31	15.93±0.62	10.62±0.56
Control antioxidant	0.44±0.01	736.2±3.28	16.30±0.41	10.63±0.32
Control anti-inflammatory	0.45±0.02	744.3±3.512	16.47± 0.50	10.37±0.47
Alzheimer model	0.28±0.01***	840.5±3.31***	40.90±1.30***	20.40±0.60***
Alzheimer's +Antioxidant	0.31±0.01 (70%)	832.4±2.72 (112%)	38.60±1.11 (242%)	19.10±0.48 (180%)
Alzheimer's + anti-inflammatory	0.34±0.02 (77%) #	818.6±3.01 (110%) # # #	35.20 ±1.26 (220%) # #	16.09±0.54 (151%) # #

*Values represent means ± SEM (n =10). All parameters were assessed at the end of experiment. Significant levels; *P <0.05, ***P <0.001 versus control DW (Student's t-test), # P <0.05,## P < 0.01, ### P <0.001 versus Alzheimer model group. %= percentage difference from control.*

in working memory and among days 30 and 45 in reference memory. Also, $AlCl_3$ significantly increased the completion time started at day 30 (figure 2).

B) Oxidative biomarkers, AChE activity, aluminum concentration, brain growth factors and inflammatory cytokine: Biochemical assays of frontal cortex and hippocampus of Alzheimer model rats revealed a significant increase of lipid peroxidation products, nitrite concentration (figure 3); AChE activity, the aluminum concentration (figure 4); levels of pro-inflammatory cytokines (IL2 and TNF-α) and TGF-ß (table 2) relative to the control DW group. However, Alzheimer model group showed depletion of reduced

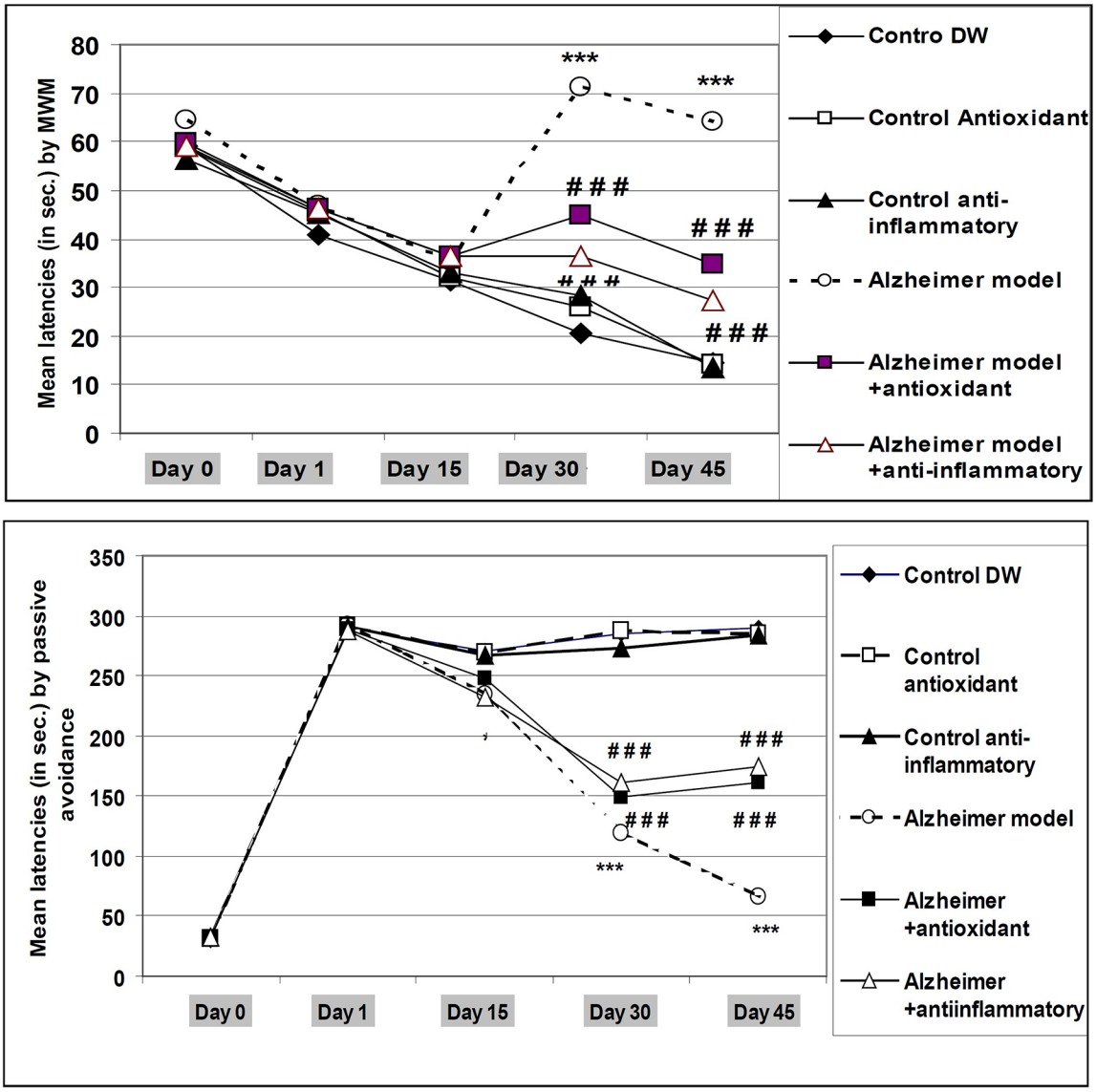


Figure 1. Mean latencies (in sec.) recorded by Morris water maze (MWM) and by passive avoidance in all studied groups. The initial training latencies were measured on day 0 and retention latencies on day 1, 15, 30 and 45 following $AlCl_3$ administration. *P < 0.05 and ***P < 0.001 relative to control DW group. # # #P < 0.001 relative to Alzheimer model group.

glutathione levels, superoxide dismutase, catalase (figure 3), and levels of BDNF (table 2) in both brain areas, with the most obvious effects appearing in the hippocampus area.

C) **Histopathological study**: Gross histopathological changes in the frontal cortex and hippocampus, including heavy loss of cortical neurons, lacunae spaces cells, and vacuolated cytoplasm were observed in Alzheimer model rats (figure 5, 6).

Effects of antioxidant and anti-inflammatory agents on Alzheimer model rats

A) **Body weight, survival rate, learning, and memory tests:** Administration of antioxidant or anti-inflammatory agents significantly increased the survival rate (table 1), latencies recorded by step-through passive avoidance (Figure 1) on day 30 and 45, with better response in the anti-inflammatory group than antioxidant; their percentage differences from control DW on day 45 were 56% and 60% respectively. However, both agents significantly

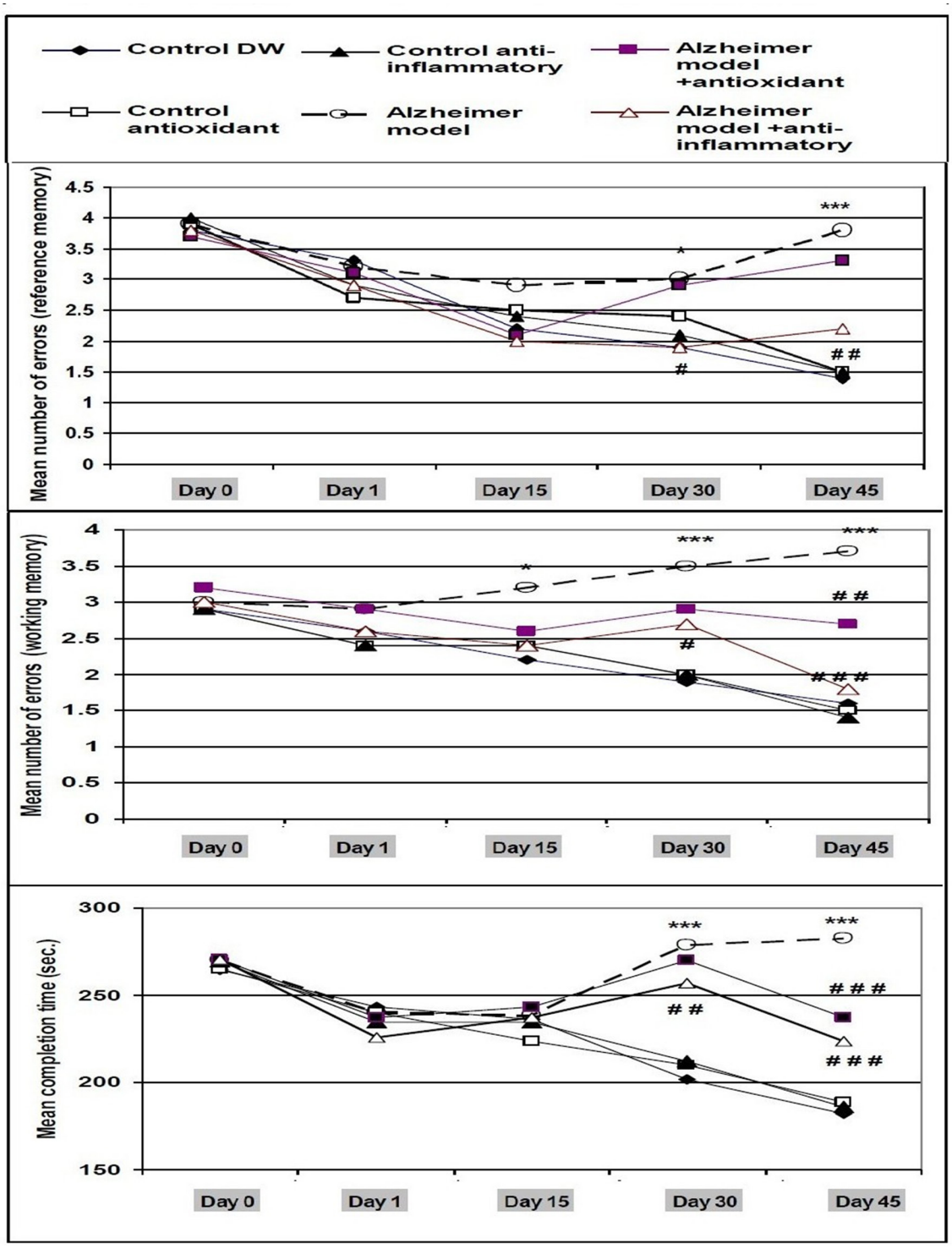


Figure 2. Time course of mean number of errors in measuring working memory (a) & reference memory (b) and the time required to complete the task in radial arm maze (c). Tests were performed at day 0, 1, 15, 30 and 45 following $AlCl_3$ administration. *P < 0.05 and ***P < 0.001 relative to control DW. #P < 0.05, # #P < 0.01 and # # #P < 0.001 relative to Alzheimer model group.

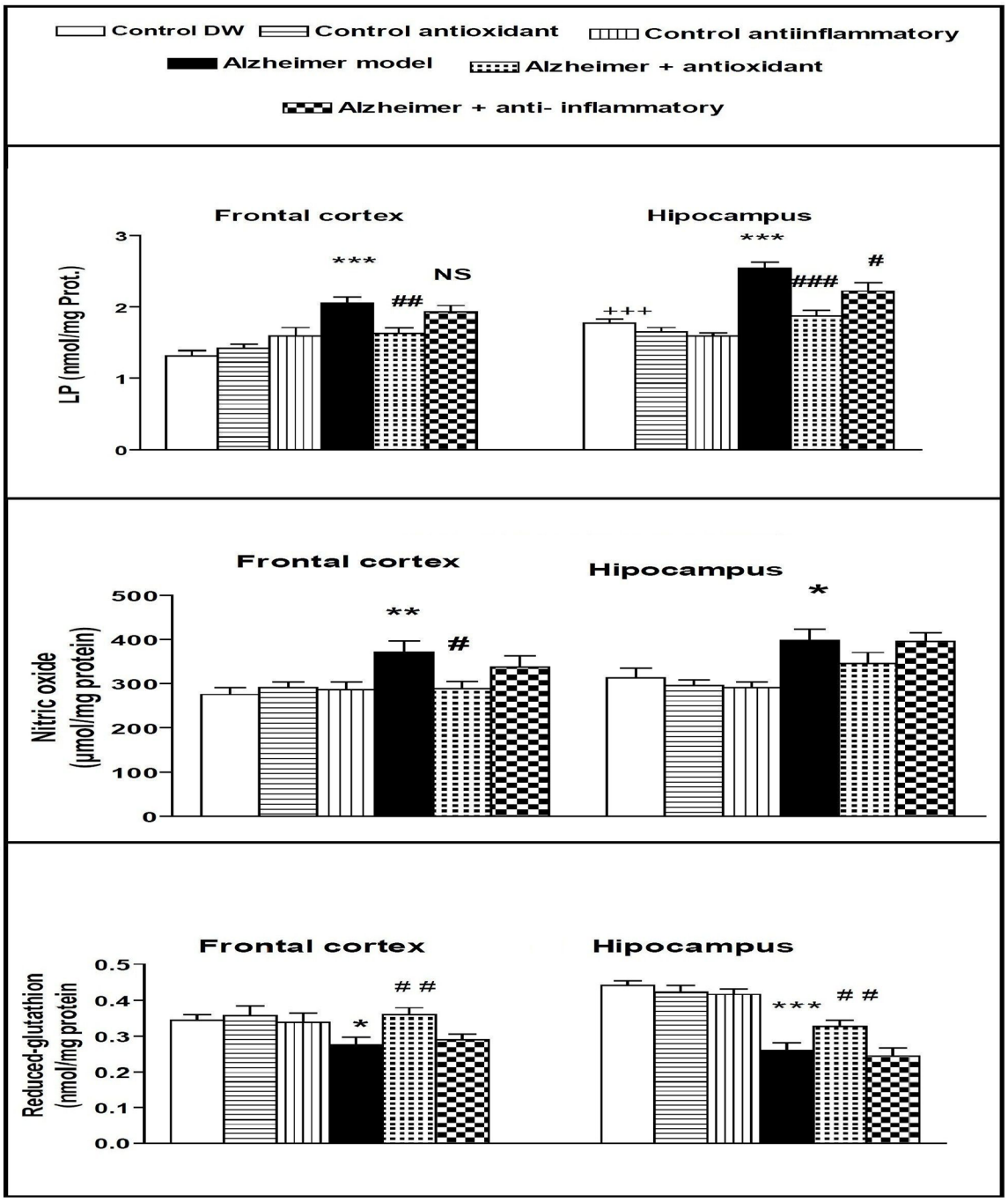


Figure 3. Effects of antioxidant and anti-inflammatory agents on oxidative biomarkers (lipid peroxidation products, nitric oxide, reduced glutathione, Superoxide dismutase, catalase) in frontal cortex and hippocampus of Alzheimer model rats. Values represent means ± SEM (n =10). *P < 0.05, **P < 0.01 and ***P < 0.001 relative to control DW. #P < 0.05, # #P < 0.01 and # # #P < 0.001 relative to Alzheimer model group.

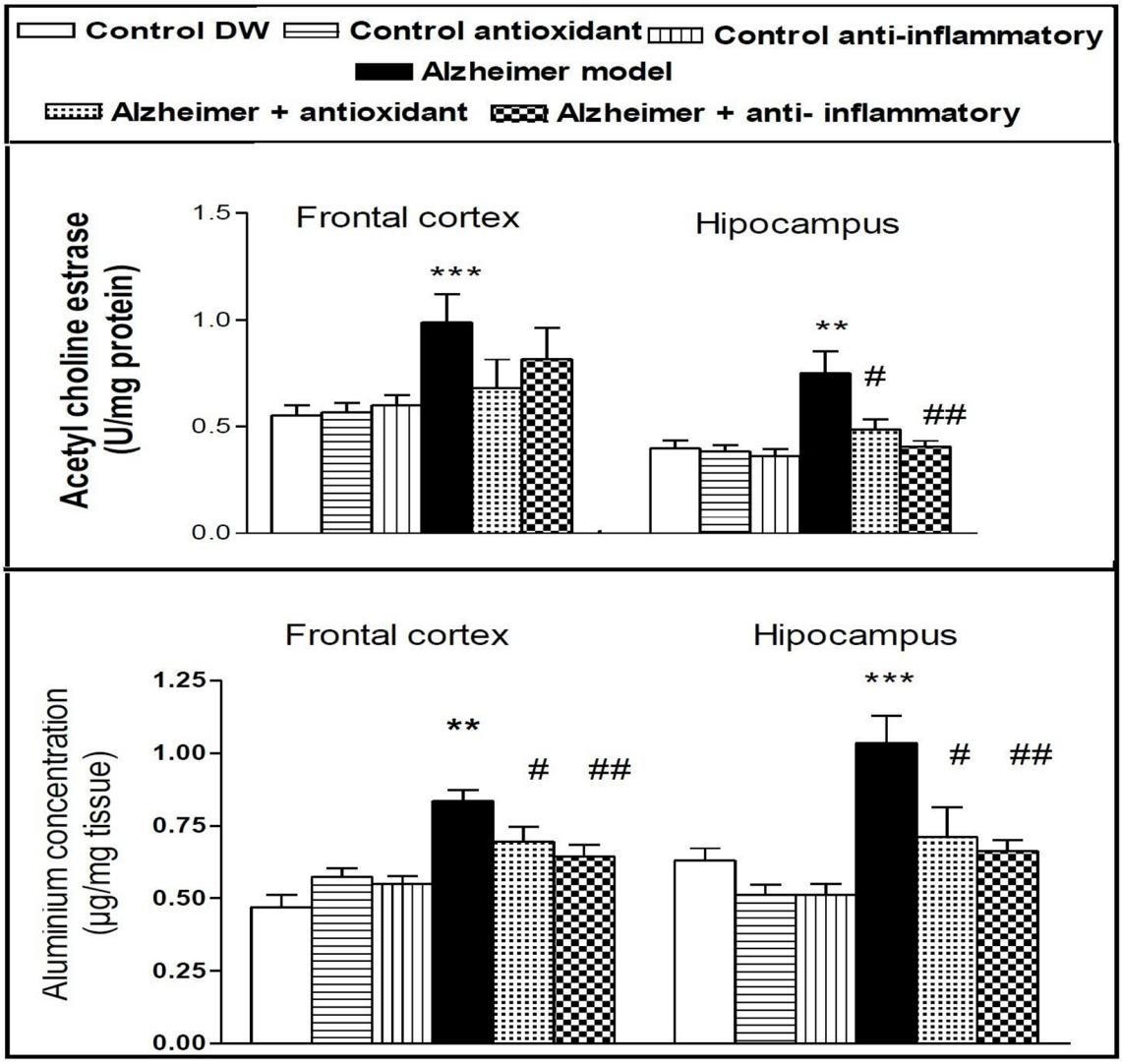
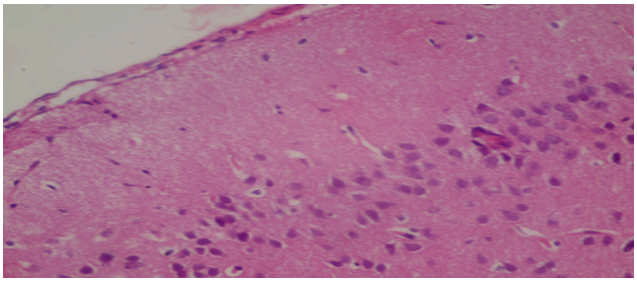


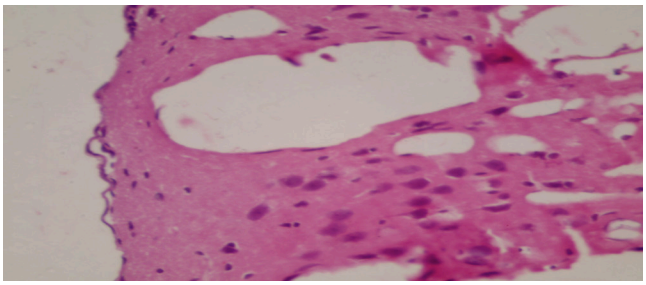
Figure 4. Effects of antioxidant and anti-inflammatory agents on Acetyl choline esterase activity and Aluminun concentration in frontal cortex and hippocampus of Alzheimer model rats. Values represent means ± SEM (n =10). Significant levels; **P< 0.01, ***P < 0.001 versus control (Student's t-test), # P <0.05 and ## P < 0.01 versus $AlCl_3$.

lowered the latencies of MWM (figure 1), the number of errors of working & reference memories, and also the completion time (figure 2). Anti-inflammatory agent has more amelioration effect than antioxidant, and their percentage difference of latencies in MWM from control DW at day 45 were 188% and 239% respectively. The percentage differences from control of anti-inflammatory and antioxidant groups in working, reference memories and completion time at day 45 were 169% &113%, 236% & 157%, and 130% & 123% respectively.

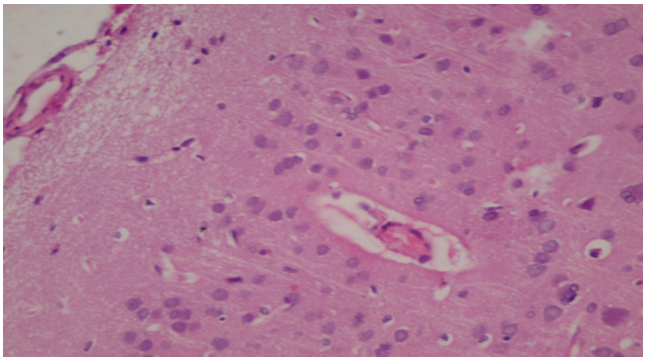
B) Oxidative biomarkers, AChE activity, aluminum concentration, brain growth factors and inflammatory cytokine: Co-administration of antioxidant or anti-inflammatory agents significantly prohibited the changes in the previously measured parameters (figure 3, 4). The percent differences of antioxidant & anti-inflammatory from control DW levels were: (LP 123%, 146%, and 106%, 124%), (nitrites 105%, 123%, and 110%, 126%), (reduced glutathione 105%, 84%, and 75%, 55%), (SOD 19%, 9%, and 19%, 5%) and (Catalase 34%, 27%, and



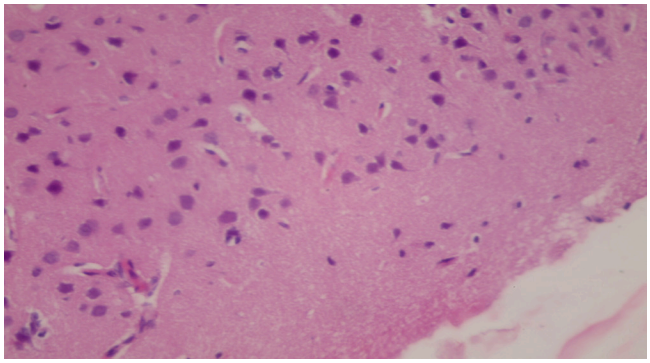
Control



Alzheimer model



Alzheimer + antioxidant



Alzheimer + anti-inflammatory

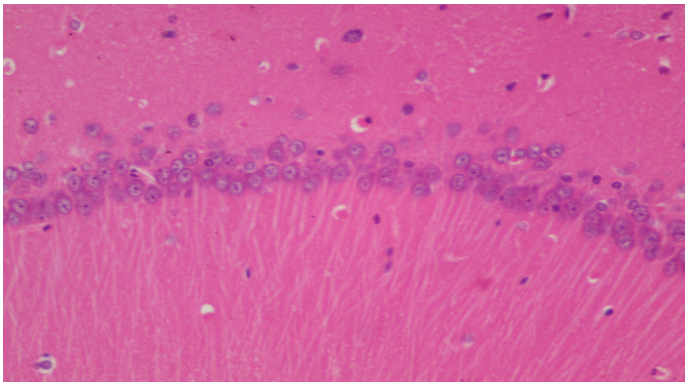
Figure 5. Histopathological changes in frontal cortex of Alzheimer model rats showing heavy loss of cortical neurons, lacunae spaces cells and vacuolated cytoplasm were observed in Alzheimer model animals. On the other hand, remarkable improvement was observed in the Alzheimer model rats treated with antioxidant or anti-inflammatory agent . Normalization of most of neuronal structure was observed after application of anti-inflammatory agent.

35%, 32%) in frontal cortex and hippocampus areas respectively. Similarly, treatment with antioxidant and anti-inflammatory agents significantly attenuated the AChE activity only in hippocampus area and declined the elevated concentration of $AlCl_3$ in both brain areas. (figure 4). The percent of differences of AChE from control, in antioxidant and anti-inflammatory groups, were 123%, 149% in frontal cortex and 122.5%, 100% in hippocampus respectively, while that for aluminum concentration were 110%, 103% in frontal cortex and 149%, 140% in hippocampus respectively. Statistical analysis revealed that administration of anti-inflammatory to Alzheimer's model rats reduced significantly the elevated levels of pro-inflammatory cytokines (IL2 and TNF-α) and TGF-ß while it increased levels of BDNF in hippocampus area only (table 2).

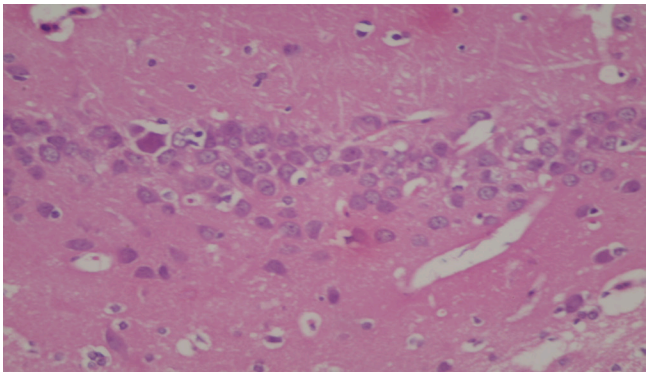
C) Histopathological results: Microscopic examination of cortical tissue shows remarkable improvement in the anti-inflammatory + Alzheimer's model and antioxidant + Alzheimer's model groups. Normalization of most neuronal structures was obvious after administration of an anti-inflammatory agent (figure 5). Similar changes were observed after microscopic examination of hippocampal tissue (figure 6).

Discussion

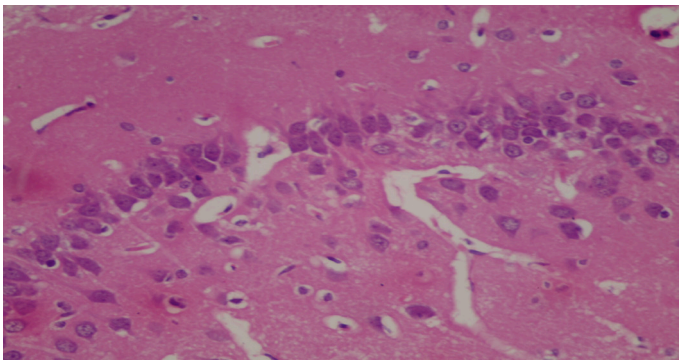
In the present study, time course monitoring of memory and learning tests of control animals verifies the development of spatial learning and memory, however, active learning was significantly impaired in Alzheimer's model group which manifested by increasing latencies in MWM associated with increasing reentry frequency of the animals and prolongation of completion time in radial arm maze, adding to shorten latencies to enter shocking compartment in passive avoidance. The recorded negative effects of $AlCl_3$ on memory and learning functions in the current study concur with the results of Thirunavukkarasu et al (27). These findings harmonized with marked neuronal loss and vacuolated cytoplasm proved by histopathological study of frontal cortex and hippocampus of Alzheimer's model rats.



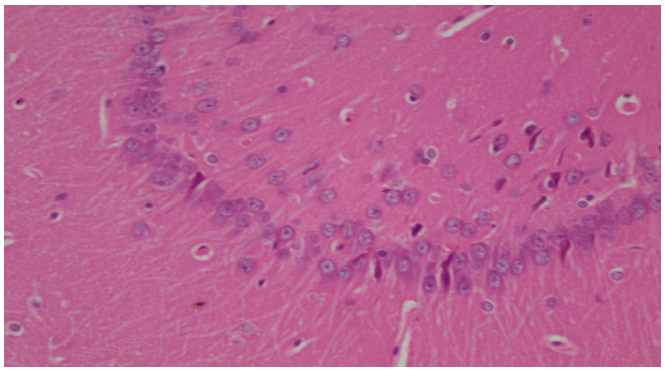
Control



Alzheimer model



Alzheimer + antioxidant



Alzheimer + anti-inflammatory

Figure 6. Histopathological changes in hippocampus of Alzheimer model rats showing loss of normal structural arrangement of pyramidal cells with frequent appearance of degenerated cells H&E x400. Many degenerated pyramidal cells with darkly stained cytoplasm and pyknotic nuclei were still observed after administration of antioxidant agent. Returning the normal arrangement of the layers with recovery of most of neuronal structure were noticed in Alzheimer model treated with anti-inflammatory agent.

These results validate the success of orally administered 100 mg/kg $AlCl_3$ to reach the brain and induce model of AD with cognitive dysfunction that manifested mostly after thirty days of $AlCl_3$ exposure. This finding could be attributed to the ability of aluminum to interfere with cyclic GMP, involved in long-term potentiation and memory (28) that could explain the recorded memory impairment.

Administration of antioxidant or anti-inflammatory agents to Alzheimer's model animals induced a significant improvement of learning and memory tests with obvious response recorded after anti-inflammatory application that goes in line with the results of Lee et al (29). Anti-inflammatory and antioxidant agents began to improve the performance of the Alzheimer model rats on day 30, which verifies the ability to retain and retrieve information after treatment with both agents (29,30). Anti-inflammatory Cilostazol can protect against cognitive impairment induced by intracerebroventricular injection of Aβ (25-30) in C57BL/6J mice (31) as well as in human patients with AD (32).

In the current study, Alzheimer's model showed a reduction in body weight and the number of living rats that were associated with deterioration of cognitive function. The physiologic explanation of this outcome may be contributed to the ability of $AlCl_3$ to depress general brain function including the appetite center that runs parallel with suppression of cognitive function (33). Neither the anti-inflammatory nor antioxidant agent used can increase significantly the body weight; however, they increased the suppressed survival rate.

Cholinergic neurons are positive markers for the

progression of memory, so disturbance of acetylcholine and AChE activity may be one of the mediators of cognitive dysfunction in AD (34). $AlCl_3$ has a biphasic effect on AChE activity, with an initial increase in its activity followed by a marked decrease. This biphasic effect has been attributed to the accumulation of aluminum in the brain (34), which coincides with the recorded elevation of $AlCl_3$ concentration, that parallels increasing AChE activity in the frontal cortex and hippocampus areas as noted in the current investigation. The results presented here showed that anti-inflammatory and antioxidant agents were able to attenuate the increased concentration of aluminum and AChE activity in both regions of the rats' brain.

The brain is an organ that is in particular more susceptible to peroxide damage. Increased production of reactive oxygen species (ROS) seen as lipid peroxidation and nitrite concentration, as well as decreased levels of antioxidant enzymes in frontal cortex and hippocampus of Alzheimer's model group, may be a vital factor attributed to neuronal dysfunction. Thus, it can be hypothesized that oxidative stress could be one of the contributing factors for aluminum-induced cognitive dysfunction. Interestingly, the oxidative changes were observed more in the hippocampus than frontal cortex, which agrees with its fundamental role in the recorded memory impairment. Brain imaging studies confirmed additionally that decreases in the function of the hippocampus is a main contributor to the decline in memory during advanced age and in AD (5). Moreover, Zhihao et al., (2) reported that $AlCl_3$ is a potent pro-oxidant known to enhance lipid peroxides in the hippocampus.

Because oxidative stress and cognitive dysfunction are closely related, agents that modulate reactive oxygen species may be potentially protective against impaired memory, which is in accordance with the current results. Administration of anti-inflammatory and antioxidant agents was found to improve not only the memory retention but it also reduced oxidative damage induced in Alzheimer model. In the present work, antioxidant agents appears to more potently attenuate the changes in oxidative biomarkers than the anti-inflammatory agents. In fact, antioxidant (NAC) is known to increase the intracellular stores of glutathione thereby enhancing the endogenous antioxidant level (9). Also its thiol group interacts directly with reactive oxygen species leading to cellular protection against oxidative damage (9).

In the present study, exposure of Alzheimer's model rats to $AlCl_3$ increased proinflammatory cytokines (IL2 and TNF-α) in the hippocampus and frontal cortex with obvious effects in the hippocampus. $AlCl_3$ activated microglia that induced overproduction of these mediators (35). Also, $AlCl_3$ causes cell depletion in the hippocampus (3), thereby inducing learning deficits as it may interfere with glutamatergic neurotransmission and impair hippocampal long-term potentiation. This is a form of synaptic information storage and memory formation. Therefore, reduction of proinflammatory mediators, and controlling microglia activation, could attenuate the severity of the memory impairment (35). The present data are inconsistent with results of the current study, as anti-inflammatory treatment reduced elevated levels of proinflammatory cytokines (IL 2 and TNF-α), especially in hippocampus. Meanwhile co-administration of antioxidant, NAC with $AlCl_3$ did not significantly improve most of the measured proinflammatory cytokines. These results validate the superior role of anti-inflammatory agents which can explain the amelioration that happened in cognitive function and neuronal structure after its administration which agree with previous reports (7,8).

TGF-ß plays a central role in brain response to injury, and increases in AD (11). Findings in the present study revealed that exposure of Alzheimer's model rats to $AlCl_3$ caused a significant elevation of TGF-ß while it reduced the levels of BDNF in the frontal cortex and hippocampus, with more noticeable effects in the hippocampus. This result agrees with observations of Zetterberg et al., (36) as they reported increased TGF-ß in senile plaque in the brains of AD patients. Also, deposition of amyloid plaque (AP) in the brain seems to induce the inflammatory cascade that activates microglia to produce excess growth factors and cytokines. The other possible explanation of increasing TGF-ß after $AlCl_3$ exposure in the current study is it may increase the clearance of AP by microglia. Therefore, it represents a self-limiting defense mechanism against further accumulation of AP.

Administration of an anti-inflammatory agent (Cilostazol) to Alzheimer's model animals in the present study reduced the high levels of TGF-ß in the hippocampus area. Herein, the lowered hippocampal levels of TGF-ß may propose to be a result of the anti-inflammatory action of Cilostazol, as it can increase the intracellular cAMP level. This can inhibit the mitogen-activated protein kinase (MAPK) and interfere with the inflammatory cascade and the release of cytokines (37). Nagahara and Tuszynski (38) demonstrated

that growth factors strongly influence neuronal survival and function as they have a great efficacy against a variety of pathogenic insults. These findings concur with the present results of the anti-inflammatory agent, Cilostazol. It significantly raised the reduced BDNF, especially in the hippocampus. These results agree with the results reported by other investigators (39). Moreover, anti-inflammatory Cilostazol not only modulated inflammatory responses but also potently affected brain growth factors. Therefore, it may be a potential therapeutic candidate for the treatment of various inflammatory and neurodegenerative diseases (40).

In conclusion, the results from the present study confirm that the selective antioxidant agent (NAC) and anti-inflammatory agent (Cilostazol) can ameliorate the memory deficits in Alzheimer's model rats. The improvement of cognitive function may be attributed to the reduction of $AlCl_3$ concentrations in the brain hippocampus and frontal cortex, interference with the cholinergic dysfunction, as well as the prevention of oxidative damage. Additionally, ant-inflammatory agent Cilostazol superiorly modulated inflammatory responses and induced recovery of hippocampal and cortical neurons through adjustment of brain growth factors as BDNF and TGF-ß. This conclusion supports the predominant role of inflammation and brain growth factors in the pathophysiology of AD. The effects of both agents; antioxidant (NAC) and anti-inflammatory, Cilostazol; need to be simulated in more physiological models of AD. The involvement of additional molecular mechanisms needs to be clarified as DNA fragmentation and an anti-apoptotic effect. The shift to clinical trials using therapies that prevent inflammation and generate high levels of growth factors should be applied, as they will eventually have a useful role in the treatment of AD.

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