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Modulatory effect of Zeolite in an experimental rat model of Alzheimer's disease

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Abstract: In our study, the potential modulatory effect of zeolite on aluminum chloride used to induce Alzheimer's disease was investigated. Rats were divided into four groups (control group, aluminum chloride group, aluminum chloride and zeolite group, zeolite group). Aluminum chloride treatment characterized by decreasing latency for animals to step into the dark section in passive avoidance test, increasing escape latency to climb the platform and spent significantly less time in the defined part in Morris water maze test .Also, increase acetylcholinesterase content, Amyloid beta content, oxidative stress markers and inflammatory mediators, also decrease phosphorylated glycogen synthase kinase 3 beta content ,and increase free cytosolic beta catenin and brain derived neurotrophic factor content (downregulation of Wnt/ β-catenin pathway). Also, in histopathological examination shows pyknosis and degeneration in the neurons of cerebral cortex, hippocampus and striatum indicating induction of Alzheimer's disease. On the other hand, Treatment with zeolite increases latency for animals to step to the dark section of passive avoidance test, decreasing escape latency and spent significantly more time in the defined part in Morris water maze test .Also, decrease acetylcholinesterase content, Amyloid beta content, oxidative stress markers and the release of inflammatory mediators, increase phosphorylated glycogen synthase kinase 3 beta content, and decrease cytosolic free beta catenin and brain derived neurotrophic factor content (upregulation of Wnt/ β-catenin signaling pathway) decreases pyknosis and degeneration in histopathological examination indicating zeolite attenuation of aluminum chloride induction of Alzheimer's disease .

Keywords: Alzheimer's disease; zeolite; acetylcholinesterase; oxidative stress; inflammatory mediators; brain derived neurotrophic factor; glycogen synthase kinase 3 beta; (Wnt/ β-catenin signaling pathway).

1. INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease, is a type of dementia occurs usually in elderly and worsen over the time and characterized by cognitive decline and memory impairment[1].

Alzheimer's pathology characterized by phosphorylated proteins of tau and 1–42 amino acid of amyloid beta (Aβ)[2], Mutations in amyloid precursor protein (APP) gene lead to familial AD, Aβ aggregation led to free radicals like reactive oxygen species (ROS) to react with many proteins and lipids, which converted to oxidized toxic proteins and per oxidized lipids[3].

Peroxidation of lipids leads to toxic products that migrate to the neurons and make several alterations of its functions. Disrupt the neuronal cell signaling and finally leads to neuronal cell death[4].

Also, leads to inflammatory response, and release inflammatory mediators like cytokines, which prevent removal of Aβ by microglia and promote the neuronal cell death and loss of neuronal cell signaling by microglia, a main cause of AD pathogenesis.

Amyloid beta aggregation leads to neurofibrillary tangles (NFT) formation inside neurons which formed of hyperphosphorylated proteins called tau proteins [6].

Nuclear factor kappa B (NF-κB) is transcription factor that involved in memory, plasticity of the synapse and in improvement of learning abilities, AD is accompanied with increased nuclear expression of NF-κB, increased Ca^{+2} intracellularly, TNFα, H_2O_2, ROS considered as stimulators of NF-κB [6].

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Also, inhibition of PI3K/AKT signal pathway is linked to the appearance and development of AD so its stimulation may be a promising field in protecting neuronal cell against Aβ formation and aggregation and so against AD [7,8]

As inhibition of PI3K/AKT pathway will increase activity of GSK-3β that increase phosphorylation of tau proteins and formation of neurofibrillary tangles (NFT), and so its activation will lead to phosphorylation of GSK-3β and so decrease NFT formation and AD progression[7,8]. GSK-3β also decreases acetylcholine formation which is a great cause for development of AD[9]. Acetylcholine neurotransmitter play an important role in cognition, attention, memory and motivation [10].

WNT signaling is expressed in different brain region and that's important for development of the neurons and synapse [11]. When GSK-3β activity increased, prevent WNT/β-catenin signaling pathway and so impaired the memory, and increases Tau protein phosphorylation [12]and decreasing β-catenin and its gene expression [13,14].

Aluminum metal is one of the great causes of AD[15,16], Aluminum accumulates in the cortex, cerebellum, and hippocampus, which are responsible for memory and cognition [16].

Natural zeolites originate from volcanoes. The hot lava from the volcano reaches the sea reacts with the salt and water and form crystalline solid called zeolite[17,18].

zeolites composed of aluminosilicate structure with silicon (Si^{4+}) and aluminum (Al^{3+}) that surrounded by four oxygen atoms (O^{2-}) zeolites classified to natural or synthetic[19].

Natural zeolite Clinoptilolite is constant during ion exchange process and after twelve hours of heating at 750°C and in acids making it suitable for *in vivo* applications used in veterinary and human medicine[20]it can act as adsorbent, cation exchanger, catalyst[21].

This experiment aimed to clarify the protective effect of zeolite (clinoptilolite) against aluminum chloride-induced Alzheimer's disease in rat and investigating the mechanisms underlying these protective effects.

2. METHODS

2.1. animals

Adult male rats, (300-340 g) in weight were brought from (NODCAR) National Organization for Drug Control and Research animal house, Giza, Egypt.

2.2. Chemicals

(a) $AlCl_3.6H_2O$ a yellow-colored powder obtained from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. We dissolve aluminum chloride in dist.water and daily intraperitoneally (IP) injected at a dose 70 mg/kg [22]for 5 weeks. Micronized Zeolite (clinoptilolite) purchased as An off white powder obtained from Gongyi_xiangrui eco material Co.Itd (gongyi, China) . We dissolve it in dist. water and as 100 mg/kg/orally,daily [23]for 4 weeks.

2.3. Experimental design

We divided 32 rats to four groups with 8 rats in each group. First group: Rats given water (orally) for 4 weeks and saline (IP) daily for 5 weeks as a control group. Second group: Rats received $AlCl_3$ (70 mg/kg, IP.) daily for 5 weeks. Third group: Rats received $AlCl_3$ (70 mg/kg, IP.) daily for 5 weeks[22]and zeolite (100mg/kg, oral) daily for 4 weeks[23] starting from the second week till the end of experiment. Fourth group: Rats received zeolite (100mg/kg, oral) daily for four weeks. In the last day animals weighed, hypnotized and sacrificed, two brains obtained from each group for microscopic histological analysis then we separate the hippocampus and cortex from the rest brains and make homogenate for further biochemical analysis and storing it in -80 °C till analysis.

2.4. Morris water maze

Rats learns to find a platform during swimming in certain swimming pool apparatus [24], it is diameter 150 cm and its height 60 cm the tank is divided to four parts with a platform in middle of a fixed part[25]. On the first day, two trials were performed, the platform was placed protruding one inch above the water surface. Each animal received two consecutive trials, each 180 sec, At the beginning of the training session, animal continued to search for a way out. Eventually, they learned to find the platform in the fixed part and climbed it.[20]. three min is the latency time on the second, third, and fourth day of the experiment, Probe test: After completion of the 4 days training [21]the platform was removed each rat swim for 180 sec for 2 trials and we recorded the time spent by the rat in the part of the platform that were removed.

2.5. Passive avoidance test

A Simple and rapid tool of memory testing [26] In this test, the animal learns that a specific place should be avoided owing to an aversive effect[27]. We put each rat at the lighted room of the apparatus for three min to explore it with open sliding door between light and dark room, acquisition trial in the second day the rat placed again at the lighted room and when pass through the dark room the sliding door shut and apply electric shock for 5 sec. After twenty-four hour and forty-eight hour we make retention test after the acquisition trial with an open sliding door. for a maximum period of 3 min to test the latency to avoid the shock-associated compartment[28].

2.6. Assessment of acetylcholinesterase

Acetylcholinesterase content was assessed by colorimetric method (absorbance at 412 nm) using a kit according to manufactured instructions (Sigma Aldrich Co., St Louis, MO, USA), catalog number (MAK119)[29].

2.7. Assessment of Aβ

Amyloid beta content was assessed by ELISA kits according to manufactured instructions (biotech instruments, VT, USA), catalog number (MBS702915).

2.8. Assessment of oxidative stress biomarkers

Lipid peroxidation is assessed colourmetrically by the reaction of malonaldehyde (MDA) with thiobarbituric acid as MDA is a product of lipid peroxidation (at 535nm) [28] ,kits obtained from (Sigma-Aldrich Co., St Louis, MO, USA) ,catalog number(MAK085C) .Total antioxidant capacity (TAC) is assessed colourmetrically by its reaction with hydrogen peroxide and the residual hydrogen peroxide is react with certain enzyme and the formed colored product is determined (at 510 nm)[31], kits obtained from (Bio diagnostic, Giza, Egypt),catalog number (TA 25 12).

2.9. Assessment TNF-α

Tumor necrosis factor alpha content was assessed by ELISA kits according to manufactured instructions (biotech instruments, VT, USA), catalog number (MBS355371).

2.10. Assessment of NF-κB

Neurotrophic factor kappa B content was assessed by ELISA kits according to manufactured instructions (biotech instruments, VT, USA), catalog number (MBS722386).

2.11. Assessment of BDNF:

Brain derived neurotrophic factor content was assessed by ELISA kits according to manufactured instructions (biotech instruments, VT, USA) catalog number (MBS355345).

2.12. Western blotting analysis

Phosphorylated GSK-3β and β-catenin were detected using Western blotting as previously described. [31] Briefly, first blotting done, then we made blocking with 5% (BSA), then the membrane incubated with 1ry antibody (1:1000) dilution with TBST-buffer over the night at 4 °C then washed four times with the same buffer, then the membrane incubated with the 2ry antibody (1:10000) dilution with TBST-buffer for thirty minutes then we use enhanced chemiluminescence (ECL) system for signal detection,p-Gsk3β at ser9 and β-catenin 1ry antibody obtained from (cell signaling technology MA, USA) ,catalog number (9336)and control sample beta actin obtained from (Thermo Fisher Scientific, MA, USA) ,catalog number (A1-91399).

2.13. Histopathological Examination

Brain tissue put in 10% buffered formalin, dehydrated ethanol serial dilutions, then put it in xylene paraffin. Ventricular sections cut and stained it with hematoxylin and eosin (H&E), then visualized on a microscope at 40× magnification [33].

2.14. Statistical Analysis:

Statical analysis were done by one way ANOVA then Multiple comparison test, express the data as ±S.E.M and significant value at $p< 0.05$, using computer program GraphPad Prism software (version 8) (ISI, USA).

3. RESULTS

3.1. Morris water maze test

$AlCl_3$ group showed marked decrease in learning abilities as compared to control group on the second to the fourth, hence indicating cognitive deficits. However, treatment of rats with $AlCl_3$ and zeolite showed a clear improvement in their ability in seeking the platform with $AlCl_3$ alone treated group on the second to the fourth day as shown in (figure 1 A).

Probe trial: on the fifth day during performing MWM test, in the probe trial, $AlCl_3$ group spent less time in the defined part compared to control group. However, treatment of rats with $AlCl_3$ and zeolite showed a clear improvement in memory compared with $AlCl_3$ alone treated group as shown in (figure 1 B).

3.2. Passive avoidance test

$AlCl_3$ group showed marked decrease in latency by on the third and fourth days of the experiment compared to control group. However, treatment of rats with $AlCl_3$ and zeolite showed a clear elevation in latency on the third and fourth days of the experiment compared with $AlCl_3$ alone treated group as shown in (figure 2 A, B).

3.3. Effect of zeolite on acetylcholinesterase and beta amyloid content in aluminum chloride treated rats.

$AlCl_3$ group showed marked increase in AChE and Aβ content compared to control group. However, treatment of rats with $AlCl_3$ and zeolite showed a clear reduction in AChE and Aβ content compared with $AlCl_3$ alone treated group as shown in (figure 3 A, B)

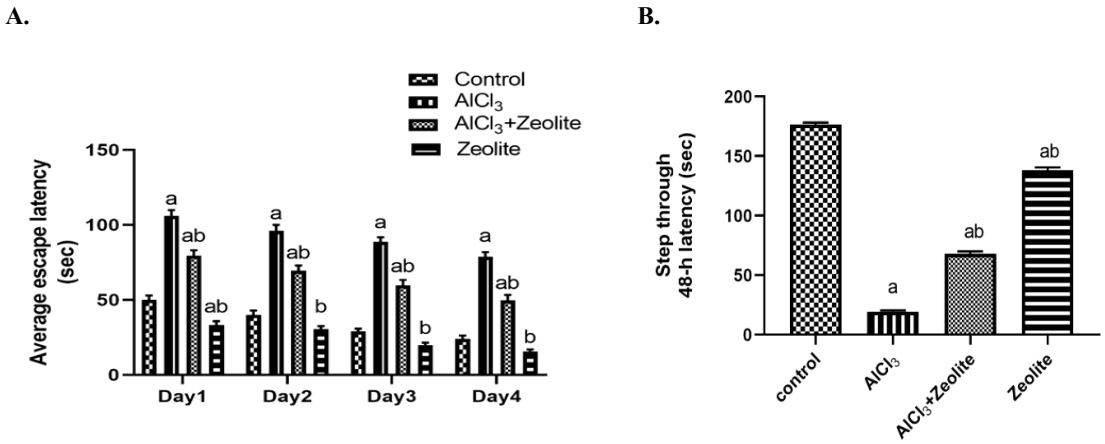


Figure 1: Zeolite (100 mg/kg body weight, oral) for 4 weeks decreases the escape latency induced by $AlCl_3$ (70mg/kg body weight, IP) for 5 weeks treated rats during MWM test from second to fourth day of training (A). Also, Zeolite (100 mg/kg body weight, oral) for 4 weeks increases the time spent in defined part that declined by $AlCl_3$ (70me/kg body weight, IP) for 5 weeks treated rats in probe trial on the fifth day of MWM test. Data was expressed as means ± S.E.M.(N=8), significance a ($P<0.05$) against control, significance b ($P<0.05$) against $AlCl_3$

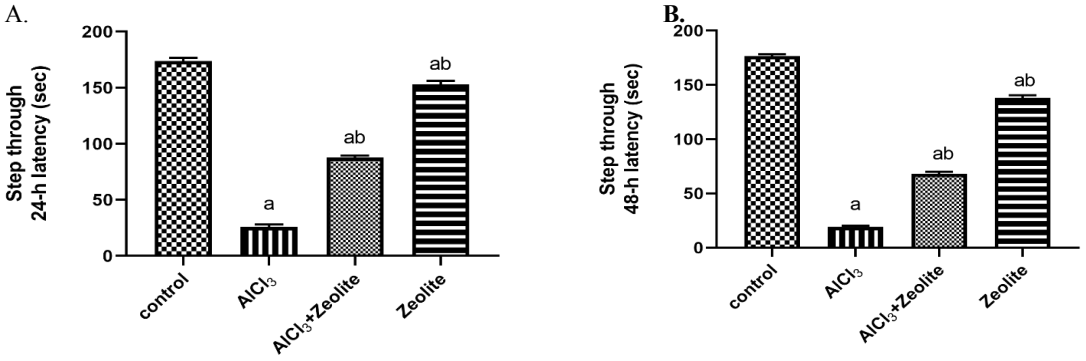


Figure 2: Zeolite (100 mg/kg body weight, oral) for 4 weeks decrease the step through latency which induced by $AlCl_3$ (70mg/kg body weight, IP) for 5 weeks treated rats using passive avoidance test (PA) twenty-four (24) (A) & forty-eight (48) hour (B) post training. Data was expressed as means ± S.E.M.(N=8), significance a ($P<0.05$) against control, significance b ($P<0.05$) against $AlCl_3$.

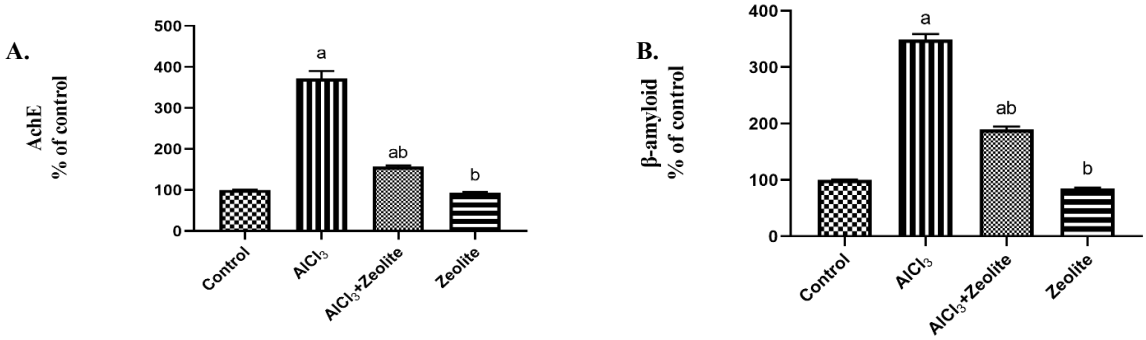


Figure 3: Zeolite (100 mg/kg body weight, oral) for 4 weeks decreases AChE (A) and Aβ (B) content induced by $AlCl_3$ (70mg/kg body weight, IP) for 5 weeks in treated rats. Data was expressed as means ± S.E.M.(N=8), significance a ($P<0.05$) against control, significance b ($P<0.05$) against $AlCl_3$.

3.4. Effect of zeolite on malondialdehyde and total antioxidant capacity content in aluminum chloride treated rats.

$AlCl_3$ group showed marked increase in MDA content and marked decrease in TAC content compared to Control group. On the other hand, $AlCl_3$ and zeolite treated groups showed a significant decrease in MDA content and a clear elevation in TAC content compared with $AlCl_3$ alone treated group as shown in (figure 4 A, B).

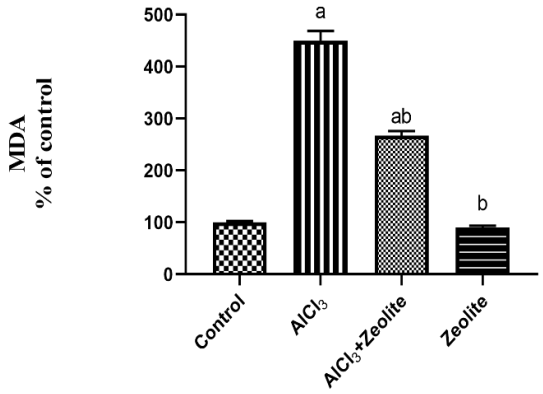
3.5. Effect of zeolite on tumor necrosis factor alpha and nuclear factor kappa B in aluminum chloride treated rats.

$AlCl_3$ group showed marked increase in TNF-α and NF-κB content compared to control group. However, treatment of rats with $AlCl_3$ and zeolite showed a clear reduction in TNF-α and NF-κB content compared with $AlCl_3$ alone treated group as shown in (figure 5 A, B).

3.6. Effect of zeolite on brain derived neurotrophic Factor, glycogen synthase kinase 3 beta and beta catenin content in aluminum chloride treated rats.

$AlCl_3$ group showed marked decrease in BDNF and P-GSK-3β content and showed marked increase in β-Catenin content compared to control group. However, treatment of rats with $AlCl_3$ and zeolite showed a clear elevation in BDNF and P-GSK-3β content, compared with $AlCl_3$ alone treated group, and showed a clear reduction in β-Catenin content compared with $AlCl_3$ alone treated group (figure 6 A, B, C and D).

A.



B.

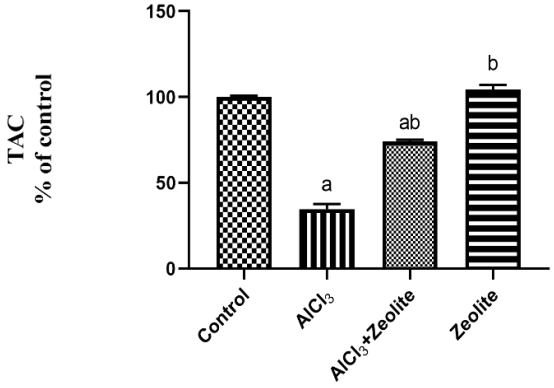
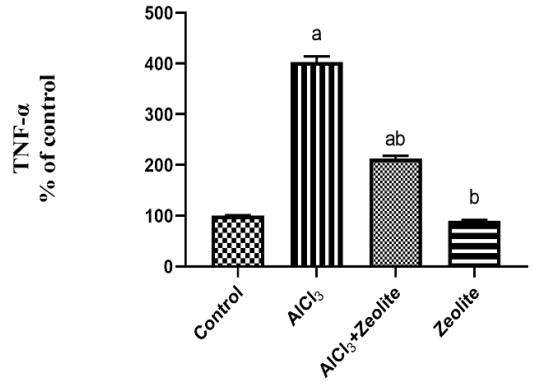


Figure 4: Zeolite (100 mg/kg body weight, oral) for 4 weeks decreases MDA content (A)induced by $AlCl_3$ (100 mg/kg body weight, oral) for 4 weeks in treated rats, and increase TAC content (B) that decreased by $AlCl_3$ (70mg/kg body weight, IP) for 5 weeks in treated rats. Data was expressed as means ± S.E.M.(N=8), significance a ($P<0.05$) against control, significance b ($P<0.05$) against $AlCl_3$.

A.



B.

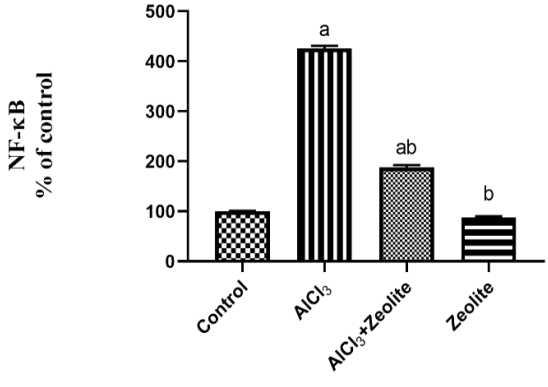
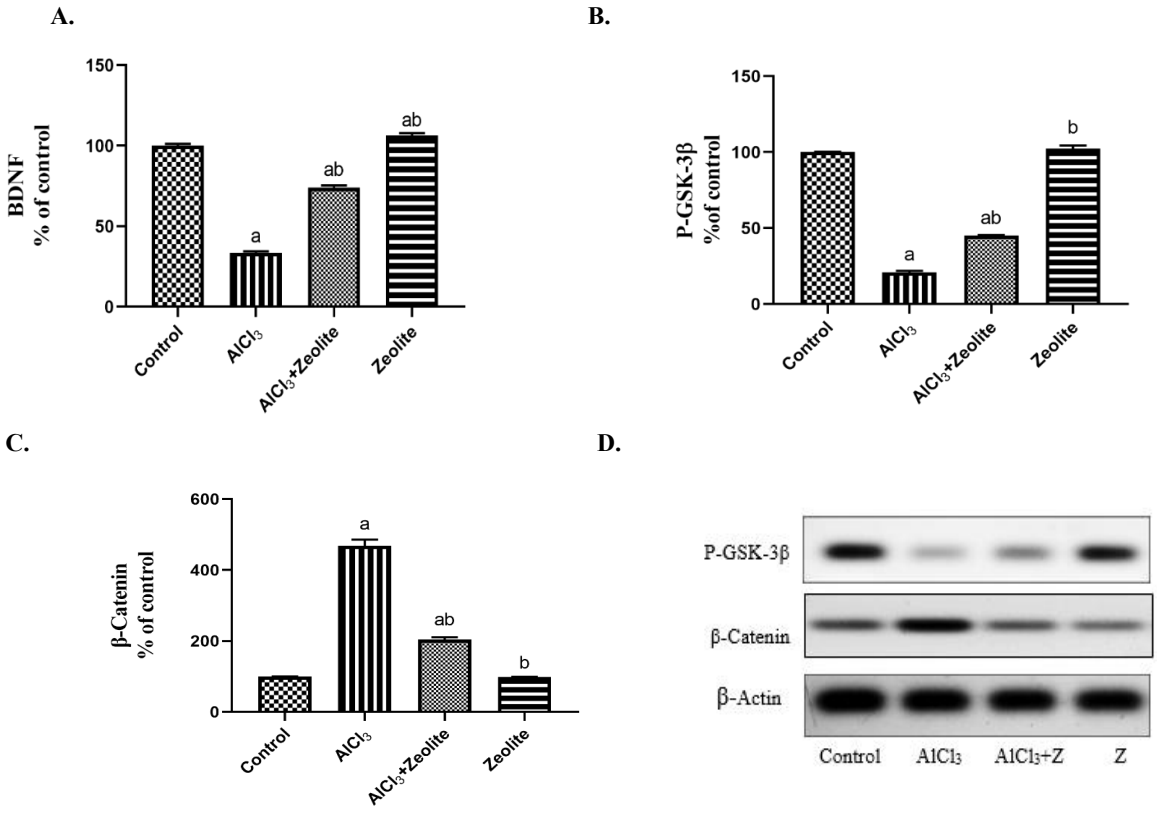


Figure 5: Zeolite (100 mg/kg/oral) for 4 weeks decreases TNF-α (A) and NF-κB (B) induced by $AlCl_3$ (70mg/kg body weight, IP) for 5 weeks in treated rats. Data was expressed as means ± S.E.M.(N=8), significance a ($P<0.05$) against control, significance b ($P<0.05$) against $AlCl_3$.



Figures 6: Zeolite (100 mg/kg body weight, oral) for 4 weeks increase BDNF content by ELISA (A)and increases P-GSK-3β content by western blotting technique (B&D) decreased by $AlCl_3$ (70mg/kg body weight, IP) for 5 weeks treated rats but decreases β-Catenin content by western blotting technique (C&D) induced by $AlCl_3$ (70mg/kg body weight, IP) for 5 weeks treated rats. Data was expressed as means ± S.E.M.(N=8), significance a ($P<0.05$) against control, significance b ($P<0.05$) against $AlCl_3$

3.7. Effect of zeolite on histopathological alteration of different brain regions in aluminum chloride treated rats Using hemoxylin and eosin staining.

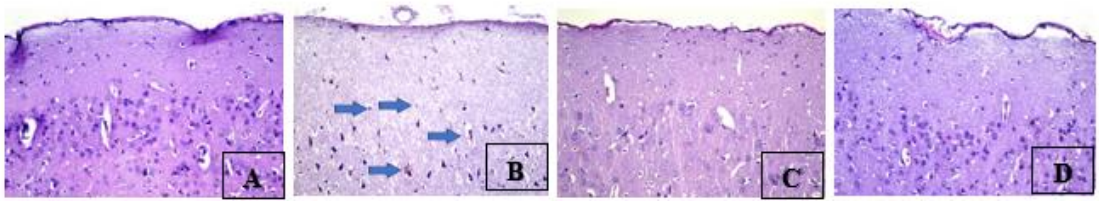


Figure 7: Zeolite (100 mg/kg body weight, oral) for 4 weeks improve histopathological alteration induced by $AlCl_3$ (70mg/kg body weight, IP) for 5 weeks in cerebral cortex. No histopathological alteration in control group(A) nuclear pyknosis and degeneration in all neurons of $AlCl_3$ treated group(B) no histopathological alteration in $AlCl_3$ and zeolite treated group(C) no histopathological alteration in zeolite alone treated group(D) magnification power of X 40.

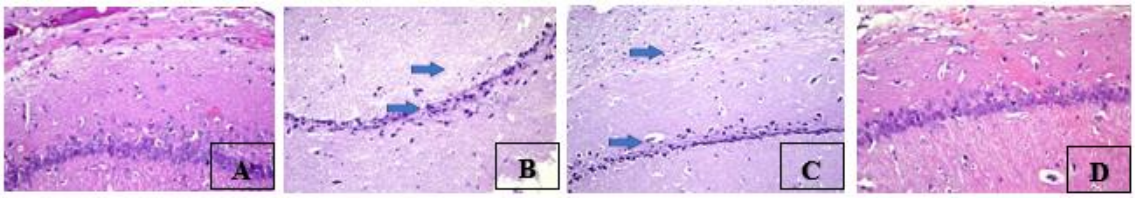


Figure 8: Zeolite (100 mg/kg body weight, oral) for 4 weeks improve histopathological alteration induced by $AlCl_3$ (70mg/kg body weight, IP) for 5 weeks in hippocampus (subiculum). No histopathological alteration in control group(A)nuclear pyknosis and degeneration in all neurons of $AlCl_3$ treated group(B) nuclear pyknosis and degeneration in $AlCl_3$ and zeolite treated group(C) no histopathological alteration in only zeolite treated group(D) magnification power of X 40.

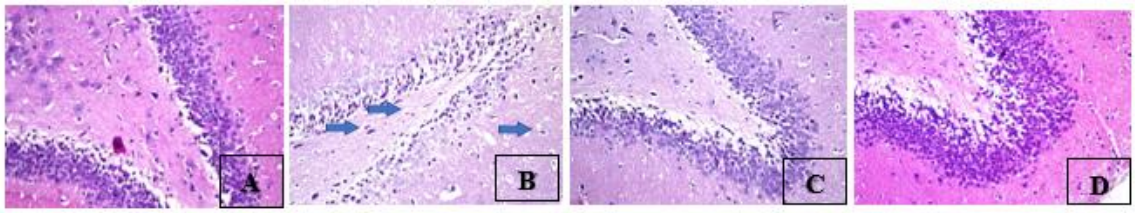


Figure 9: Zeolite (100 mg/kg body weight, oral) for 4 weeks improve histopathological alteration induced by $AlCl_3$ (70mg/kg body weight, IP) for 5 weeks in hippocampus (fascia dentata, hilus). No histopathological alteration in control group(A) nuclear pyknosis and degeneration in all neurons of $AlCl_3$ treated group(B) no histopathological alteration in $AlCl_3$ and zeolite treated group(C) and also, no histopathological alteration in only zeolite treated group(D) magnification power of X 40.

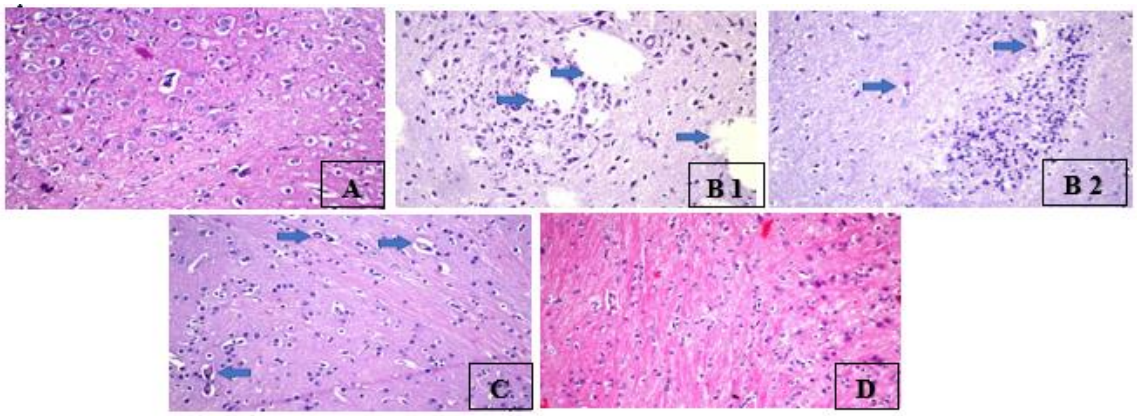


Figure 10: Zeolite (100 mg/kg body weight, oral) for 4 weeks improve histopathological alteration induced by $AlCl_3$ (70mg/kg body weight, IP) for 5 weeks in striatum. No histopathological alteration in control group(A) multiple focal areas of neuronal damage with diffuse gliosis in $AlCl_3$ treated group(B1) focal areas of gliosis in neurons of $AlCl_3$ treated group(B2) diffuse gliosis in between the intact neurons in $AlCl_3$ and zeolite treated group (C) no histopathological alteration in only zeolite treated group(D) magnification power of X40.

4. DISCUSSION

Aluminum metal is the biggest causes of the development of AD [15,16], aluminum accumulates in the cortex, cerebellum, and hippocampus, which are responsible for memory and cognition [16]. In our study administration of $AlCl_3$ increases escape latency and decrease time spent in the target part in probe test in Morris water maze (MWM) test and decreases latency in passive avoidance (PA) test which indicate memory and cognitive decline that's confirmed by study in which $AlCl_3.6H_2O$ decrease escape latency in MWM and increase step through latency in PA test[34].

Aluminum increased levels of APP by up-regulated gene expression for APP and so increased formation of abnormal amyloid peptides and enhance their aggregation and formation of amyloid plaques [35], Aβ aggregation led to free radicals like ROS to react with many proteins and lipids, which converted to oxidized toxic proteins and per oxidized lipids. [36,3].

Peroxidation of lipids leads to toxic product that migrate to the neurons and make several alterations of its functions. Disrupt the neuronal cell signaling and finally leads to neuronal cell death[37,4].

In our study, administration of $AlCl_3$ increased Aβ that's confirmed by study in which rats given $AlCl_3$,where $AlCl_3$-induced Aβ and tau formation [38] also increase AChE activity, oxidative stress marker as MDA and decrease in TAC that's confirmed by study in which $AlCl_3$ increased oxidative stress as increase in MDA level[39,34]. It also increases inflammatory mediators' markers (TNF-α and NF-κB) confirmed by study in which rats given $AlCl_3$ (100 mg/kg, oral) for 6 weeks lead to Activation of astrocytes and microglia and production of cytokines (TNF-α and NF-κB) [38].

When GSK-3β activity increased, prevent WNT/β-catenin signaling pathway and so impaired the memory, and decrease level of phosphorylated GSK-3β and so increase degradation of β-catenin of which increases Tau protein phosphorylation[12] and decreasing β-catenin and its gene expression [13,14].

WNT/β-catenin signaling pathway activation is neuroprotective in mouse AD model, as it reduces Aβ accumulation in cortex and hippocampus, improve the memory, and decrease the synapse protein level[40].

In our study, administration of $AlCl_3$ decreases phosphorylated GSK-3β content, and increase β-catenin and decrease BDNF content (down regulation and inhibition of PI3K/AKT signaling pathway and WNT/ β-catenin signaling pathway) that's confirmed by study in which Aluminum reduced phosphorylation of GSK-3β and decreases the expression of β-catenin and BDNF [41].

Histopathological examination of certain brain areas in our study $AlCl_3$ showed nuclear pyknosis and degeneration in neurons of cerebral cortex and hippocampus that's confirmed by study in which $AlCl_3$ (70mg/kg) for five weeks administration showing atrophy and neuronal degeneration in the hippocampus, associated with encephalomalacia in the striatum [42].

The aim of our study to explore the protective effect of zeolite (clinoptilolite) in $AlCl_3$-induced neuronal changes in rats. In our study we found that zeolite (clinoptilolite) will attenuate the increase in AChE activity that's confirmed by study in which Tuff zeolite (composed of 61% of clinoptilolite) showed a significant reduction of the cholinesterase activity in all the tissues after increased by administration of VX substance [43]

The decrease in Aβ confirmed by study in which the hippocampus of treated mice shows significant 54% decrease of Aβ level if comparing to control group [44].

Also, zeolite decrease oxidative stress marker as MDA and increase TAC content that's confirmed by study in which after partial hepatectomy and Clinoptilolite taken orally two times daily for 10 days in 5 mg dose decreases plasma and liver level of MDA than the partial hepatectomy group [45].

Also, zeolite decrease inflammatory mediators' markers (TNF-α and NF-κB) confirmed by study in which after1 and 24 hours treatment with zeolite reversed the increase in TNF-α and NF-κB level after the increase in TNF-α and NF-κB protein level by administration of Adriamycin (a hepatotoxic drug) [46].

zeolite also increase phosphorylated GSK-3β content, and decrease β-catenin and increase BDNF content (upregulation and stimulation of PI3K/AKT signaling pathway and WNT/ β-catenin signaling pathway) induced by $AlCl_3$ administration in rats.

As we previously confirmed that zeolite decreases Aβ content, oxidative stress and inflammatory mediators so for the first time we confirmed that zeolite decrease GSK-3β activity and decrease phosphorylation of β-catenin and enhance its stabilized in complex and then increase its gene expression include BDNF.

Histopathological examination of different brain tissue in our study where administration of zeolite will diminish nuclear pyknosis and degeneration stimulated by $AlCl_3$ administration in cerebral cortex and hippocampus that's confirmed by another study in which micronized zeolite treatment decreases Aβ plaque by 56% in micronized zeolite treated mice compared to control group [44].

In our study for the first time, we confirmed that zeolite attenuate increase in escape latency and the decrease in the time spent at the defined part in MWM, also, increase latency in PA test, that's also confirmed by our previous finding as we confirmed that zeolite attenuate the increase in AChE which is an important neurotransmitter involved in memory and cognition and also decrease Aβ aggregation which affect neuronal cells signaling especially in cortex and hippocampus (responsible of memory, thinking and cognition) lead to increased oxidative stress and release of inflammatory mediators from microglia affecting neuronal cell in destructive ways.

5. CONCLUSIONS

It could be concluded that Zeolite(clinoptilolite) can be used as a protective agent against aluminum chloride-induced progression of Alzheimer's disease in rats through different mechanistic pathways. Also, Zeolite(clinoptilolite) attenuates Aβ aggregation, oxidative stress, and inflammatory mediators release through modulation of PI3K/AKT signaling pathway and Wnt/ β-catenin signaling pathway.

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Conflicts of Interest no conflict of interest declared by authors.

Ethical Statement: Everything in animals' techniques was done according to the Ethics Committee of the faculty of Pharmacy Al-Azhar University, Egypt (permit number: 227/2019). Unnecessary disturbance of animals, pressure and tough maneuver was avoided.

Author Contribution: All authors were hand by hand in each part of this research

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