Neuropsychiatric Disease and Treatment

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ORIGINAL RESEARCH

Garden Cress (*Lepidium sativum*) Seeds Ameliorated Aluminum-Induced Alzheimer Disease in Rats Through Antioxidant, Anti-Inflammatory, and Antiapoptotic Effects

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Background: Bioaccumulation of aluminum in the brain is associated with adverse neuroinflammatory and neurodegenerative changes, such as those seen in Alzheimer's disease (AD).

Objective: This study aimed to assess the impact of the administration of *Lepidium sativum* (LS) extract on behavioral, biochemical, and cerebral histopathological changes in rats with $AlCl_3$-induced AD and explore the mechanism behind this effect.

Materials and Methods: This study was conducted on 40 male albino rats divided into four groups (n=10): LS (control, 20 mg/kg body weight for 8 weeks), AD ($AlCl_3$, 10 mg/kg body weight), and an LS-treated AD group. Behavioral assessment included radial armed maze and active avoidance training tests. Proinflammatory cytokines, oxidant/antioxidant markers, Aβ, AchE, tau protein, $TGF\beta_1$, homocysteine, folic acid, and vitamin B_{12} were biochemically assessed in the serum. The cerebral cortex was histopathologically examined.

Results: $AlCl_3$ administration significantly impaired rats' memory, indicating AD-like behavioral changes, significantly increased ($P<0.001$) oxidative stress markers, enhanced proinflammatory cytokines, and significantly increased AChE ($P<0.001$) adding to cytotoxic effects and neuronal loss in the cerebral cortex. LS administration significantly improved the antioxidant parameters, reduced proinflammatory cytokines, and alleviated AD-associated histopathological changes.

Conclusion: LS ameliorated $AlCl_3$-induced changes through its antioxidant, anti-inflammatory, and antiapoptotic effects, suggesting that it has a neuroprotective effect.

Keywords: *Lepidium sativum*, aluminum, neuroinflammation, antioxidant, Aβ, AchE, tau, $TGF\beta_1$

Introduction

Exposure of humans to aluminum has significantly increased worldwide. Potential sources of aluminum exposure include drinking water, food, medicines, vaccines, and aluminum cookware utensils. Aluminum bioaccumulation in the brain has been associated with neuroinflammatory and neurodegenerative changes that might predispose to Alzheimer's disease (AD).[1] Subchronic administration of Al^{3+} to rats could be used as a beneficial model for the screening of new anti-AD drugs.[2] It has been reported that although none of the currently available animal models of AD recapitulates all aspects of human AD, $AlCl_3$-induced AD is among the highly valid ones. Despite that, one should always be aware of the potential dangers of uncritically extrapolating from model organisms to a human condition that takes decades to develop and mainly involves higher cognitive functions.[3]

AD is considered the most common cause of dementia. The number of people aged ≥65 years with AD is expected to increase to 12.7 million by 2050. AD is formally listed as the sixth-leading cause of death in the US and the fifth-leading

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cause of death for those aged ≥65 years. AD is manifested by cognitive inability attributed to deposition of β-amyloid, formation of hyperphosphorylated neurofibrillary tangles, and a malfunctioning cholinergic system.[4]

The FDA has approved drugs for only the symptomatic treatment of AD. Unfortunately, the drugs available that are being used currently for treating AD cannot slow or stop the destruction of neurons that cause AD symptoms and make the disease fatal.[5] Added to that, most of these drugs have severe side effects; therefore, alternative therapies that include the use of medicinal plants for the treatment of AD attract the attention of many researchers, as many of them can enhance the memory and learning process. Numerous phytochemicals have shown promising results in AD therapy.[6,7]

It has been reported that plant-based medicines play a significant role in health care worldwide in traditional, alternative and preventive medicines, as they represent a source of biologically active components.[8] *Lepidium sativum* (LS), which belongs to the Brassicaceae family, is used as a remedy for many diseases and possesses many pharmacological properties, including anti-inflammatory, antioxidant, and antidiabetic activities.[9] However, the underlying molecular mechanisms of some medicinal uses of LS extracts are unclear. In a recent study, it was postulated that lepidine B and E, the major component of LS alkaloids, are noncompetitive inhibitors of acetylcholinesterase[10] by interacting with peripheral anionic sites of AChE and butyrylcholinesterase; therefore, they can prevent HuAChE-induced Aβ aggregation, in addition to possessing promising anti-AD activity.[11,12]

TGFβ is a pleiotropic cytokine and a potent regulator of neuroinflammation and cytotoxicity. Many beneficial effects depend on the regulation of microglial cell activity by TGFβ.[13] TGFβ orchestrates the expression of numerous genes associated with inflammation and the immunoresponse. In the nervous system, the TGFβ pathway is involved in the regulation of genes associated with the cell cycle, cell proliferation, preservation of neural progenitor cells, oligodendroglia and neuronal differentiation, neuron survival and function, and several neurotransmission-related genes.[14] Impaired expression of TGFβ signaling has been reported to be involved in the pathogenesis of AD, as AD patients show decreased plasmatic levels of $TGF\beta_1$.[15] Therefore, the expression of this cytokine was assessed in this study. This study aimed to assess the impact of the administration of LS extract on behavioral, biochemical, and cerebral histopathological changes in rats with AlCl-induced AD and explore the mechanisms behind this effect.

Materials and Methods

Chemicals

$AlCl_3$ (Sigma-Aldrich, St Louis, USA) was supplied as white powder. The powder was dissolved in distilled water and given at a dose of 10 mg/kg body weight. All chemicals used in this study were of analytical grade.

Preparation of *Lepidium Sativum* Aqueous Extract

LS seeds were purchased from a local market in Jeddah, Saudi Arabia. They were verified by a botanist at the Faculty of Sciences, King Abdulaziz University. The aqueous extract was prepared in accordance with traditional Moroccan phytotherapy.[16] Exactly 1 g of the powdered seeds was mixed with 100 mL distilled water and the mixture boiled for 10 minutes, then cooled for 15 minutes. Thereafter, the aqueous extract was filtered using a Millipore filter (0.2 mm, St Quentin en Yvelines, France) to remove particulate matter. The filtrate was then freeze-dried and the desired dose (mg lyophilized aqueous extract of LS seeds/kg body weight) was then prepared and reconstituted in 0.5 mL distilled water. The aqueous extracts were reconstituted daily, just before administration. These were then given orally to the groups of rats at a dose of 20 mg/kg body weight.

Animals

Forty male albino rats weighing from 120–140 g were purchased from the animal house at the King Fahed Medical Research Center (KFMRC). The guidelines for dealing with animals followed by the KFMRC were adopted in this study. The rats were left for 1 week to acclimatize at the behavior science laboratory at KFMRC under standard lab conditions: relative humidity 70%, temperature 24°±1°C, and exposure to light and dark cycles of 12 hours each. After 1 week, they were divided into four groups. Rats in the control group received intraperitoneal injections of normal saline and orally given normal saline through gavage for 8 weeks. Rats in the positive-control group (LS) were orally given LS water

extract at a dose of 20 mg/kg through gavage for 8 weeks.[17] Rats in the AD group received daily intraperitoneal injections of $AlCl_3$ (10 mg/kg body weight) dissolved in distilled water for 8 weeks and normal saline orally.[18] Rats in the treated group (LS-treated AD) were injected intraperitoneally with $AlCl_3$ daily for 8 weeks and received normal saline through oral gavage during the first 4 weeks of the experiment, which was replaced by LS water extract through oral gavage during the last 4 weeks of the experiment.s

Behavioral Assessment

Radial Armed Maze

This test was performed to assess spatial memory in rats. It was conducted in accordance with Tarragon et al.[19] The maze used consisted of eight chambers. During the first day of the test and after being fasted overnight, each rat was left in the center of the maze to explore the maze freely without recording. During days 2–5 of the test, each rat was placed in the maze center and given a maximum 6 minutes to find the food. If the rat found the food before this, then the watch was stopped and the elapsed time recorded.

Active Avoidance Training

This test was performed to assess memory and cognitive function in rats that had received $AlCl_3$. It was conducted in accordance with the method described by Hirakawa[20] using special apparatus (Lafayette Instrument) consisting of a closed chamber and power control. The latter was utilized for generation of a warning sound and electric shock of 1 MA. The floor of the closed chamber has metallic bars that delivered an electric shock. A plastic pole, which could be climbed by the rat, was in the center of the chamber. During the test, the rat was allowed to explore the chamber for 1 minute, then a warning sound was released for 3 seconds, followed by an electric shock for 2 seconds. A break of 7 seconds was allowed before the next warning sound and another break of 60 seconds every five sessions.

The test consisted of 15 sessions for each rat every day. During the session, three variables were recorded: "none" if the rat did not escape the electric shock by climbing the pole, "escape" if the rat climbed the pole to escape the electric shock, and "avoid" if the rat climbed the pole during the warning sound to avoid the electric shock. The avoidance percentage was calculated for each animal and the mean of the group calculated.

Biochemical Assessment

Assessment of Proinflammatory Cytokines TNFα, IL6, Amyloid β, AchE, Tau Protein, and TGFβ

Blood samples were taken from the intra-orbital sinus at the end of the experiment. Serum was obtained by centrifugation at 3000 rpm for 15 minutes and kept at −18°C. TNFα, IL6 (Abcam, Cambridge, UK), TGFβ (DRG International, Springfield, USA), Aβ, and tau (MyBioSource, San Diego, USA) were assessed using sandwich solid-phase ELISA[21] kits as per the manufacturers' instructions.

Assessment of Homocysteine, Folic Acid, and Vitamin B_{12}

Homocysteine (MyBioSource), folic acid (Cusabio Technology, Houston, USA), and vitamin B_{12} (G-Biosciences, St Louis, USA) were assessed in serum using ELISA kits as per the manufacturers' instructions.

Assessment of Malondialdehyde, Nitric Oxide, Superoxide Dismutase, and Catalase in Serum and Brain and Total Antioxidant Capacity in Serum

Levels of SOD were assessed in both serum and brain using an assay kit (Biodiagnostic, Egypt) and in accordance with the method described by Packer.[22] To quantify CAT activity, assay kits (Biodiagnostic) were used as previously described.[23] TAC (Elabscience, Texas, USA) was assessed in serum using the Biodiagnostic assay kit in accordance with the method described by Packer.[22]

Histopathological Assessment

At the end of the experiment, the rats were anesthetized using with 4% isoflurane (Sedico Pharmaceuticals, Cairo, Egypt) in 100% oxygen, then euthanized by cervical dislocation. Blood samples were immediately obtained from the heart for biochemical assessment. Centrifugation was performed at 3000 rpm for 15 minutes at 4°C to obtain serum. The whole

brain was also immediately and gently removed from the skull, then divided into two halves. The right half was fixed in 10% formaldehyde and further processed into paraffin blocks, while the left half was stored at −80°C for biochemical assessment of proteins. Small pieces of the right half of the brain were fixed in 4% gluteraldehyde, then osmium tetroxide. They were processed and embedded in Epon, then sectioned into semithin sections (0.5–1 mm) and stained with toluidine blue. Ultrathin sections (500–800 nm) were counterstained with uranyl acetate and lead citrate and examined with a transmission electron microscope (TEM; JEM-100 Cx11, JEOL, Egypt) belonging to the TEM Unit of Assiut University.

For histological examination, the paraffin blocks were sectioned at 4 μm thickness, then stained H&E, as well as immunohistochemically stained using the streptavidin–biotin–peroxidase technique. Anti-GFAP antibody (sc-33673, Santa Cruz Biotechnology, USA, dilution of 1:100) was used as a specific maker of astrocytes. Antibodies against proapoptotic proteins caspase 3 (sc-7272, Santa Cruz Biotechnology, dilution of 1:100) and caspase 7 (sc-56063, Santa Cruz Biotechnology, dilution of 1:1000) were used to detect apoptosis. Aβ (sc-28365, Santa Cruz Biotechnology, dilution of 1:100) was used for detection of β-mmyloid, which is deposited in a variety of neurological disorders, including AD. A light microscope (BX-51, Olympus, Germany) connected to a digital camera was used for photographing. Image-Pro Plus 6.0 was used for semiquantitative assessment of immunoexpression of used antibodies. Area percentages of caspase 3, caspase 7, Aβ, and GFAP were assessed in five nonoverlapping sections in each animal at magnification 200×.

Statistical Analysis

Statistical analysis was performed using SPSS 20. Results are expressed as means ± SD. Data were compared using one-way ANOVA followed by Bonforoni post hoc test to assess statistical significance. Differences were regarded as significant at $P<0.05$.

Results

Radial Armed Maze

Average elapsed time to find the baited arm was significantly longer ($P<0.001$) in the AD group than the control (186.61 ±7.47 versus 134.35±29.72 seconds). On the other hand, time was significantly shorter ($P<0.001$) in the LS-treated AD group compared to the AD group (149.42±1.82 versus 186.61±7.47 seconds). There was no significant difference in the LS-treated group from the control (Figure 1).

Active Avoidance Training

The AD group showed significantly lower ($P<0.001$) average avoidance response through the 5 days on the active avoidance test of 38.54% compared to the control. On the other hand, the LS-treated AD group showed a significantly higher ($P<0.001$) avoidance response (1.14-fold) than the AD group (Table 1). Figure 1 shows progressive increases in avoidance response along the 5 days of the active avoidance test in the control, LS-treated, and LS-treated AD groups.

Effect of LS on Oxidant/Antioxidant Profile

Levels of MDA in serum and cerebral cortex and NO in the serum were significantly higher ($P<0.001$) in the AD group than the control and significantly lower in the LS-treated AD group ($P=0.003$ and $P<0.001$, respectively) than the AD group (Table 2). Levels of SOD and CAT were significantly higher ($P=0.04$ and $P<0.001$, respectively) in the serum of the LS-treated group and significantly lower ($P<0.001$) in both serum and cerebral cortex of the AD group than the control. On the other hand, SOD and CAT levels were significantly higher in the serum ($P<0.001$ and $P=0.002$, respectively) and cerebral cortex ($P=0.02$ and $P=0.004$, respectively) of the LS-treated AD group than the AD group (Table 2).

Effect of LS on Proinflammatory Cytokines

Serum levels of TNFα and IL6 were significantly higher ($P<0.001$) in the AD group than the control and significantly lower ($P<0.001$) in the LS-treated AD group than the AD group (Table 2).

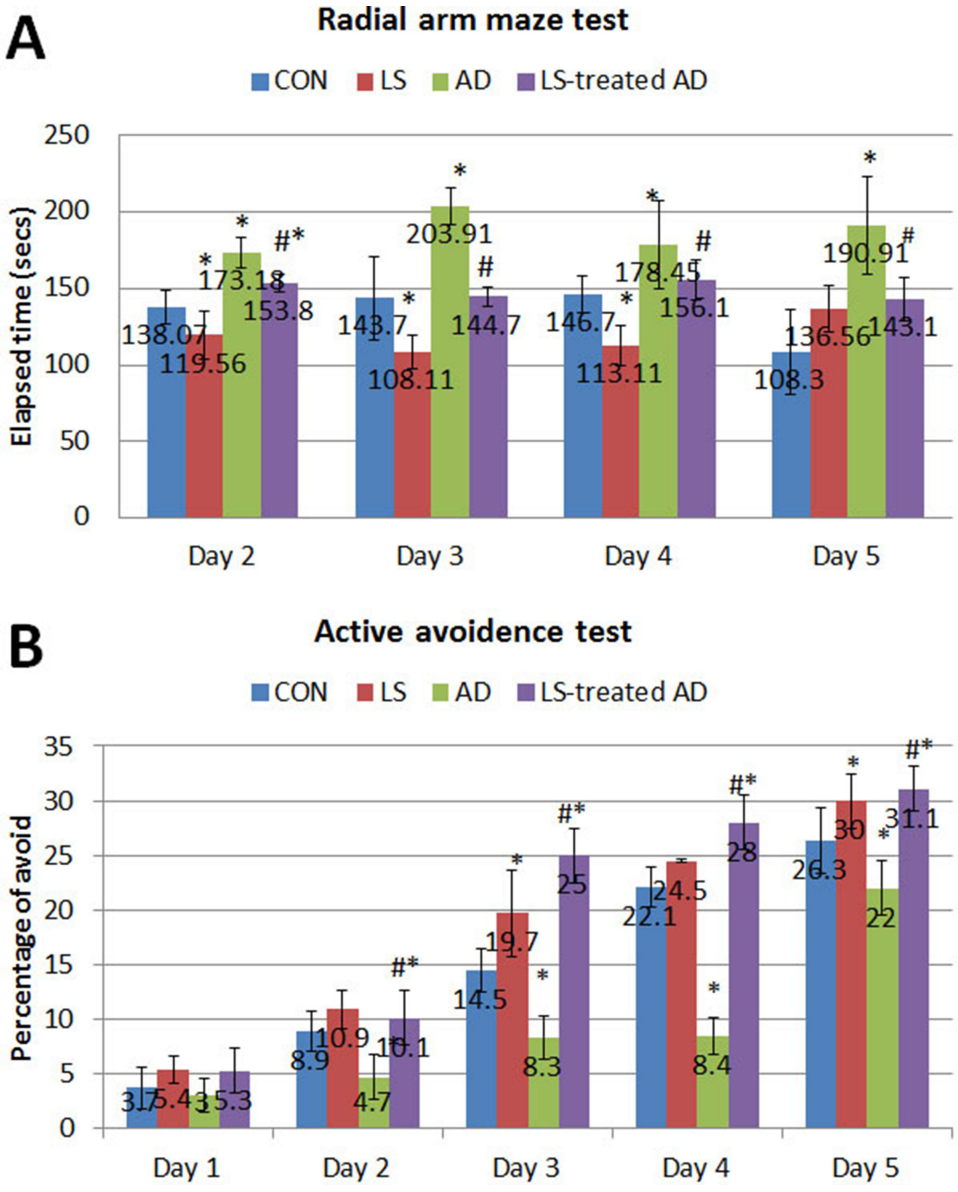

Figure 1 Effect of $AlCl_3$ and *Lepidium sativum* (LS) on radial arm maze (**A**) and active avoidance (**B**) tests. *Versus control; #versus AD group.

Effect of LS on AchE, Tau Protein, and Aβ

Serum and cerebral cortex levels of AchE were significantly higher ($P<0.001$) in the AD group than the control and significantly lower ($P<0.001$) in the LS-treated AD group than the AD group (Table 2). Serum levels of tau and Aβ protein were significantly higher ($P<0.001$) in the AD group than the control and significantly lower ($P=0.01$ and $P<0.001$, respectively) in the LS-treated AD group than the AD group (Table 2).

Effect of LS on Serum TGFβ

Levels of TGFβ in the serum were significantly higher ($P<0.001$) in the AD group than the control. Administration of LS resulted in a significant reduction ($P<0.001$) in serum TGFβ compared to the AD group (Table 2).

Effect of LS on Serum Levels of Folic Acid, Vitamin B_1, and Homocysteine

Serum levels of folic acid and vitamin B_1 were significantly higher ($P<0.001$ and $P=0.03$, respectively) in the LS-treated group and significantly lower ($P<0.001$) in the AD group than the control. On the other hand, folic acid and vitamin B_1 levels were significantly higher ($P<0.001$) in the LS-treated AD group than the AD group. Although the level of homocysteine showed no

Table 1 Effect of LS on behavioral changes

	Con (n=10)	LS (n=10)	AD (n=10)	LS-treated AD (n=10)
Mean elapsed time on radial arm maze test				
Day 2 (seconds)	138.07±11.38	119.56±16.71 *P*=0.004	173.18±9.46 *P*<0.001	153.8±5.81 *P*=0.03 *P1*=0.002
Day 3 (seconds)	143.7±27.57	108.11±11.63 *P*<0.001	203.91±12.94 *P*<0.001	144.7±6.13 *P*=0.98 *P1*<0.001
Day 4 (seconds)	146.7±12.97	113.11±13.14 *P*=0.004	178.45±29.52 *P*=0.004	145.10±13.69 *P*=0.97 *P1*=0.002
Day 5 (seconds)	108.30±28.34	136.56±15.56 *P*=0.79	190.91±32.08 *P*<0.001	137.10±14.11 *P*=0.35 *P1*=0.02
Average (seconds)	134.35±29.72	119.33±4.18 *P*=0.25	186.61±7.47 *P*<0.001	149.42±18.2 *P*=0.22 *P1*<0.001
Percentage of avoidance response in active avoidance test				
Day 1 (seconds)	3.70±1.89	5.40±2.17 *P*=0.29	3.00±1.41 *P*=0.96	5.30±1.89 *P*=0.37 *P1*=0.05
Day 2 (seconds)	8.90±1.79	10.90±1.79 *P*=0.21	4.70±1.89 *P*<0.001	10.10±2.60 *P*=0.98 *P1*<0.001
Day 3 (seconds)	14.50±2.18	19.70±4.30 *P*<0.001	8.30±2.21 *P*<0.001	25.00±2.58 *P*<0.001 *P1*<0.001
Day 4 (seconds)	22.10±1.79	24.50±2.55 *P*=0.12	8.40±1.71 *P*<0.001	28.00±2.58 *P*<0.001 *P1*<0.001
Day 5 (seconds)	26.30±2.86	30.00±2.58 *P*=0.02	22.00±2.59, *P*=0.01	31.10±2.60 *P*=0.002 *P1*<0.001
Average (seconds)	15.10±0.83	18.10±0.69 *P*<0.001	9.28±0.69 *P*<0.001	19.90±1.71 *P*<0.001 *P1*<0.001

Notes: Comparisons between the groups were performed using one-way ANOVA followed by Bonforoni post hoc test. Significance was taken as $P<0.05$. *P*, versus control; P_1, versus AD group.

significant difference in the LS-treated group **from the control**, it was significantly higher ($P<0.001$) in the AD group than the control and significantly lower ($P<0.001$) in the LS-treated AD group than the AD group (Table 2).

Histopathological Findings

Upon examination of the cerebral cortex of the studied groups using the light microscope, it was observed that the control group showed intact neurons with intact, prominent nuclei and basophilic cytoplasm. The neurons were surrounded by

Table 2 Effect of LS on biochemical changes (n=10)

	Con (n=10)	LS (n=10)	AD (n=10)	LS-treated AD (n=10)
AchE in cerebral cortex (pg/100mg protein	121.15±4.72	120.91±3.75 *P*=0.97	185.16±.97 *P*<0.001	153.00±3.36 *P*<0.001 *P1*<0.001
SOD in cerebral cortex (pg/100 mg protein)	758.73±7.63	825.92±43.17 *P*=0.38	411.16±96.47 *P*<0.001	524.01±115.75 *P*<0.001 *P1*=0.02
CAT in cerebral cortex (pg/100 mg protein)	3.45±0.28	4.02±0.79 *P*=0.44	1.11±0.68 *P*<0.001	2.24±0.85 *P*=0.002 *P1*=0.004
MDA in cerebral cortex (pg/100 mg protein)	3.44±0.32	2.66±0.66 *P*=0.61	9.62±0.97 *P*<0.001	5.70±0.97 *P*<0.001 *P1*<0.001
IL6 in the serum (pg/mL)	15.40±0.58	14.29±0.31 *P*=0.98	39.19±1.6 *P*<0.001	26.94±3.47 *P*<0.001 *P1*<0.001
TNFα in the serum (pg/mL)	174.50±1.89	170.70±2.36 *P*=0.97	484.00±2.32 *P*<0.001	376.70±1.39 *P*<0.001 *P1*<0.001
Tau protein in the serum (pg/mL)	21.75±1.46	21.36±2.30 *P*=0.96	69.31±18.42 *P*<0.001	54.68±1.91 *P*<0.001 *P1*=0.01
Aβ in the serum (pg/mL)	50.97±3.29	46.36±4.16 *P*=0.08	134.58±3.00 *P*<0.001	100.56±5.01 *P*<0.001 *P1*<0.001
AchE in serum (pg/mL)	105.84±5.38	107.21±8.14 *P*=0.95	135.09±1.69 *P*<0.001	124.49±2.55 *P*<0.001 *P1*<0.001
SOD in serum (pg/mL)	423.36±7.23	481.22±19.83 *P*=0.04	128.06±9.32 *P*<0.001	282.61±86.05 *P*<0.001 *P1*<0.001
CAT in serum (pg/mL)	1.10±0.14	1.78±0.36 *P*<0.001	0.26±0.21 *P*<0.001	0.74±0.31 *P*=0.03 *P*=0.002
TAC in serum (pg/mL)	3.79±0.57	5.05±1.38 *P*=0.02	1.20±0.70 *P*<0.001	2.69±0.68 *P*=0.05 *P*=0.004
MAD in serum (pg/mL)	1.35±0.20	0.73±0.45 *P*=0.14	2.97±0.94 *P*<0.001	1.98±0.46 *P*=0.12 *P*=0.003
NO in serum (pg/mL)	1.11±0.23	0.51±0.03 *P*=0.17	12.87±1.05 *P*<0.001	7.20±0.43 *P*<0.001 *P1*<0.001

(*Continued*)

Table 2 (Continued).

	Con (n=10)	LS (n=10)	AD (n=10)	LS-treated AD (n=10)
TGFβ in serum (pg/mL)	86.48±6.67	66.99±5.59 *P*<0.001	179.66±9.01 *P*<0.001	130.72±3.22 *P*<0.001 *P1*<0.001
Folic acid in serum (pg/mL)	63.99±3.39	82.54±3.74 *P*<0.001	40.02±1.90 *P*<0.001	50.88±1.71 *P*<0.001 *P1*<0.001
Vitamin B_1 in serum (pg/mL)	215.38±11.31	225.87±6.46 *P*=0.03	153.98±6.39 *P*<0.001	187.50±5.21 *P*<0.001 *P1*<0.001
Homocysteine in serum (nmol/mL)	3.81±0.36	3.77±0.38 *P*=0.96	10.38±0.68 *P*<0.001	7.60±0.44 *P*<0.001 *P1*<0.001

Notes: Comparisons between the groups were performed using one-way ANOVA followed by Bonforoni post hoc test. Significance was taken as *P*<0.05. *P*, versus control; P_1, versus AD group.

many glial cells with small dense nuclei together with some blood vessels. There was no difference in the structure of the cerebral cortex between the control and LS-treated groups. On the other hand, most neural and glial cells of the cerebral cortex of the AD group appeared small, shrunken, and had dark nuclei, while most of those in the cerebral cortex of the LS-treated AD group appeared intact, apart from a few degenerated neurons that showed dark cytoplasm and deeply stained nuclei (Figure 2).

Examination of the ultrastructure of the cerebral cortex using electron microscopy revealed that both the control and LS-treated groups showed intact neurons with open-face nuclei and homogeneous cytoplasm and many glial cells with small dense nuclei. On the other hand, most neural and glial cells of the cerebral cortex of AD group showed dark, irregular nuclei and many autophagic vacuoles. Most neural and glial cells of the cerebral cortex of the LS-treated AD group appeared intact, apart from a few degenerated neurons that showed dark cytoplasm and deeply stained nuclei (Figure 3). Immunohistochemical expression of caspase 3 and caspase 7 was assessed in the cerebral cortex. Both were significantly upregulated ($P<0.001$) in the AD group compared to the control and significantly lower ($P<0.001$) in the LS-treated group than the AD group. The LS group did not show a significant difference in caspase 3 or caspase 7 expression compared to the control group (Figure 2).

Immunohistochemical expression of Aβ in the AD group was significantly higher ($P<0.001$) than the control and significantly lower in the LS-treated AD group ($P<0.001$) than the AD group. The LS group did not show a significant difference in Aβ expression from the control group (Figure 2). When it came to GFAP, its immunoexpression in the cerebral cortex in the AD group was significantly lower ($P<0.001$) than the control and significantly higher ($P=0.001$) in the LS-treated AD group than the AD group. LS group did not show a significant difference in GFAP expression from the control group (Figure 2).

Discussion

AD is the most devastating, progressive type of neurodegenerative diseases, and is associated with cognitive loss and dementia, mainly in old age. Based on the records of the World Health Organization, >50 million have dementia worldwide, with around 10 million new cases each year.[1] A large body of evidence exists on the link between gradual aluminum accumulation in brain tissue and progression of AD pathology. Clinical studies have also reported significantly increased levels of aluminum in brain tissue of AD patients.[24,25] Aluminum has been reported to induce AD-like symptoms in rodents through induction of oxidative stress, neuroinflammation, and neurotransmitter imbalance.[12] Therefore, LS was selected in this study to test its efficacy as it possesses antioxidant, anti-inflammatory, neuroprotective, and anticholinesterase properties.[11] This study was designed to evaluate the efficacy of LS in alleviating behavioral, biochemical, and cerebral histopathological changes associated with $AlCl_3$-induced AD in rats and explore the mechanism of this effect.

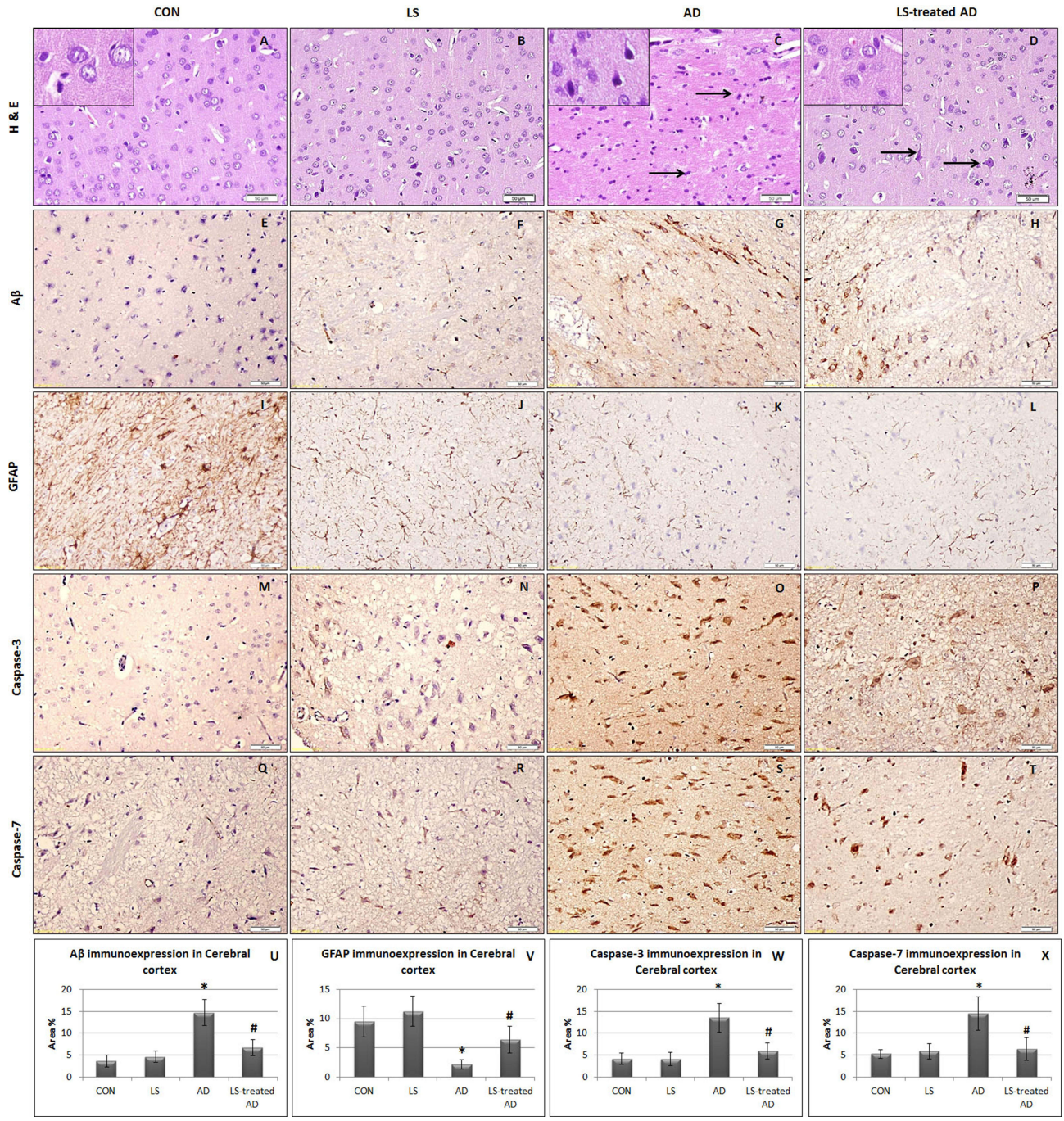

Figure 2 Sections in the cerebral cortex of the control (**A**) and LS-treated (**B**) groups stained with H&E show intact neurons with open-face nuclei and basophilic cytoplasm, as well as many glial cells that have small dense nuclei and some blood vessels. Cerebral cortex of AD group (**C**) shows that most neural and glial cells have small and dark nuclei. Most neural and glial cells of the cerebral cortex of LS-treated AD group (**D**) appear intact, apart from a few degenerated neurons that show dark cytoplasm and deeply stained nuclei (H&E [AD] ×200). Sections in the cerebral cortex stained with anti-Aβ (**E–H**), anti-GFAP (**I–L**), anti-caspase 3 (**M–P**), and caspase 7 (**Q–X**; immunohistochemistry ×200). Immunoexpression of these antibodies are shown in graphs. Comparisons between the groups were performed using one-way ANOVA followed by Bonforoni post hoc test. Significance was taken as $P<0.05$. *Versus control; #versus AD group.

Rats treated with $AlCl_3$ for 8 weeks in this study showed behavioral, biochemical, and histopathological changes relevant to AD. These changes included marked reduction in memory, learning and cognitive functions, extracellular Aβ accumulation, and significant changes in antioxidant profile and proinflammatory cytokines. These findings were in line with many previous studies.[1,12,26] Administration of LS with $AlCl_3$ for 4 weeks improved the learning and memory, significantly attenuated AChE activity and proinflammatory cytokine release, and enhanced antioxidant status.

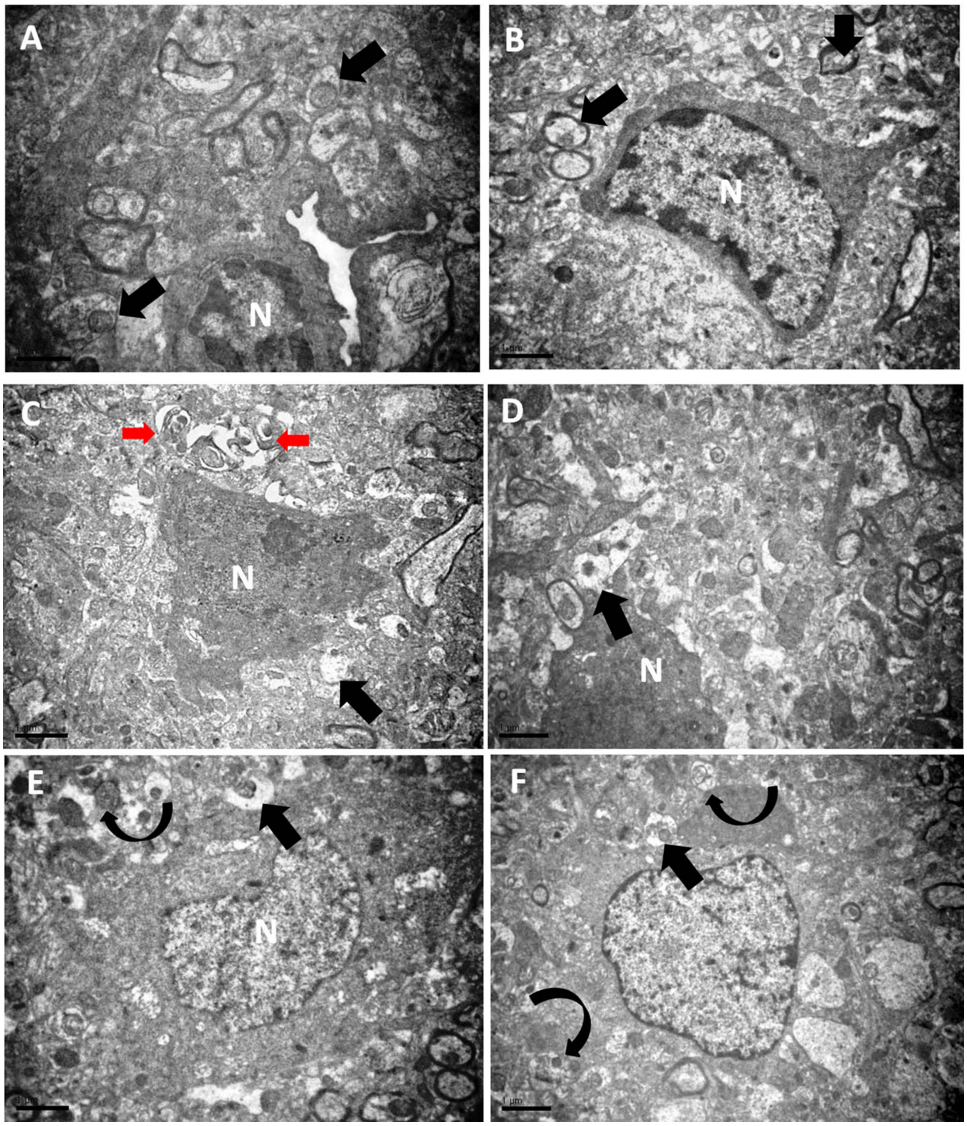

Figure 3 Electron micrographs of cerebral cortex of control (**A**) and LS-treated (**B**) rats show intact neurons with open-face nuclei and prominent nuclei (N). Many glial cells (thick arrows) with small dense nuclei appeared, surrounding these neurons (transmission electron microscopy). Cerebral cortex of AD (**C** and **D**) show small, shrunken, and deformed neural and glial cells (thick arrows) with dark, deformed, irregular nuclei (N) and some autophagosomes (red arrows). LS-treated AD (**E** and **F**) rats show most neurons intact with open-face and prominent nucleiN. Many glial cells (thin arrows) appear intact, apart from a few degenerated cells (thick arrows) that show dark vacuolated cytoplasm and deeply stained nuclei (transmission electron microscopy).

In this study, radial armed maze training was performed to assess the effect on spatial memory in rats. Active avoidance training was also performed to assess memory and cognitive functions. The AD group showed reduced spatial memory, which was evident by the significant increase in elapsed time during the radial armed maze test. This group showed also significantly decreased average avoidance response through the 5 days of the active avoidance test, indicating impaired memory and cognitive function. In accordance with that, impairment of spatial memory and cognitive functions has been observed in in male Swiss mice using a radial armed maze test[27] and Wistar rats using a Morris water maze and novel object-recognition test.[12]

The consistency in the results of both radial arm maze and active avoidance tests in this study indicated that they may evaluate memory processing in a specific region of rat brain. It has been reported that Al^{3+} can result in severe derangement of long-term potentiation in the hippocampus by hindering downstream signaling molecules' cyclic guanosine monophosphate (cGMP) and produce a disruption of the glutamate–nitric oxide cGMP pathway in the rat brain.[28] The LS-treated AD group in this study showed a significant reduction in elapsed time, indicating improved memory and learning. The memory- and learning-enhancing effects of LS observed in this study might have been due to the decreased AChE activity detected improving cholinergic neurotransmission.

Consistenly with the findings of this study, increased AChE activity in $AlCl_3$-exposed rats has been previously described.[29,30] Al^{3+} can increase the activity of AChE, leading to ACh overconsumption.[31] The increased AChE activity is possibly due to the direct neurotoxic effect of $Al3^+$, which was reported to change the kinetic properties of AChE.[32] It might also be attributed to Aβ overproduction, observed also in the current study, which promotes the activity of AChE through α7 nicotinic acetylcholine receptors.[33] One of the reported nonclassical roles of AChE is its ability to accelerate the assembly of the Aβ peptide into amyloid fibrils, and hence AChE–Aβ complexes show enhanced neurotoxicity compared with fibrils containing only Aβ.[34] This could explain the cellular neurotoxicity and enhanced cell death observed in the cortex in $AlCl_3$-induced AD in the current study.

Protein misfolding and aggregation are considered key players in the pathogenesis of neurodegeneration. Specifically, abnormal expression of Aβ that occurs as part of Al-induced chronic neuroinflammation is triggered by glial cell activation.[35] $AlCl_3$-induced AD in this study was associated with a significant increase in neurodegeneration-associated proteins, including Aβ and tau in the serum, and this was confirmed immunohistochemically in the cerebral cortex. These findings are in accordance with Zaky et al.[36] In a previous study, the calculated drastic decrease in the insoluble-to-soluble infrared spectroscopy (IR) protein ratio (A1673:A1695 cm) in $AlCl_3$ intoxication was behind the neurotoxicity observed, and this ratio may be used as an excellent indicator of it.[37] In the same study, the researchers reported that protein renaturation occurred after LS administration and added that LS could help in breaking the hydrogen bonding that stabilizes the structure of the Aβ tau protein that indicates its resolubilization.[37]

TGFβ secreted by microglia decreases the release of the inflammatory mediators O_2 and NO by microglia. The lack of TGFβ-mediated inhibition of microglia inflammatory activation could result in cytotoxicity and neurodegenerative changes, such as observed in AD.[13] In this study, $AlCl_3$-induced AD was associated with a significant increase in serum $TGF\beta_1$ while LS administration significantly reduced it. Increased production of $TGF\beta_1$ in response to inflammatory conditions is considered a regulatory mechanism secondary to cell activation that limits the temporal and spatial extent of neuroinflammation and neurotoxicity.[38] Tesseur et al reported that the level of TGFR2 in AD brains was 50% less in the prefrontal cortex than controls. They added that reducing neuronal TGFβ signaling in a mouse model of AD resulted in age-dependent neurodegeneration and promoted Aβ accumulation and dendritic loss.[21] Based on these study findings and the observations of previous studies, we postulated that the level of $TGF\beta_1$ would be increased in $AlCl_3$-induced AD. It was not functioning, and could not induce its neuroprotective effects because of the marked reduction in TGFR2.

Recent reports have shown that Al^{3+} administration to rodents significantly enhanced oxidative stress and inflammatory mediator levels and neuroinflammation in the hippocampus.[2,30,31,39] Aluminum activates glial cells, which release proinflammatory cytokines. Al^{3+} also activates the transcription factor NFκB and HIF1, which boost the inflammatory process (Cao et al, 2016). In this study, significant upregulation of TNFα and IL6 was confirmed in the AD group while LS significantly reduced it. Therefore, it could be postulated that LS modulated its effect at the molecular level by acting as a natural inhibitor of NFκB. One explanation for the downregulation of TNFα and IL6 levels in the cerebral cortex is the capability of LS to inhibit the NFκB pathway, as previously described.[40] NFκB regulates the expression of various genes, such as *COX2*, *MMP9*, *NOS2*, and *IL8*, and antiapoptotic proteins.[41]

Chronic administration of AlCl3 in this study resulted in a notable increase in lipid peroxidation, manifested by significant increases in serum and brain MDA and NO and significant reductions in the enzymatic antioxidants SOD, CAT, and TAC, while LS alleviated this effect. Therefore, $AlCl_3$ altered neuronal redox potential and promoted cytokine release, which resulted in neurotoxicity in rodents and AD-like symptoms, in line with previous findings.[36,39] The antioxidant activity of LS was documented in this study and many previous ones.[42]

Based on the Bradford Hill criteria, a consensus statement was reached, stating that "elevated plasma total homocysteine, which reflects the functional status of three B vitamins (folate, vitamins B_{12}, B_6), is a modifiable risk factor for development of cognitive decline, dementia, and AD in older persons." In addition, homocysteine-lowering treatment with B vitamins have reported in interventional trials to markedly slow the rate of whole- and regional brain atrophy and slows cognitive decline.[43] This fact is further confirmed by this study. The AD group showed a significant increase in homocysteine, while LS significantly reduced it. On the other hand, the LS-treated AD group showed significantly higher levels of vitamin B_1 and folic acid. It was

found that LS contains significant amounts of folic acid[44] and vitamin B_1 and others (B_2, B_3, B_5, B_6).[45] This interesting finding might focus the light on another possible mechanism of action of LS as a neuroprotective element in AD.

$AlCl_3$-induced AD in this study was associated with degenerative changes, evident by the presence of small, shrunken neural and glial cells with dark and deformed nuclei and many autophagic vacuoles. This was confirmed immunohistochemically by the significant upregulation of caspase 3 and caspase 7 in the cerebral cortex, indicating increased apoptosis and subsequent neuronal loss. These findings were also observed by Zaky et al.[36] Neuronal loss following aluminum exposure was attributed to prooxidant and proinflammatory effects of aluminum that cause oxidative imbalance.[46] The loss of neurons in the hippocampus was reported to affect memory functions.[47] In our previous study, it was observed that $AlCl_3$ influenced Aβ aggregation and plaque formation, which in turn interfered with and disrupted membrane phospholipid structure due to free-radical attacks caused by oxidative stress mediated by $AlCl_3$. On the other hand, LS significantly overcame the signs of $AlCl_3$-induced oxidative stress on membrane lipid and restoreed protein misfolding.[48]

$AlCl_3$-induced AD in this study was also associated with a significant reduction in GFAP immunohistochemistry in the cerebral cortex, which indicated a reduced astrocyte population. This is supported by Wang et al,[49] who added that a reduced astrocytic population due to aluminum toxicity resulted in an alteration in GABA and glutamate levels. Although it has been shown that caspase 3, rather than caspase 7, is the major functional executioner protease during the destruction phase of apoptosis,[50] both of these apoptotic markers were used in this study. They revealed a significant increase in apoptosis in the cerebral cortex by Al^{3+}, which has been previously reported in rats and mice.[51] An antiapoptotic effect of LS based on caspase 3 expression in a d-galactosamine/lipopolysaccharide-induced liver damage model has been reported, and was described to be mostly TNFα-dependent.[40] This could explain the antiapoptotic effect of LS.

In this study, LS induced significant neuroprotection against the $AlCl_3$-induced changes. This might be attributed to its components, which include sinapic acid and silicon.[52] Silicon has been described to significantly reduce Al absorption from the human digestive track and its accumulation in the brain, besides its ability to potentially reduce AD risk and greatly support cognitive function.[53,54] Sinapic acid was found to have antioxidant, anti-inflammatory, and antianxiety activity. With its derivative sinapoyl choline, it acts as an acetylcholinesterase inhibitor, which might have therapeutic uses in diseases like AD,[55] and this was evident in this current study. Among the limitations of this study was the inability to assess the gene-expression levels of the proinflammatory cytokines, which will be conducted in coming work.

In conclusion, this study revealed that chronic $AlCl_3$ administration significantly impaired rats' memory, increased oxidative stress, promoted neuroinflammation, increased expression of AChE enzymes, and induced cytotoxic effects and neuronal loss in the cerebral cortex. $AlCl_3$ seems to induce these effects through cellular oxidative stress and neuroinflammatory reactions. LS ameliorated these effects through its antioxidant, anti-inflammatory, and antiapoptotic effects, suggesting that it has a neuroprotective effect.

Data Sharing

The original contributions presented in the study are publicly available.

Ethics

The animal study was reviewed and approved by the Biomedical Research Ethics Committee, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia (2021-23-2).

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Disclosure

The author has no conflicts of interest to disclose.

References

1. Dey M, Singh RK. Neurotoxic effects of aluminium exposure as a potential risk factor for Alzheimer's disease. *Pharmacol Rep*. 2022;74:439–450. doi:10.1007/s43440-022-00353-4
2. Ravi SK, Ramesh BN, Mundugaru R, Vincent B. Multiple pharmacological activities of Caesalpinia crista against aluminium-induced neurodegeneration in rats: relevance for Alzheimer's disease. *Environ Toxicol Pharmacol*. 2018;58:202–211. doi:10.1016/j.etap.2018.01.008
3. Van Dam D, De Deyn PP. Animal models in the drug discovery pipeline for Alzheimer's disease. *Br J Pharmacol*. 2011;164:1285–1300. doi:10.1111/j.1476-5381.2011.01299.x
4. Ovais M, Zia N, Ahmad I, et al. Phyto-therapeutic and nanomedicinal approaches to cure Alzheimer's disease: present status and future opportunities. *Front Aging Neurosci*. 2018;10:284. doi:10.3389/fnagi.2018.00284
5. Monica Moore M, Díaz-Santos M, Vossel K. Alzheimer's association 2021 facts and figures report; 2021.
6. Sadiq A, Zeb A, Ullah F, et al. Chemical characterization, analgesic, antioxidant, and anticholinesterase potentials of essential oils from Isodon rugosus Wall. ex. Benth. *Front Pharmacol*. 2018;9:623. doi:10.3389/fphar.2018.00623
7. Zohra T, Ovais M, Khalil AT, et al. Extraction optimization, total phenolic, flavonoid contents, HPLC-DAD analysis and diverse pharmacological evaluations of Dysphania ambrosioides (L.) mosyakin & clemants. *Nat Prod Res*. 2019;33:136–142. doi:10.1080/14786419.2018.1437428
8. Chawla R, Thakur P, Chowdhry A, et al. Evidence based herbal drug standardization approach in coping with challenges of holistic management of diabetes: a dreadful lifestyle disorder of 21st century. *J Diabetes Metab Disord*. 2013;12:1–16. doi:10.1186/2251-6581-12-35
9. Attia ES, Amer AH, Hasanein MA. The hypoglycemic and antioxidant activities of garden cress (Lepidium sativum L.) seed on alloxan-induced diabetic male rats. *Nat Prod Res*. 2019;33:901–905. doi:10.1080/14786419.2017.1413564
10. Miller RG, Costacou T, Orchard TJ. Risk factor modeling for cardiovascular disease in type 1 diabetes in the Pittsburgh epidemiology of diabetes complications (EDC) study: a comparison with the diabetes control and complications trial/epidemiology of diabetes interventions and complications study (DCCT/EDIC). *Diabetes*. 2019;68:409–419. doi:10.2337/db18-0515
11. Talia S, Benarous K, Lamrani M, Yousfi M. Lepidine B from lepidium sativum seeds as multi-functional anti- alzheimer's disease agent: in vitro and in silico studies. *Curr Comput Aided Drug Des*. 2021;17:360–377. doi:10.2174/1573409916666200302120305
12. Kaur S, Raj K, Gupta YK, Singh S. Allicin ameliorates aluminium- and copper-induced cognitive dysfunction in Wistar rats: relevance to neuroinflammation, neurotransmitters and Aβ(1–42) analysis. *J Biol Inorg Chem*. 2021;26:495–510. doi:10.1007/s00775-021-01866-8
13. Herrera-Molina R, Flores B, Orellana JA, von Bernhardi R. Modulation of interferon-γ-induced glial cell activation by transforming growth factor β1: a role for STAT1 and MAPK pathways. *J Neurochem*. 2012;123:113–123. doi:10.1111/j.1471-4159.2012.07887.x
14. Kandasamy M, Lehner B, Kraus S, et al. TGF-beta signalling in the adult neurogenic niche promotes stem cell quiescence as well as generation of new neurons. *J Cell Mol Med*. 2014;18:1444–1459. doi:10.1111/jcmm.12298
15. Juraskova B, Andrys C, Holmerova I, et al. Transforming growth factor beta and soluble endoglin in the healthy senior and in Alzheimer's disease patients. *J Nutr Health Aging*. 2010;14:758–761. doi:10.1007/s12603-010-0325-1
16. Eddouks M, Maghrani M, Zeggwagh N-A, Michel J. Study of the hypoglycaemic activity of Lepidium sativum L. aqueous extract in normal and diabetic rats. *J Ethnopharmacol*. 2005;97:391–395. doi:10.1016/j.jep.2004.11.030
17. Sharma S, Agarwal N. *Nourishing and Healing Prowess of Garden Cress (Lepidium Sativum Linn.)-A Review*. NISCAIR-CSIR, India; 2011.
18. Li Z, Zhao G, Qian S, et al. Cerebrovascular protection of β-asarone in Alzheimer's disease rats: a behavioral, cerebral blood flow, biochemical and genic study. *J Ethnopharmacol*. 2012;144:305–312. doi:10.1016/j.jep.2012.09.013
19. Tarragon E, Lopez L, Ros-Bernal F, et al. The Radial Arm Maze (RAM) for the Evaluation of Working and Reference Memory Deficits in the Diurnal Rodent Octodon degus. In: Proceedings of Measuring Behavior 2012, 8th International Conference on Methods and Techniques in Behavioral Research; August 28-31, 2012; Utrecht, The Netherlands. Tests for Mild Cognitive Impairment:98-100.
20. Hirakawa M, Tamura A, Nagashima H, Nakayama H, Sano K. Disturbance of retention of memory after focal cerebral ischemia in rats. *Stroke*. 1994;25:2471–2475. doi:10.1161/01.str.25.12.2471
21. Tesseur I, Zou K, Esposito L, et al. Deficiency in neuronal TGF-beta signaling promotes neurodegeneration and Alzheimer's pathology. *J Clin Invest*. 2006;116:3060–3069. doi:10.1172/JCI27341
22. Packer L. *Superoxide dismutase*. Elsevier; 2002.
23. Gamal M, Moawad J, Rashed L, Morcos MA, Sharawy N. Possible involvement of tetrahydrobiopterin in the disturbance of redox homeostasis in sepsis–Induced brain dysfunction. *Brain Res*. 2018;1685:19–28. doi:10.1016/j.brainres.2018.02.008
24. Exley C, Clarkson E. Aluminium in human brain tissue from donors without neurodegenerative disease: a comparison with Alzheimer's disease, multiple sclerosis and autism. *Sci Rep*. 2020;10:1–7. doi:10.1038/s41598-020-64734-6
25. Exley C, Mold MJ. Aluminium in human brain tissue: how much is too much? *J Biol Inorg Chem*. 2019;24:1279–1282. doi:10.1007/s00775-019-01710-0
26. Kawahara M, Kato-Negishi M. Link between aluminum and the pathogenesis of Alzheimer's disease: the integration of the aluminum and amyloid cascade hypotheses. *J Alzheimers Dis*. 2011;2011:1.
27. Abdulmalek S, Suliman M, Omer O. Possible neuroprotective role of pomegranate juice in aluminum chloride induced Alzheimer's like disease in mice. *J Alzheimers Dis Parkinsonism*. 2015;5:2161–2460.
28. Liang RF, Li WQ, Wang XH, et al. *Aluminium-Maltolate-Induced Impairment of Learning, Memory and Hippocampal Long-Term Potentiation in Rats*. *Industrial health*. 2012;50(5):428–436.
29. Rather MA, Thenmozhi AJ, Manivasagam T, et al. Neuroprotective role of Asiatic acid in aluminium chloride induced rat model of Alzheimer's disease. *Front Biosc*. 2018;10:262–275. doi:10.2741/s514
30. Khalaf NEA, El Banna FM, Youssef MY, et al. Clopidogrel combats neuroinflammation and enhances learning behavior and memory in a rat model of Alzheimer's disease. *Pharmacol Biochem Behav*. 2020;195:172956. doi:10.1016/j.pbb.2020.172956
31. Justin Thenmozhi A, William Raja TR, Manivasagam T, Janakiraman U, Essa MM. Hesperidin ameliorates cognitive dysfunction, oxidative stress and apoptosis against aluminium chloride induced rat model of Alzheimer's disease. *Nutr Neurosci*. 2017;20:360–368. doi:10.1080/1028415X.2016.1144846
32. Zatta P, Zambenedetti P, Bruna V, Filippi B. Activation of acetylcholinesterase by aluminium (III): the relevance of the metal species. *Neuroreport*. 1994;5:1777–1780. doi:10.1097/00001756-199409080-00023

33. Fodero L, Mok SS, Losic D, et al. α7-Nicotinic acetylcholine receptors mediate an Aβ1− 42-induced increase in the level of acetylcholinesterase in primary cortical neurones. *J Neurochem*. 2004;88:1186–1193. doi:10.1046/j.1471-4159.2003.02296.x
34. Lin W-T, Chen R-C, Lu -W-W, Liu S-H, Yang F-Y. Protective effects of low-intensity pulsed ultrasound on aluminum-induced cerebral damage in Alzheimer's disease rat model. *Sci Rep*. 2015;5:1–7.
35. Zaky A, Mohammad B, Moftah M, Kandeel KM, Bassiouny AR. Apurinic/apyrimidinic endonuclease 1 is a key modulator of aluminum-induced neuroinflammation. *BMC Neurosci*. 2013;14:1–12. doi:10.1186/1471-2202-14-26
36. Zaky A, Bassiouny A, Farghaly M, El-Sabaa BM. A combination of resveratrol and curcumin is effective against aluminum chloride-induced neuroinflammation in rats. *J Alzheimers Dis*. 2017;60:S221–S235. doi:10.3233/JAD-161115
37. Balgoon MJ, Ahmed GA-R, Qusti SY, Shaker S. Transit phases of β-amyloid and tau proteins formation and re-solubilisation in AD rat hippocampus tissue as probed by ATR-IR spectroscopy. *Neurol Psychiatry Brain Res*. 2019;31:1–8. doi:10.1016/j.npbr.2018.11.001
38. Saud K, Herrera-Molina R, Von Bernhardi R. Pro-and anti-inflammatory cytokines regulate the ERK pathway: implication of the timing for the activation of microglial cells. *Neurotox Res*. 2005;8:277–287. doi:10.1007/BF03033981
39. Cao Z, Yang X, Zhang H, et al. Aluminum chloride induces neuroinflammation, loss of neuronal dendritic spine and cognition impairment in developing rat. *Chemosphere*. 2016;151:289–295. doi:10.1016/j.chemosphere.2016.02.092
40. Raish M, Ahmad A, Alkharfy KM, et al. Hepatoprotective activity of Lepidium sativum seeds against D-galactosamine/lipopolysaccharide induced hepatotoxicity in animal model. *BMC Complement Altern Med*. 2016;16:501. doi:10.1186/s12906-016-1483-4
41. Karunaweera N, Raju R, Gyengesi E, Münch G. Plant polyphenols as inhibitors of NF-κB induced cytokine production—a potential anti-inflammatory treatment for Alzheimer's disease? *Front Mol Neurosci*. 2015;8. doi:10.3389/fnmol.2015.00024
42. Ahmad A, Nabi R, Mishra A. A Panoramic Review on Lepidium sativum L. *Bio Prosp Therapeut*. 2021;71:233–242. doi:10.1055/a-1334-4101
43. Smith AD, Refsum H, Bottiglieri T, et al. Homocysteine and dementia: an international consensus statement. *J Alzheimers Dis*. 2018;62:561–570. doi:10.3233/JAD-171042
44. Malar J, Chairman K, Singh AR, et al. Antioxidative activity of different parts of the plant Lepidium sativum Linn. *Biotechnol Rep*. 2014;3:95–98. doi:10.1016/j.btre.2014.05.006
45. Shail MD, Neeraj K, Gupta L. Nutritional importance of Lepidium sativum L. (Garden cress/Chandrashoor): a review. *Int J Pharm Anal Res*. 2016;5:152–160.
46. Mesole SB, Alfred OO, Yusuf UA, Lukubi L, Ndhlovu D. Apoptotic inducement of neuronal cells by aluminium chloride and the neuroprotective effect of eugenol in wistar rats. *Oxid Med Cell Longev*. 2020;2020:1–7. doi:10.1155/2020/8425643
47. Erazi H, Ahboucha S, Gamrani H. Chronic exposure to aluminum reduces tyrosine hydroxylase expression in the substantia nigra and locomotor performance in rats. *Neurosci Lett*. 2011;487:8–11. doi:10.1016/j.neulet.2010.09.053
48. Balgoon MJ, Raouf GA, Qusti SY, Ali SS. ATR-IR study of the mechanism of aluminum chloride induced Alzheimer's disease; curative and protective effect of Lipidium sativum water extract on hippocampus rats brain tissue. *Int J Med Health Biomed Bioengi Pharma Eng*. 2015;9:782–792.
49. Wang L, Wang Y, Zhou S, et al. Imbalance between glutamate and GABA in Fmr1 knockout astrocytes influences neuronal development. *Genes*. 2016;7:45. doi:10.3390/genes7080045
50. Walsh JG, Cullen SP, Sheridan C, et al. Executioner caspase-3 and caspase-7 are functionally distinct proteases. *Proc Natl Acad Sci*. 2008;105:12815–12819. doi:10.1073/pnas.0707715105
51. Said MM, Abd Rabo MM. Neuroprotective effects of eugenol against aluminiuminduced toxicity in the rat brain. *Arh Hig Rada Toksikol*. 2017;68:27–36. doi:10.1515/aiht-2017-68-2878
52. Raouf GA, Gashlan H, Khedr A, Hamedy S, Al-jabbri H. In vitro new biopolymer for bone grafting and bone cement. *Int J Lat Res Sci Technol Res*. 2015;4:46–55.
53. Gillette-Guyonnet S, Andrieu S, Vellas B. The potential influence of silica present in drinking water on Alzheimer's disease and associated disorders. *J Nutr Health Aging*. 2007;11:119.
54. Jurkić LM, Cepanec I, Pavelić SK, Pavelić K. Biological and therapeutic effects of ortho-silicic acid and some ortho-silicic acid-releasing compounds: new perspectives for therapy. *Nutr Metab*. 2013;10:1–12. doi:10.1186/1743-7075-10-2
55. Nićiforović N, Abramovič H. Sinapic acid and its derivatives: natural sources and bioactivity. *Compr Rev Food Sci Food Saf*. 2014;13:34–51. doi:10.1111/1541-4337.12041