WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 10, 124-140. Research Article ISSN 2277– 7105

VITAMIN E PROTECT AGAINST NEUROTOXICITY ON RAT MODEL OF ALZHEIMER'S DISEASE

Eslam M. A. El-Nahrery*

Department of Chemistry (Biochemistry Branch), Faculty of Science, Suez University.

Article Received on
28 July 2015,

Revised on 17 Aug 2015,
Accepted on 08 Sep 2015

***Correspondence for Author**
Eslam M. A. El-Nahrery
Department of Chemistry (Biochemistry Branch), Faculty of Science, Suez University, Suez, Egypt.

ABSTRACT

This study aimed to investigate the protective effect of vitamin E (VitE) on aluminum chloride ($AlCl_3$) induced neurotoxicity in male rats. Twenty six Wistar albino rats were divided into three groups including; normal control group (n=6), Alzheimer's disease (AD) induced (n=10) group and AD induced protected (AD-Vit E) group (n=10) orally with vitamin E dose of 300 I.U/kg b.w. daily for 12 weeks. The antioxidant status determination (TAC), lipid peroxidation (MDA), serum and brain acetylcholinesterase (AchE) activity, serum and brain homogenate C-reactive protein (CRP) assays along with histological examination were performed. The $AlCl_3$ administration affected the brain structure and functions as it significantly decrease TAC ($P < 0.001$) and significantly increase AchE, CRP and MDA levels ($P < 0.001$). VitE administration, significantly enhanced the AD-reduced TAC levels ($P < 0.001$) and significantly decrease AchE, MDA and CRP levels ($P < 0.001$, $P < 0.05$, $P < 0.001$ respectively). Administration of VitE remarkably attenuated the AD-induced damage in hippocampus and congestion in the blood vessels of the cerebrum.

KEYWORDS: vitamin E, aluminum chloride, acetylcholinesterase.

1. INTRODUCTION

Alzheimer's disease (AD) is the most common neurodegenerative disease in elders which characterized by loss of cognitive functions associated with pathological changes in the brain.[1] Alzheimer's disease is associated with microtubule dysfunction and characterized by the appearance of specific cytoskeletal cellular abnormalities, which are associated with cognitive impairment.[2] These cognitive disabilities include the impairment of behavior, visual-spatial skills, speech and motor ability, depression, delusions, hallucinations,

aggressive behavior and ultimately, increasing dependence upon others before death.[3]

Aluminum (Al) exposure has been implicated in the development of AD[4], Animal studies have shown that Al exposure causes neuropathological and neurobehavioral changes resulting in impaired learning ability; thus, Al can be used as an experimental model for inducing AD.[5] Al exposure could produce inflammation in a rat brain.[6] Neuropathological evidence suggests that the accumulation of amyloid-b (Ab) peptides in the brain is a key event in the pathophysiology of AD. Amyloid fibrils are an ordered protein aggregate with a lamellar cross-beta structure.[7]

The hippocampus, a key region of the medial temporal lobe, is a frequent target in many neurological diseases and most forms of dementia. It is well established that damage to the hippocampus accounts for many of the cognitive deficits observed in AD, particularly those concerned with long term memory.[8]

AD is caused by reduced synthesis of the neurotransmitter acetylcholine (ACh).[9] Acetylcholinesterase inhibitors (AChEIs) increase the availability of ACh through inhibition of its destruction, hence an enhancement of cholinergic transmission in the brain and improvement in the symptoms of AD.[10]

Colchicine is a microtubule-disrupting agent that produces marked destruction of hippocampal granule cells, mossy fibers and septoippocampal pathways. It induces neurofibrillary degeneration by binding to tubulin, the structural protein of the microtubule, which is associated with loss of cholinergic neurons and decrease in acetylcholine transferase, thereby, resulting in impairment of learning and memory. Colchicine-induced cognitive impairment has been established as an animal model of sporadic dementia of Alzheimer's type.[11]

Oxidative stress, due to increased free radical generation and impairment of the endogenous antioxidant mechanisms, is an important factor that has been implicated in neuronal damage of AD and cognitive deficits seen in elderly.[12] Traditionally, Alpha tocopherol (vitamin E) major function appears to be as an antioxidant that prevents the propagation of free-radical reactions, results from recent studies provide a role of vitamin E as neuroprotective effects and prevents neuronal apoptosis significantly.[13]

2. Aim of the work

Therefore, the aim of the present work was to investigate and assess the possible neuro-protective effect of the active form of vitamin E in $AlCl_3$ induced AD in rats.

3. MATERIALS AND METHODS

3.1. Materials

- **Chemicals**

$AlCl_3$ were purchased from Laboratory Rasayan Co (Germany).

- **Animals and protection**

Wistar albino rats, weighing 150–200 g, were obtained from the animal house colony of the National Research Center, Cairo, Egypt. . They were housed in polypropylene cages (with stainless steel grill top) under hygienic and standard environmental conditions (28± 2°C, humidity 60–70%, 12 h light/dark cycle). The rats were allowed a standard diet and water ad *libitum*. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals and approved by the Institutional Animal Ethical Committee of the Ain shams university

- **Groups**

A total number of 26 adult rats with a weight range between 150 gm and 200 gm, rats were classified into 3 main groups.

Group I (n= 10): {AD-induced rats (AD)} received 1 ml $AlCl_3$ (100mg/kg body weight / day orally) for 12 weeks,

Group II (n= 10): {AD- induced protected with Vitamin E (AD-Vit E)} received 1 ml $AlCl_3$ (100mg/kg body weight / day orally) and 1 ml vitamin E (300 I.U /kg body weight / day orally) at the same time for 12 weeks

Group III (n= 6): {normal control group} received 1 ml H_2O served as the negative control (N).

3.2. METHODS

- **Induction and Protective Methods**

AD-like model was achieved in rats by the oral administration of $AlCl_3$ in a dose of 100 mg/kg B w[14] daily for 90 days.

Groups 2 animals were induced like group 1 and protected by using vitamin E purchased from pharco pharmaceuticals Alexandria, Egypt. The protective AD group was orally administered with daily 300 I.U. of vitamin E also for 90 days.

- **Sample collection**

At the end of the experiment, blood samples were collected after a 12-h fast using the orbital sinus technique, under light anesthesia by diethyl ether. Each blood sample was left to clot in clean, dry test tubes, and then centrifuged at 1800 g for 10 min at 4^{o}C to obtain serum. The clear serum was frozen at – 20^{o}C for biochemical analysis.

After blood collection, the rats were sacrificed by decapitation and the whole brain of each rat was rapidly dissected, thoroughly washed with isotonic saline, dried, and then weighed.One half of each brain was homogenized immediately to give 0.1 g/ml homogenate in an ice-cold medium containing 50mM Tris-HCl and 300mM sucrose (pH 7.4).[15] The homogenate was centrifuged at 1800 g for 10 min at 4^{o}C and the supernatant (10%) was separated for the different biochemical analyses. The second half of each brain was fixed in a formalin buffer (10%) for histological investigation.

- **Biochemical analysis**
- **Total protein (TP)**

Quantitative estimation of the total protein levels in the brain homogenate was carried out according to the method of[16] to express the concentration of different brain parameters per mg protein. [17]

- **Cholinesterase (AchE)**

Brain cholinesterase activity was determined kinetically using a kit purchased from Biostc Co (Cairo, Egypt), according to the method of Young.[18]

- **High-sensitivity C-reactive protein (hCRP)**

hCRP levels were assayed according to[19] using the ELISA kit purchased from Bio Check (Foster City, CA, USA).

- **Total antioxidant capacity (TAC)**

We measured total antioxidant capacity (TAC) in brain homogenates using Biodiagnostic Kit, Egypt, according to.[20]

- **Malondialdehyde (MDA)**

MDA as an indirect index of lipid peroxidation was determined in brain homogenates following the method described by.[21]

- **Histological studies**

Brain tissues previously fixed in formalin buffer (10%) for 24 h were washed under tap water for 20 min. Then, the serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56^0C in hot air oven for 24 h. Paraffin beeswax tissue blocks were prepared for sectioning at 4-mm-thick using sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained with hematoxylin and eosin stains[22] for histological examination through the light microscope.

- **Statistical analyses**

In the present study, all results are expressed as mean ± standard deviation of the mean. Data were analyzed by one-way analysis of variance and paired sample T Test by using the Statistical Package for the Social Sciences (SPSS) program (version 22) followed by the least significant difference technique to compare the significance between groups. The difference was considered significant when the P value was >. 05. The percentage difference representing the percent of variation with respect to the corresponding control group.

4. RESULTS

4.1. AchE

AD induced rats showed a significant decrease in AchE/g protein when compared to AD-Vit E group, and a high significant change when it compared to normal group, and a high significant change when AD-E group was compared with normal group Table (1), Figure (1).

Serum level of AchE was non-significantly changed in AD group when compared to AD-E group and there is a significant change in AchE serum levels in both AD and AD-E when compared with normal group Table (1), figure (2).

4.2. TAC and MDA

The effects of Vitamin E against AD changes in total antioxidant capacity (TAC) and the levels of lipid peroxidation end product malondialdehyde (MDA) are shown in figures (3, 4). Rats received Vitamin E recorded in a high significant increase ($P < 0.001$) in TAC

when compared to AD group, and there were a high significant decreased (P < 0.001) in AD and AD-E when compared with normal control group. TAC levels in brain extract with the normal control. On the other hand, animals intoxicated with $AlCl_3$ exhibited a significant increase in MDA level (p < 0.001) concomitant with a significant decrease (p < 0.001) in TAC level in the brain when were compared to normal control. Administration of vitamin E to rats succeeded to prevent significantly the $AlCl_3$-induced changes in the mentioned parameters. In MDA levels of brain extract show a high significant increase in both AD and AD-E groups when compared to the normal control, also the protective effect of vitamin E is shown by high significant decrease in AD-E group when compared with AD animals Table (5), figure (4).

4.3. hs-CRP

The AD brain extract showed a high significant increase (<0.001) in CRP/Protein when compared to a normal control group, also there were a high significant increase in AD when compared to AD-E Figure (5). In the same wile a high significant increase (<0.001) in CRP/Protein when AD-E group was compared to a normal control group. In serum also there were a high significant increase (<0.001) in CRP/Protein in AD group when compared to AD and AD-E groups Figure (6).

4.4. Histological studies

Photomicrographs of brain sections of control rats showed normal nerve cells (Figur 7A).

Table 1. Brain extract and serum AchE results, descriptive statistical data is presented as mean ± SD.

Groups	AchE	
	Brain Extract / protein (U/g protein)	**Serum (U/ L)**
AD AD-E Normal	99.25 ± 27.44 62.84 ± 4.175 49.40 ± 7.29	36.32 ± *6.83* 31.89 ± *11.79* 19.18 ± *0.723*

Table 2. MDA all data presented in Mean ± SD.

Groups (nmol/mg protein)	**MDA**
AD	9.98 ± 0.97
AD-E	6.51 ± 0.62
Normal	5.65 ± 0.21

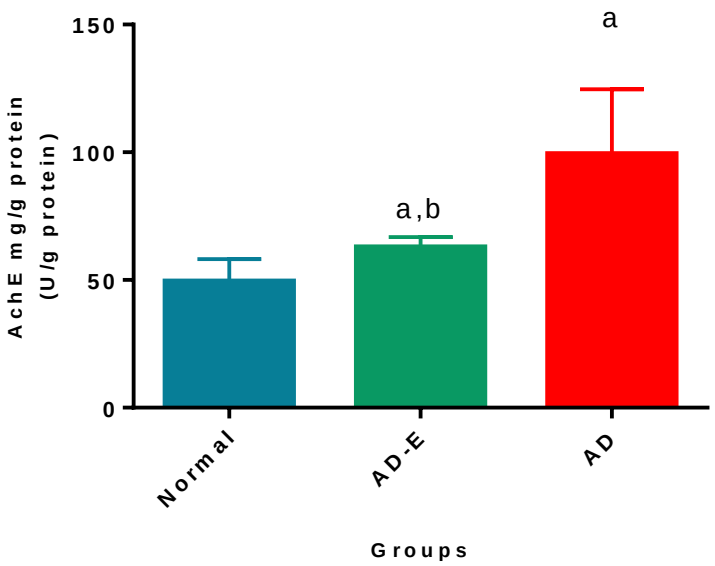


Figure 1: AchE brain extract/par in results show (a) high significant increase in AchE <0.001 in both AD and AD-E g the control group mpared with control group; (b) a significant increase in AchE in AD-E <0.05 when compared to AD group.

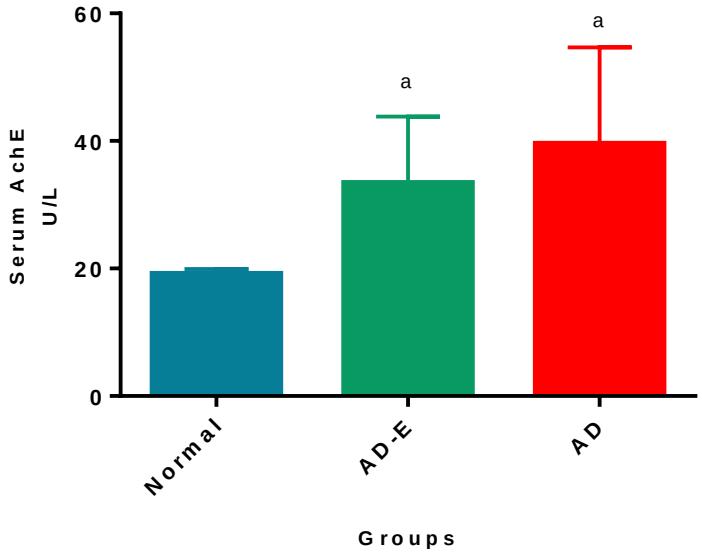


Figure 2 Serum AchE results show a non-significant change in AD-E group when compared with AD group while (a) a significant change <0.05 in both groups when compared to the normal control.

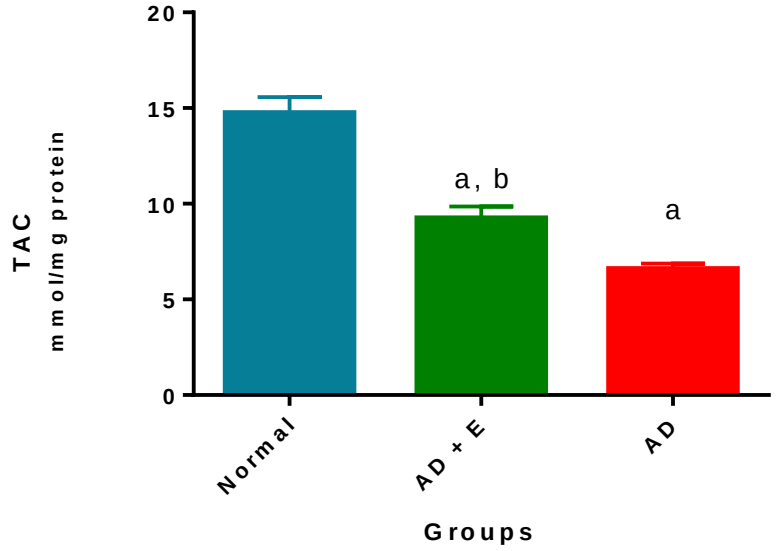


Figure 3. Mean ± SD of the brain extract levels of MDA. vitamin E administration decrease brain extract level of MDA versus non-protected induced animals AD. Represent high significant differences between different test groups (N = 10) for each group) with each other and control. P < 0.05 was considered as significantly different.

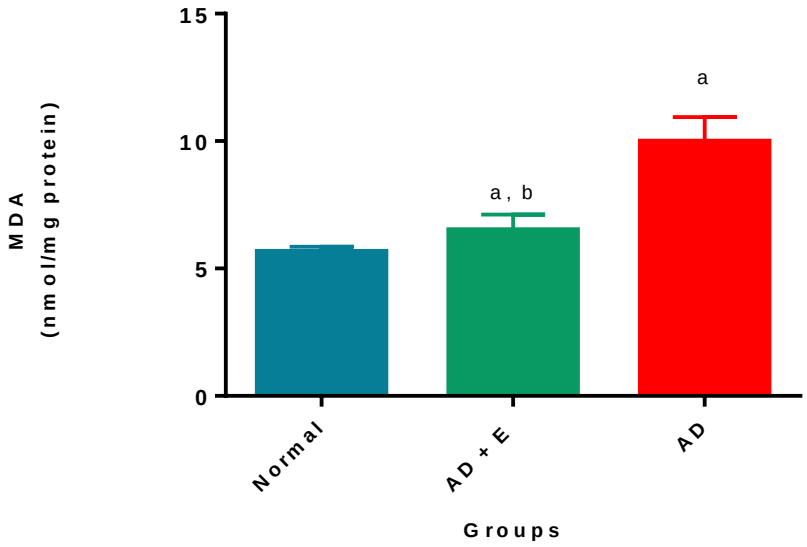


Figure 4 Mean ± SD of the brain extract levels of MDA. Vitamin E administration decrease brain extract level of MDA versus non-protected induced animals AD. Represent high significant differences between different test groups (N = 10) for each group) with each other and control. P < 0.05 was considered as significantly different.

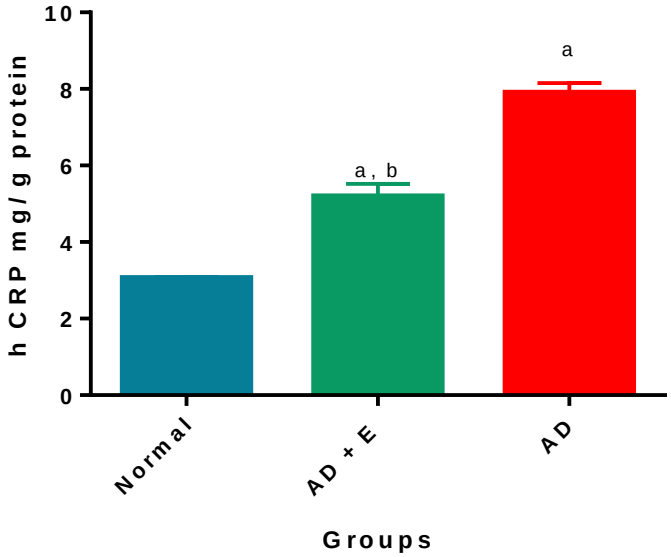


Figure 5. Mean ± SD of the brain extract levels of hCRP. Vitamin E administration decrease brain extract level of hCRP versus non-protected induced animals AD. Represent high significant differences between different test groups (N = 10) for each group) with each other and control. P < 0.05 was considered as significantly different.

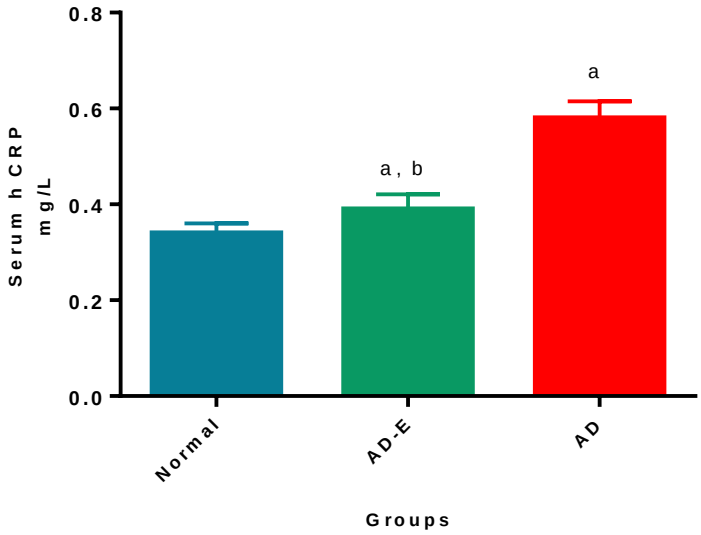


Figure 6. Mean ± SD of the serum levels of hCRP. Vitamin E administration decrease serum level of hCRP versus non-protected induced animals AD. Represent high significant differences between different test groups (N = 10) for each group) with each other and versus control. P < 0.05 was considered as significantly different.

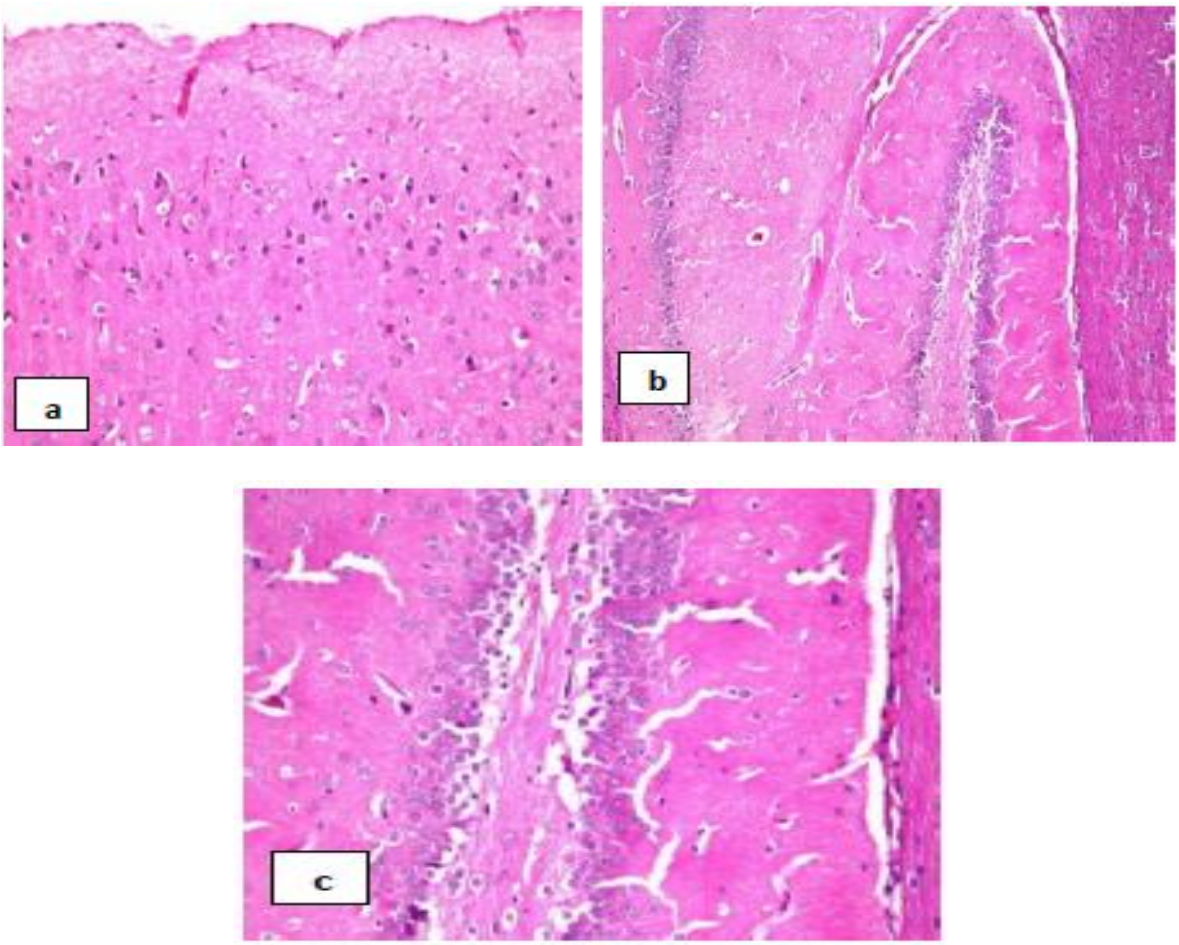
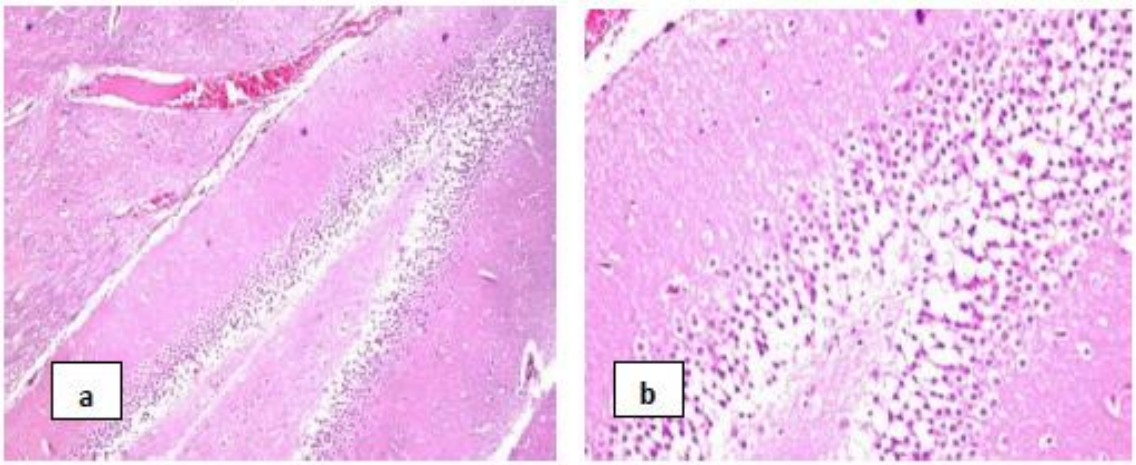


Figure 7: There was no histopathological alteration and the normal histological structure of the meninges, cerebral cortex, cerebrum striatum and hippocampus were recorded in Fig.5 (a, b & c).



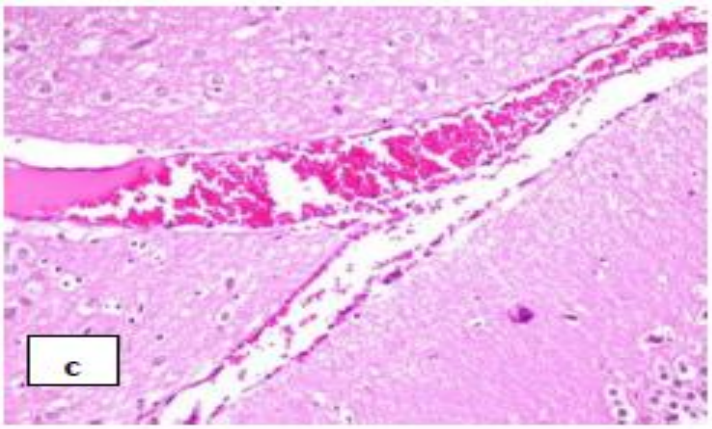


Figure 8 Image of the brain section of an Alzheimer's disease-induced rat (group 2) show degeneration was detected in all of the neuronal cells of the hippocampus associated with congestion in the blood vessels of the cerebrum striatum Fig.8. (a, b & c).

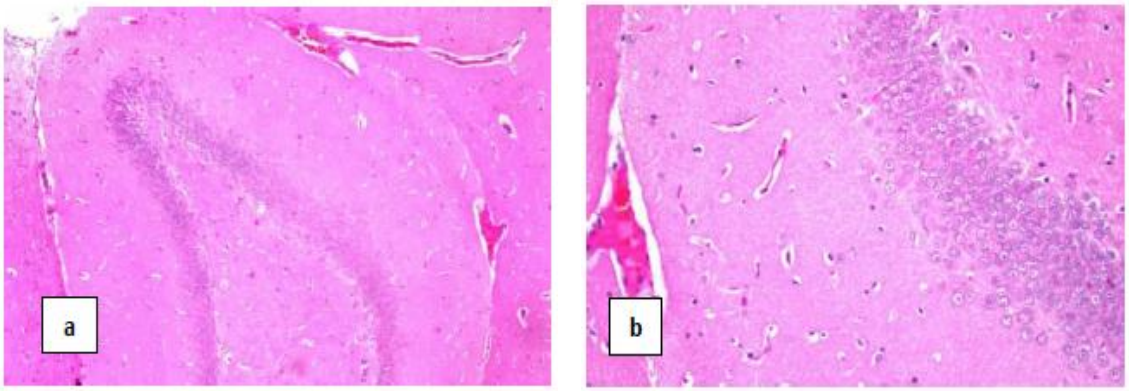


Figure 9. Image of the brain section of an Alzheimer's disease-induced protected with vitamin E rat (group 3) show congestion in the cerebral blood vessels while the neuronal cells in the hippocampus were histologically intact Fig.9. (a,& b).

5. DISCUSSION

The present study demonstrated that the aluminum chloride administration (AD-like pathology model) shown a significant increase in brain acetylcholinesterase (AchE) activity. This result coincides with that of revealed a significant increase in brain acetylcholinesterase (AchE) activity, this results are in agree with **Fadl et al, 2013**[23] how fined a significant increase in brain acetylcholinesterase (AchE) activity after oral administration of aluminium chloride ($AlCl_3$) in a dose of 17 mg/kg body weight (bw) daily for 45 days. And also with **Zhang et al**,[34] There are two proposed mechanisms for aluminium-induced stimulation of AchE activity in the brain are: (1) aluminium can induce

caspase-1 activation and IL-1b secretion.[35] IL-1b has been found to promote the activity and expression of AchE both in cell cultures and in vivo.[36] (2) aluminium can augment the accumulation of insoluble Ab protein. [37] Ab induced elevation in the AchE activity through the induction of lipid peroxidation in neuronal membranes due to the production of hydrogen peroxide (H2O2).[24] H2O2 may have a direct action on the AchE enzyme as it acts as a modulator (may be allosteric) in the activity of functionally important proteins, receptors and enzymes.[25] Based on these findings, aluminium's action on AchE activity may be related to the involvement of aluminium in the aetiology of AD pathological process,[26] as the impairment of cholinergic neurotransmission became a well-established fact in AD.[27]

The present data show that brain AchE show a significant decrease in the vitamin E protected group when compared with AD model group, these results confirmed by Iraj et al 2015[28] how prove that administration of vitamin E improve the memory in AD induced rat with Lead ($Pb2^{+}$) is a neurotoxin substance.

Our data revealed that plasma CRP was significantly elevated in AD induced group than that of normal or protected groups. These findings are in harmony with those of Zaciragic et al[29] and Ho et al.[30]In brain tissue, the pathophysiology of CRP accumulation is complex because of the requirement of systemically produced CRP to cross the brain barrier (BBB). However, it has been established that during inflammatory conditions, the BBB becomes dysfunctional, enabling proteins normally only found in serum to enter the cerebrospinal fluid, so the transit of circulating CRP across the BBB is the most likely potential source of cerebral CRP.[31]

Strang et al[32] demonstrated that amyloid plaques induced the dissociation of pentameric CRP to individual monomers, which possess strong pro-inflammatory properties not shared with pentameric CRP, and localizing inflammation to Alzheimer's plaques.[33] Hyperhomocysteinemia can induce tau hyperphosphorylation and interrupt DNA repair in hippocampal neurons and make neurons more vulnerable to amyloid toxicity.[34] Demethylation of the PS-1 (an important component of γ-secretase) promoter gene stimulates PS-1 expression, which in turn increases the production of Aβ. Zhang et al[35] found that the levels of PS-1 mRNA and protein were increased in rats with hyperhomocysteinemia, suggesting that high Hcy may selectively disrupt the methylation of PS-1 promoter,

leading to increased Aβ production. ADMA is an endogenous competitive inhibitor of nitric oxide synthase. It was found that Hcy inhibits DDAH, thereby increasing ADMA accumulation and reducing NO production in cultured neurons. This finding may explain the relationship between increased circulating Hcy and the development of cognitive dysfunction in AD.[36]

The current study demonstrate that lipid peroxidation end product malondialdehyde MDA increase significantly in AD induced group than normal, while results of protected group report show a significant decreased than AD induced group.

Lipid peroxidation (LPO) is one of the main manifestations of oxidative damage and has been found to play an important role in the toxicity of many xenobiotics. Aluminum--- has been reported to induce LPO, and to alter physiological and biochemical characteristics of biological systems.[37,38] There is a high susceptibility of brain to oxidative insult because it contains a large amount of polyunsaturated fatty acids and consumes 20% of the body's oxygen. Moreover, in spite of the high rate of oxidative metabolism, the brain has a relatively low antioxidant defense system.[37,38]

The present results showed that $AlCl_3$ promotes oxidative stress by significant reduction of TAC level which was confirmed by the significant increase in the levels of MDA in AD induced group than normal and protected groups. While the effect of vitamin E seemed by a significant increase of TAC and decrease of MDA in protective group than AD induced one.

These results agreed with El-Demerdash, (2004)[39] how suggest participation of free radical-induced oxidative cell injury in mediating the toxicity of aluminum. Also the data are in agreement with those obtained by Yousef (2004)[40] who observed that aluminum administration lowered antioxidant enzyme activities and increased lipid peroxidation in the brains of rabbits Increased reactive oxygen species (ROS) were reported in previous studies during aluminum exposure, which was attributed to electron leakage, enhanced mitochondrial activity and increased electron chain activity. ROS subsequently attack almost all cell components including membrane lipids and producing lipid peroxidation.[41] Therefore, it can be hypothesized that oxidative stress may be one of the contributing factors for aluminum-induced neurotoxicity.[28] These effects may also be due to the accumulation of metal in the brain and blood.[43] Some studies have

shown that aluminum can bind amino acids such as glutamate to form aluminum–glutamate complexes that allow it to reach the blood of the brain.[44]

The biochemical finding was confirmed with histological investigation of brain tissue of rat model of AD which showed neuronal degeneration in the hippocampus and protection of induced rats with vitamin E result a congestion in the blood vessels without neurological degradation.

CONCLUSION

In summary, vitamin E was successful in repairing affected regions and improvement of metabolic disturbances in $AlCl_3$- protected rats. So vitamin E can use as natural save as a protective agent to protect or decrease the severity of Alzheimer's. However, further clinical studies are needed for extrapolating the experimental animal results to humans.

6. REFERENCES

1. Böttger D, Ullrich C, Humpel C. Monocytes deliver bioactive nerve growth factor through a brain capillary endothelial cell-monolayer in vitro and counteract degeneration of cholinergic neurons. Brain Res., 2010; 1312: 108–19.
2. Kumar A, Dogra S, Prakash A. Protective effect of narining, a citrus flavonoid against colchicine- induced cognitive dysfunction and oxidative damage in rats. J Med Food., 2010; 13(4): 976–84.
3. Voisin T and Vellas B. Diagnosis and treatment of patients with severe Alzheimer's disease. Drug Aging., 2009; 26: 135–144.
4. Walton JR: An aluminum-based rat model for Alzheimer's disease exhibits oxidative damage, inhibition of PP2A activity, hyperphosphorylated tau, and granulovacuolar degeneration. J Inorg Biochem., 2007; 101: 1275–1284.
5. Wisniewski HM, Sturman JA, Shek JW: Aluminium chloride induced neurofibrillary changes in the developing rabbit: a chronic animal model. Ann Neurol., 1980; 8: 479–490.
6. Li Y, Liu W, Oo TF, Wang L, Tang Y, Jackson-Lewis V, Zhou C, Geghman K, Bogdanov M, Przedborski S, Beal MF, Burke RE, Li C: Mutant LRRK2 (R1441G) BAC transgenic mice recapitulate cardinal features of Parkinson's disease. Nat Neurosci., 2009; 12: 826–828.
7. Hsu RL, Lee KT, Wang JH, Lee LY and Chen RP. Amyloid-degrading ability

of nattokinase from Bacillus subtilis natto. J Agric Food Chem., 2009; 57: 503–508

8. Götz J, Ittner M. Animals models of Alzheimer's disease and frontotemporal dementia. Nat Rev Neurosci., 2008; 9(7): 532–44.
9. Cummings JL, Cole G. Alzheimer disease. JAMA., 2002; 287: 2335–2338.
10. Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. Lancet., 2006; 368: 387– 403.
11. Kumar A, Dogra S, Prakash A: Protective effect of curcumin (Curcuma longa) against aluminum toxicity: possible behavioral and biochemical alterations in rats.Behav Brain Res., 2009; 205: 384–390.
12. Torres LL, Quaglio NB, de Souza GT, Garcia RT, Dati LM, Moreira WL, et al. Peripheral oxidative stress biomarkers in mild cognitive impairment and Alzheimer's disease. J Alzheimer's Dis., 2011; 26(1): 59–68.
13. Reisi P, Dashti GR, Shabrang M, Rashidi B. The effect of vitamin E on neuronal apoptosis in hippocampal dentate gyrus in rabbits fed with high-cholesterol diets. Adv Biomed Res. 2014; 3: 42. Published online Jan 24, 2014. doi: 10.4103/2277-9175.125731
14. Krasovskii GN, Vasukovich LY and Chariev OG. Experimental study of biological effects of leads and aluminium following oral administration. Environ Health Perspect 1979; 30: 47–51.
15. Tsakiris, S., K.H. Schulpis, K. Marinou, and P. Behrakis. Protective effect of L- cysteine and glutathione on the modulated suckling rat brain Na+-K+ ATPase and Mg+2-ATPase activities induced by the "in vitro" galactoseaemia. Pharmacological Research, 2004; 49: 475-479.
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the folin phenol reagent. J Biol., 1951; 193: 265–275.
17. Karthikeyan K, Bai BR, Devaraj SN: Cardioprotective effect of grape seed proanthocyanidins on isoproterenol-induced myocardial injury in rats. Int J Cardiol., 2007; 115: 326–333.
18. Szasz G.: Serum cholinesterase determination with acetyl- and butyrylthiocholine as substrate Clin Chim Acta., 1968 Feb; 19(2): 191-204
19. Roberts WL, Sedrick R, Moulton L, Spencer A, Rifai N: Evaluation of four automated high-sensitivity C-reactive protein methods: implications for clinical and epidemiological applications. Clin Chem., 2000; 46: 461–468.
20. D Koracevic, G Koracevic, V Djordjevic, S Andrejevic, V Cosic, Method for the

measurement of antioxidant activity in human fluids, J Clin Pathol., 2001; 54: 356–361.

21. Satoh, K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta., 1978; 1: 37-43
22. Banchroft JD, Stevens A and Turner DR. Theory and practice and practice of histoloical techniques. 4th ed. New York, London, San Francisco, Tokyo: Churchill Livingstone, 1996.
23. NN Fadl, HH Ahmed, HF Booles3 and AH Sayed: Serrapeptase and nattokinase intervention for relieving Alzheimer's disease pathophysiology in rat model. Human and Experimental Toxicology., 2013; 32(7): 721–735.
24. Melo JB, Agostinho P and Oliveira CR. Involvement of oxidative stress in the enhancement of acetylcholinesterase activity-induced by amyloid-beta peptide. Neurosci Res., 2003; 45: 117–127.
25. Kamster A and Segal M. Hydrogen peroxide as a diffusible signal molecule in synaptic plasticity. Mol Neurobiol., 2004; 29: 167–178.
26. Kaizer RR, Correa MC, Gris LRS, Da Rosa CS, Bohrer D, Morsch VM, et al. Effect of long term exposure toaluminum on the acetylcholinesterase activity in the central nervous system and erythrocytes. Neurochem Res., 2008; 33: 2294–2301.
27. Zivin Mand Pregelj P. Prolonged treatment with donepezil increases acetylcholinesterase expression in the central nervous system. Psychiatr Danub., 2008; 20: 168–173.
28. Iraj Salehi; Maryam Sahab Soleimani; Mahsa Poorhamze; Fahimeh Ghasemi Moravej; Alireza Komaki 1; Sara Soleimani Asl.
29. Zaciragic A, Lepara O, Valjevac A, et al. Elevated serum C-reactive protein concentration in Bosnian patients with probable Alzheimer's disease. J Alzheimers Dis., 2007; 12: 151–156.
30. Ho YS, Yu MS, Yang XF, So KF, Yuen WH, Chang RC. Neuroprotective effects of polysaccharides from wolfberry, the fruits of Lycium barbarum, against homocysteine induced toxicity in rat cortical neurons. J Alzheimers Dis., 2010; 19: 813–827.
31. Stolp HB, Dziegielewska KM. Role of developmental inflammation and blood–brain barrier dysfunction in neurodevelopmental and neurodegenerative diseases. Neuropathol Appl Neurobiol., 2009; 35: 132–146.
32. Strang F, Scheichl A, Chen YC, et al. Amyloid plaques dissociate pentameric to monomeric C-reactive protein:A novel pathomechanism driving cortical inflammation in Alzheimer's disease? Brain Pathol., 2012; 22: 337–346.

33. Kamath AF, Chauhan AK, Kisucka J, et al. Elevated levels of Hcy compromise blood brain barrier integrity in mice. Blood., 2006; 107: 591–593.
34. Kruman II, Kumaravel TS, Lohani A, et al. Folic acid deficiency and Hcy impair DNA repair in hippocampal neurons and sensitize them to amyloid toxicity in experimental models of AD. J Neurosci., 2002; 22: 1752–1762.
35. Zhang CE, Wei W, Liu YH, Peng JH, Tian Q, Handy DE. Hyperhomocysteinemia increases β amyloid by enhancing expression of γ-secretase and phosphorylation of amyloid precursor protein in rat brain. Am J Pathol., 2009; 174: 1481–1491.
36. Selley ML. Homocysteine increases the production of asymmetric dimethylarginine in cultured neurons. J Neurosci Res., 2004; 77: 90–93.
37. Dua, Raina, and Kiran Dip Gill. "Aluminium phosphide exposure: implications on rat brain lipid peroxidation and antioxidant defence system." Pharmacology & toxicology., 2001; 89(6): 315-319.
38. Sharma, Virender K., Ria A. Yngard, and Yekaterina Lin. "Silver nanoparticles: green synthesis and their antimicrobial activities." Advances in colloid and interface science., 2009; 145(1): 83-96.
39. El-Demerdash FM. Antioxidant effect of vitamin E and selenium on lipid peroxidation, enzyme activities and biochemical parameters in rats exposed to aluminium. J Trace Elem Med Biol., 2004; 18(1): 113-21.
40. Yousef, M. I., Aluminum induced changes in hematobiochemical parameters, lipid peroxidation and enzyme activities of male rabbits: protective role of ascorbic acid. Toxicology, 2004; 199: 47-57.
41. Flora, S.J.S., A. Mehta, K. Satsangi, G. M. Kannan, and M. Gupta. Aluminum induced oxidative stress in rat brain: response to combined administration of citric acid and HEDTA. Comp. Biochem. Physiol., 2003; 134: 319-28.
42. Yousef, M. I. and A.F. Salama. Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. Food and Chemical Toxicology, 2009; 47: 1168-1175.
43. Sharma, P. and K.P. Mishra, Aluminum-induced maternal and developmental toxicity and oxidative stress in rat brain: response to combined administration of Tiron and glutathione. Reprod. Toxicol., 2006; 21: 313-21.
44. Shrivastava, S., Combined effect of HEDTA and selenium against aluminum induced oxidative stress in rat brain. Journal of Trace Elements in Medicine and Biology, 2012; 26: 210-214.